

New therapies for hereditary angioedema: Disease outlook changes dramatically

Michael M. Frank, MD,^a and Haixiang Jiang, MD, PhD^b Durham, NC

Hereditary angioedema (HAE) is an autosomal dominant disease associated with episodic attacks of nonpitting edema that may affect any external or mucosal body surface. Attacks most often affect the extremities, causing local swelling, the GI tract, leading to severe abdominal pain, and the mouth and throat, at times causing asphyxiation. Most patients with HAE have low levels of the plasma serine protease inhibitor C1 inhibitor. The edema in these patients is caused by unregulated generation of bradykinin. Effective chronic therapy of patients with impeded androgens or plasmin inhibitors has been available for decades, but in the United States, we do not have therapy for acute attacks. Five companies have completed or are in the process of conducting phase 3 clinical trials, double-blind, placebo-controlled studies of products designed to terminate acute attacks or to be used in prophylaxis. Two companies, Lev Pharmaceuticals and CSL Behring, have preparations of C1 inhibitor purified from plasma that have been used in Europe for decades (trade names Cinryze and Berinert P, respectively). One company, Pharming, has developed a recombinant C1 inhibitor preparation. One company, Dyax, is testing a kallikrein inhibitor (ecallantide), and one company, Jerini, is completing testing of a bradykinin type 2 receptor antagonist (Icatibant). Although little has been published thus far, all of these products may prove effective. It is likely that HAE treatment will change dramatically within the next few years. (J Allergy Clin Immunol 2008;121:272-80.)

Key words: Hereditary angioedema, angioedema, bradykinin system antagonists, C1 inhibitor

The prospects for specific therapy in hereditary angioedema (HAE) in America have changed dramatically in the last several years, and it appears that our approach to therapy in just a few years will be quite different from that used currently. The first attempts to treat patients with HAE were entirely empirical. An agent was tried because it was available and thought to be nontoxic (methyltestosterone).¹ There was essentially no rationale for its

Abbreviations used

EACA: Epsilon aminocaproic acid
FDA: US Food and Drug Administration
FFP: Fresh frozen plasma
HAE: Hereditary angioedema

use, but a double-blind study of its use in 1 family showed it to be effective. Because a reasonable rationale was absent, the value of the therapy never became recognized and was not used. A second effective therapy, epsilon aminocaproic acid (EACA), also was origin/ally was used with relatively little rationale. Its value was suggested in a wide screening study, examining the usefulness of the drug in a great many unrelated diseases.² It was then tried as specific therapy for HAE.^{3,4} The first investigators examining its use in HAE all performed limited open trials and found it to be effective. There was some rationale for its use in that it was possible that the enzymes involved in fibrinolysis played a part in HAE attacks, and EACA was a fibrinolysis inhibitor. Interestingly, there is still no clear evidence presented that it functions in HAE as a fibrinolysis inhibitor. These articles led to a double-blind study that proved its effectiveness.⁵ A more conveniently used chemical cousin of EACA, tranexamic acid, also proved effective, although this drug is not available for oral use in America.^{6,7}

Subsequent therapies were developed to test specific hypotheses about the basis of disease. One important group of studies was based on the clinical observation that endocrine factors appeared to be of critical importance in the course of the disease.⁸ The disease was made worse by estrogens. It usually began in childhood but was more severe at puberty. It tended to improve in pregnancy. The individuals who began the studies of impeded androgens were unaware of the original study of methyltestosterone. In a series of double-blind studies, it was shown that the "impeded" (or chemically weakened) androgens were very effective in therapy of HAE, and the surprising finding was made that the drugs tended to normalize the blood levels of C1 inhibitor and C4.⁹⁻¹² It was also realized that clinical response often occurred before normalization of blood values. These agents required chronic administration and did not have a clinical effect for about 48 hours.

With the seminal observation in 1963 that patients with HAE were deficient in the plasma protein C1 inhibitor,¹³ and with the gradual development of better techniques for purifying proteins from donor plasma, it seemed reasonable to replace this missing plasma protein. Currently this is the approach of 3 companies: Lev Pharmaceuticals, New York, NY; CSL Behring, King of Prussia, Pa; and Pharming, Leiden, The Netherlands. With a more concrete understanding of pathophysiology of the disease, more directed rational therapy of attacks has been forthcoming

From ^aPediatrics, Medicine, and Immunology, Duke University School of Medicine; and ^bPediatrics, Duke University Medical Center.

Disclosure of potential conflict of interest: M. M. Frank has consultant arrangements with Lev Pharmaceuticals, CSL Behring, Jerini, and Dyax and has received research support from Lev Pharmaceuticals and CSL Behring. H. Jiang has declared that he has no conflict of interest.

Received for publication October 29, 2007; revised November 26, 2007; accepted for publication November 27, 2007.

Reprint requests: Michael M. Frank, MD, Department of Pediatrics, Duke University Medical Center, Box 3352, Durham, NC 27710-0001. E-mail: frank007@mc.duke.edu.

0091-6749/\$34.00

© 2008 American Academy of Allergy, Asthma & Immunology
doi:10.1016/j.jaci.2007.11.019

(Ecallantide; Dyax, Cambridge, Mass; and Icatibant; Jerini, Berlin, Germany). It appears that the therapeutic armamentarium for the treatment of HAE in America is soon to be expanded markedly. It is of vital importance to patients that this expansion occurs.

Although chronic therapy has markedly altered the lives of patients with HAE, there are many reasons why this therapy is not adequate for some patients and must be considered halfway therapy. Currently in America, there is no adequate treatment for acute attacks; the care available for patients with acute attacks of HAE requires chronic therapy. The new drugs are all rapidly effective and are needed to deal with acute attacks for a number of reasons.

The first of these is that androgens, the mainstay of chronic therapy and useful in prophylaxis are not effective in acute attacks. Impeded androgens are thought to have little clinical effect for at least 48 hours after they are taken. Moreover, effective impeded androgens are not available in intramuscular or intravenous preparations. Only the methylated androgen derivatives have proven effective in treatment; testosterone itself is ineffective as therapy. The effective androgens must be given orally. This is inappropriate in patients who often have attacks of abdominal angioedema with swelling of the bowel, vomiting, and unreliable gastrointestinal absorption. Although at times they have been used, the androgens cannot be used safely in children as chronic treatment and will not be used if other alternatives are available.¹⁴ They may promote premature closure of bony epiphyses with resulting short stature and may cause other chronic problems such as hyperlipidemia, seen in some athletes taking chronic androgen treatment, and liver abnormalities.

Impeded androgens are not appropriate therapy in pregnancy, because ambiguous genitalia have been described in offspring of women taking these agents,¹⁵ although reports in the literature suggest that they do not always cause these side effects. Furthermore, it is clear that a subset of patients does not respond to these drugs. Finally, the androgens have many additional side effects, which, although usually mild, preclude their use in some people. These include mood changes, weight gain, hirsutism, occasional hair loss, bladder telangiectasia, abnormalities of liver function, and so forth.¹⁶ Hepatic adenomas and even carcinomas have been noted in patients on long-term attenuated androgens, although these are rare occurrences.¹⁷⁻¹⁹ Although the side effects are generally mild and often do not preclude administration of the androgens, safer drugs not given chronically are more appealing for proper management.

Like the androgens, the second class of agents used in chronic therapy, the plasmin inhibitors such as EACA and tranexamic acid, are not effective in acute attacks. EACA, the only plasmin inhibitor available for oral use in the United States, for unknown reasons requires about 48 hours after administration to begin to have an effect, at least in our hands. The high doses of EACA required (about 8 g/d in adults) lead to inconvenient administration, and toxic side-effects are common with prolonged use. Tranexamic acid, a similar, more potent product that is available in Europe for oral chronic treatment of HAE, is not available for oral use in this country. Although it is easier to use, it also is felt to have a delayed onset of effect.

Fresh frozen plasma (FFP) has been reported to be used widely in therapy of acute attacks.²⁰ Usually FFP is efficacious; it cures acute attacks in the vast majority of patients by supplying needed C1 inhibitor, but in some patients it may prove dangerous. We

now believe that attacks are a result of uncontrolled activation of the kinin-generating mediator system.²¹ Bradykinin is formed by cleavage of the circulating protein high-molecular-weight kininogen. In infusion of FFP, high-molecular-weight kininogen, present in normal plasma, is administered along with C1 inhibitor. All of those who have taken care of many patients in acute attacks of HAE have found that the rare patient gets worse, presumably because of the cleavage of infused high-molecular-weight kininogen and the release of additional bradykinin. In this country currently, if a patient's angioedema progresses, there is no fall back position; we have no other therapy.

Currently, 5 companies are attempting to make available therapy for acute attacks of HAE. Two of these companies, Lev Pharmaceutical and CSL Behring, are introducing purified C1 inhibitor protein prepared from pooled plasma of thousands of donors. C1 Inhibitor is partially deficient in these patients; therefore, the goal of this therapy is to replace the missing plasma protein. One company, Pharming, is introducing a recombinant C1 inhibitor protein preparation. Dyax is introducing a kallikrein inhibitor, DX-88 or Ecallantide. Because the plasma enzyme kallikrein, acting on the substrate high-molecular-weight kininogen, releases bradykinin, kallikrein inhibition prevents bradykinin generation and therefore is likely to prevent attacks of HAE. Finally, a fifth company, Jerini, is testing a bradykinin type 2 receptor antagonist, Icatibant. Icatibant blocks the bradykinin receptor, thought to be responsible for initiating the plasma leakage associated with the angioedema attack, and therefore is likely to prevent attacks. All the companies are enthusiastic about their products, and preliminary data suggest all will be effective in treating acute attacks. All have gone through phase I and II testing and are in or have completed phase III trials. All have an appropriate rationale for their action.

A major advantage in the development of new therapies for HAE is that studies can be performed relatively rapidly, because attacks are episodic and on the average have a duration of only about 3 days. Many patients on no therapy have an attack frequency of about 1 or more per month. All the current studies are placebo-controlled, double-blind studies with each subject receiving either drug or placebo once. All have a preliminary screening visit at which the diagnosis is confirmed. Patients are to have either low circulating C1 inhibitor protein, true of 85% of patients, or normal levels of C1 inhibitor antigen but low levels of functional C1 inhibitor, low C4, and normal C1q. A complication is that some patients are on danazol or other androgen that tends to normalize their C4 and C1 inhibitor blood levels. Some studies have made allowance for this and allow blood levels performed in the past in evaluation of the patients to be considered. There usually is a requirement for normal blood levels of C1q to rule out entry of patients with acquired C1 inhibitor deficiency.²² All these studies enroll individuals who are relatively early in attacks. Patients usually must enter the study 4 to 6 hours from the start of an attack, because none of the companies want to enter patients whose attacks may resolve spontaneously. If such were the case, it would confound the study by suggesting efficacy of placebo. All studies stipulate that patients continue the medications they have been on chronically. The dose of androgen is not changed once the study starts. Given the potential for life-threatening attacks, it is not considered ethical to discontinue current therapy. Most suggest that narcotic treatment for abdominal pain is not acceptable or is deemed a treatment failure. The type of attack acceptable for each treatment protocol varies from study to study. Some allow

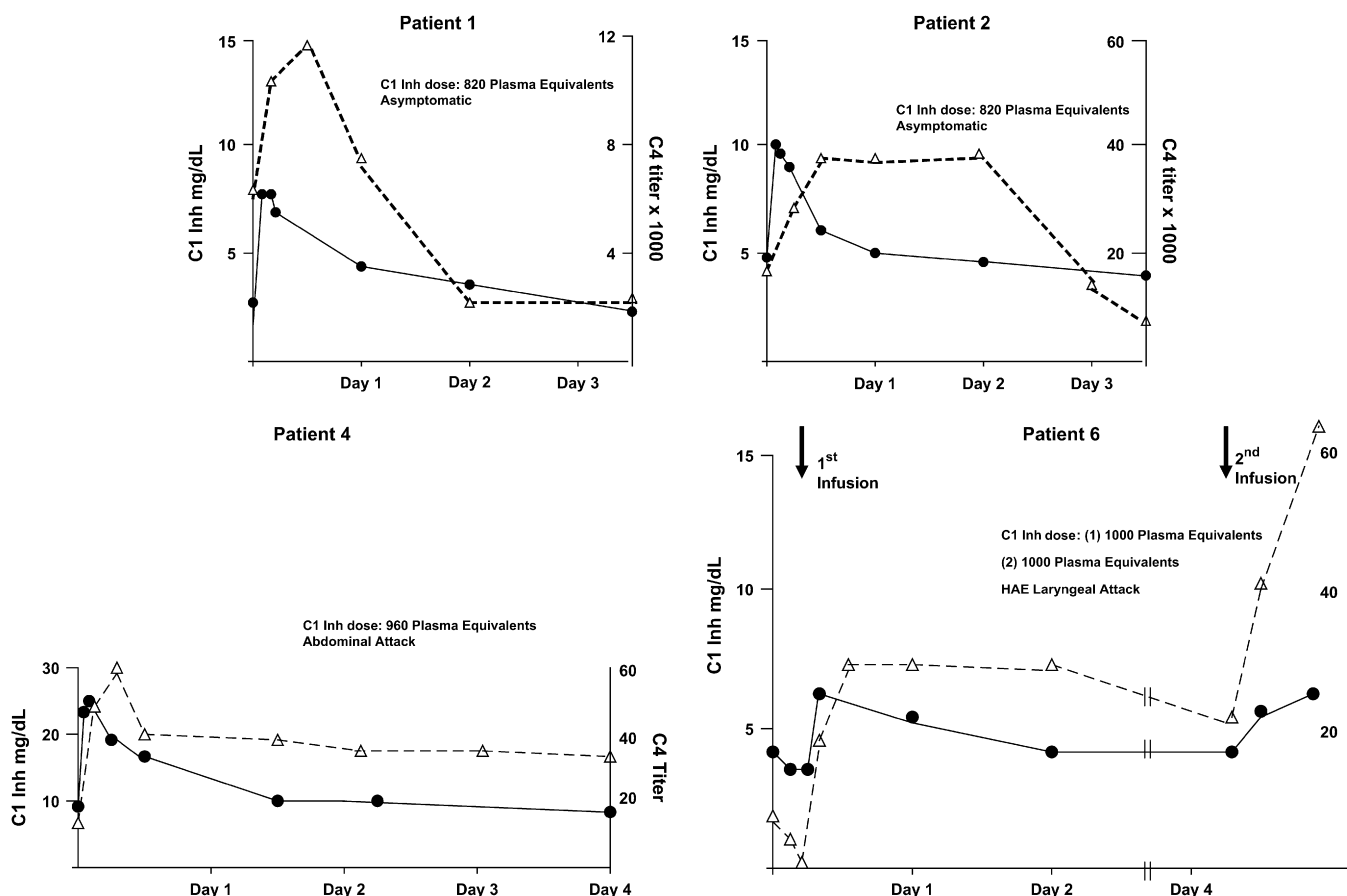


FIG 1. The treatment of 4 patients with HAE with purified C1 inhibitor (C1 Inh) prepared by the American Red Cross. The panels labeled *Patient 1* and *Patient 2* illustrate blood levels of C1 inhibitor and C4 after intravenous infusion of 2 patients who were asymptomatic at the time of infusion. The panels labeled *Patient 4* and *Patient 6* show the response in 2 patients who were treated during an attack. The patient on the right was having a laryngeal attack, and the dose was repeated. Both patients responded to treatment with clearing of symptoms. Adapted from Gadek JE, Hosea SW, Gelfand JA, Santaella M, Wickerhauser M, Triantaphyllopoulos DC, et al. Replacement therapy in hereditary angioedema: successful treatment of acute episodes of angioedema with partly purified C1 inhibitor. *N Engl J Med* 1980;10:542-6.²³

peripheral edema attacks to be included for the protocol; some do not. Some allow facial attacks; some do not. For some studies, the US Food and Drug Administration (FDA) has allowed purified serum C1 inhibitor to be used as a rescue medication if the patient remains in difficulty after the study drug has been used and found to be ineffective. In others, the rescue medication is the drug under investigation used in an unblind fashion. One factor that has slowed drug development is that 5 companies are attempting to enter patients into double-blind trials of a rare disease simultaneously, which has slowed recruitment. Each of the studies involves a relatively small number of patients, and all propose to treat about 70 patients with drug or placebo.

C1 INHIBITOR PURIFIED FROM PLASMA

As mentioned, 2 companies are testing purified C1 inhibitor from plasma. This product has the advantage of having been used successfully in Europe for decades. As noted, Donaldson and Evans¹³ described the deficiency of C1 inhibitor in patients with HAE in 1963, and by the 1970s, multiple organizations that had ongoing purification procedures for the isolation of IgG from plasma turned their attention to isolating C1 inhibitor protein.

In particular, the Dutch Red Cross, Behring Pharmaceuticals in Germany, and the American Red Cross set out to make pilot runs of purified C1 inhibitor. From 1974 to 1976 in the United States, the American Red Cross prepared experimental batches of C1 inhibitor from plasma in a small-scale research facility. Gadek et al²³ reported the biochemical effect of the Red Cross preparation in 8 patients with HAE and documented the effectiveness of the preparation in the treatment of HAE attacks in 5 patients. One patient was treated successfully on 3 separate occasions. Years later, it was discovered that one of the asymptomatic patients treated did not have HAE but had acquired angioedema with circulating monoclonal anti-C1 inhibitor autoantibody. This autoimmune disease that mimics HAE was not described until almost a decade later.²² It is interesting that this patient was the only one who did not have a significant biochemical response to the infusion. With the onset of the AIDS epidemic starting in the United States in 1980, preparation of this plasma protein by the American Red Cross was halted, and the Red Cross never got back to preparing large batches for treatment.

Fig 1 shows the effect of C1 inhibitor in 4 patients with HAE.²³ Two of the patients were having HAE attacks at the time of the infusion. It is not surprising that these data, reported 27 years

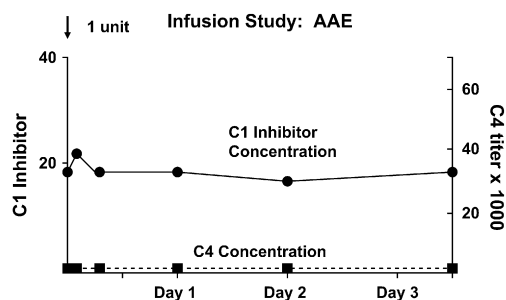


FIG 2. As part of the study illustrated in Fig 1, a patient later found to have acquired C1 inhibitor deficiency and high titer monoclonal anti-C1 inhibitor antibody was infused with C1 inhibitor at a time when the patient was asymptomatic. An increase in C1 inhibitor level was noted only in the first sample obtained, and there was no change in C4 titer. AAE, Acquired angioedema.

ago, are similar to the data being generated now. On intravenous infusion, the plasma C1 inhibitor level rose rapidly, with the C4 level also rising but far later than the C1 inhibitor. The elevated C4 level was sustained longer than that of the C1 inhibitor. The blood level of the C1 inhibitor fell rapidly, reflecting its short half-life in the circulation. In patient 6 illustrated in Fig 1 and treated during an HAE laryngeal attack, a second infusion was given on day 4, again with a rapid rise in C1 inhibitor and a slower rise in C4. All of the patients with HAE having attacks responded to therapy. The lack of biochemical response to therapy in a patient with acquired C1 inhibitor deficiency who was not having an attack is shown in Fig 2. In this HAE-like syndrome, the monoclonal anti-C1 inhibitor antibody led to rapid inactivation and loss of the infused C1 inhibitor. Here, there was no rise in C4, and the level of C1 inhibitor present in the patient showed only a small increase in level just after the beginning of the infusion. The case provides an excellent control showing that functional C1 inhibitor is required for the increased level of C4.

At about this same time, the Dutch Red Cross became interested in purifying plasma C1 inhibitor.²⁴ It is a derivative of this preparation that is being studied in this country currently by Lev Pharmaceuticals. Their first batches of C1 inhibitor were prepared from plasma as early as 1972. Agostoni et al²⁴ reported that the preparation was effective in the treatment of HAE in a case report in 1978 and in a longer report in 1980. In that publication, no detailed biochemical or clinical data were reported. The plasma protein manufacturing arm of the Dutch Red Cross merged into the company Sanquin in 2003. Over the years, the manufacturing techniques used by the company and by all companies preparing serum products have improved. For example, all companies now treat plasma protein preparations with detergents designed to destroy the membranes of enveloped viruses. In addition, during test purification runs, both known enveloped and nonenveloped viruses are added to crude preparations and taken through the purification procedure to be certain that they are removed. In 1989, heat treatment was added to plasma preparation to further reduce the possibility of viral contamination, and more recently, a nanofiltration step has been added by Sanquin to remove agents larger than 15 nm. In some companies, column chromatography is used. These various steps are added to ensure that preparations have little or no danger of contaminating viruses.

Table I shows the effect of nanofiltration and the other steps in purification on a plasma preparation that is purposely contaminated with a series of lipid enveloped viruses or nonlipid

TABLE I. Remarkable fall in titer of several viruses added to a C1 inhibitor preparation during the purification process

Step	Virus reduction during purification				
	Lipid-enveloped virus			Nonlipid-enveloped virus	
	BVDV	HIV	PRV	CPV	HAV
PEG precipitation	>4.5	>5.1	>6.0	>5.4	>2.8
Pasteurization	>6.7	>6.1	>6.7	0.1	>2.8
Nanofiltration	>5.5	>5.6	>6.4	>4.5	>4.9
Total	>16.7	>16.8	>19.1	>10.0	>10.5

BVDV, Bovine diarrheal virus; CPV, canine parvovirus; HAV, hepatitis A virus; PRV, pseudorabies virus; PEG, polyethylene glycol.

In this case, a nanofiltration step was responsible for the fall in titer. Provided by Lev Pharmaceuticals.

enveloped viruses such as canine parvovirus or hepatitis A. Many logs of purification are achieved.

All companies dealing with donated plasma go through relatively similar procedures to prove the efficacy of purification procedures. An FDA requirement is that all plasma used for preparation of C1 inhibitor to be sold in the United States must be obtained from American donors. The plasma is tested for a variety of viruses and antibodies to viruses. All units of donated plasma are stored for several months so the donor can be retested for the development of antibody, for example to HIV-1. In other words, if the donor was infected with a virus and was still in the period before he or she had made antibody, this would be detected by the current testing procedure, and the donated plasma would be discarded before it entered the donor pool.

CSL Behring in Germany also began to prepare C1 inhibitor in the 1970s. It was first licensed as a nonpasteurized product in Germany in 1979 and as a pasteurized product in 1985. It has been approved either as a licensed product or for compassionate use in many European and South American countries since the early 1980s. In normal subjects, it is reported to have a half-life of about 4.5 days.²⁵ In subjects with mild HAE, it has a half-life of about 46.5 hours, and in patients with severe attacks, a half-life of about 31.75 hours. Bork²⁶ first mentioned the use of C1 inhibitor to terminate an attack in a case report in 1979, although no biochemical data were given at that time. Since then, he and his colleagues have reported on its use extensively, but most of the studies are retrospective studies of case reports. Bork directs a center that collects many patients from all over Germany, and large numbers of patients are treated. Possibly because the German physicians tend to not use danazol but tend to treat patients with C1 inhibitor, they have treated enormous numbers of attacks per patient in their population. For example, Bork and Barnstedt²⁷ reported that they had compiled data from a variety of centers that treated 193 attacks of laryngeal edema occurring in 18 patients with 500 to 1000 units of C1 inhibitor, and all responded. Bork et al²⁸ have also reported treatment of 4874 abdominal attacks in 75 patients with a marked shortening of the period of vomiting and diarrhea and a mean time to relief of symptoms of 53.5 minutes. Similarly, Cicardi and Zingale²⁹ have treated large numbers of attacks successfully.

It is important to note that the CSL Behring and Lev products have now been given to hundreds of patients over decades with no infections reported.

The Lev product (Cinryze), manufactured in The Netherlands, has been in phase III clinical trials of treatment of acute attacks. Recently it was announced that these clinical trials have shown a

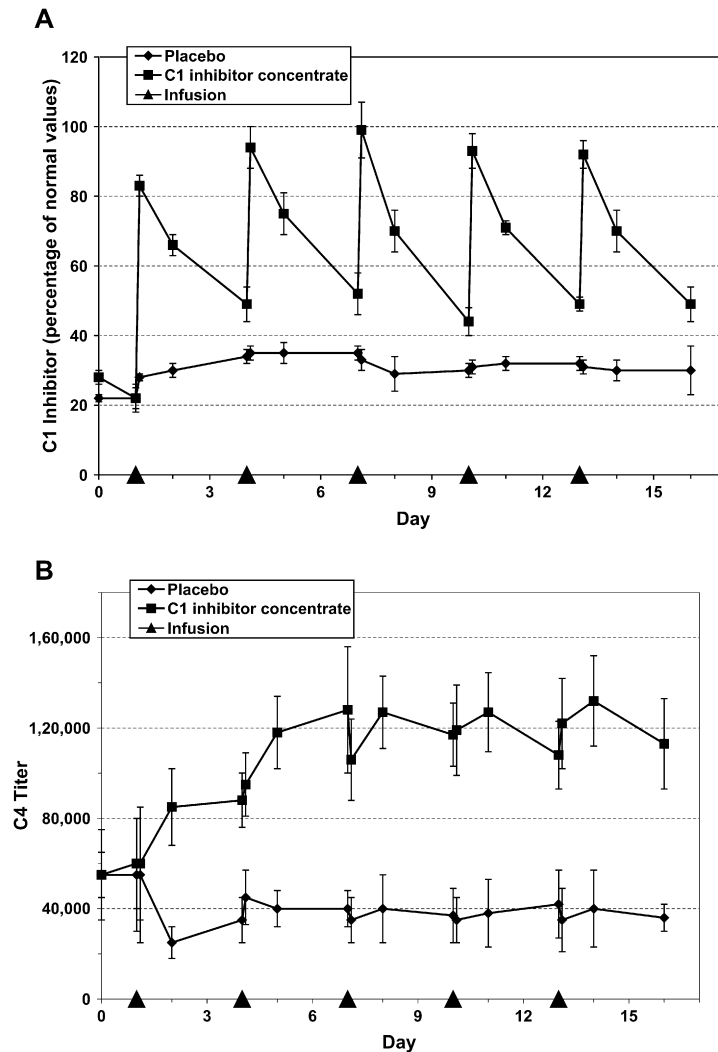


FIG 3. The effect of intravenous infusion of C1 inhibitor or placebo given intravenously every 3 days on the level of C1 inhibitor antigenic protein (**A**) and C4 functional activity (**B**) in a group of patients.

TABLE II. Outpatient infusion data obtained in Waytes et al³⁰

Location of edema	Length of time to response with C1 inhibitor or placebo			
	Response in less than 30 min		Response in less than 240 min	
	C1 inhibitor	Placebo	C1 inhibitor	Placebo
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)

Shown are responses to various types of attacks over time, comparing C1 inhibitor with placebo.

statistically significant treatment difference between drug and placebo. Moreover Lev has also recently reported that in a double-blind trial, a statistically significant difference between drug and placebo in attack frequency was seen in a group of patients using the drug twice a week for prophylaxis. It is believed that the data supporting these findings will be published in the near future. The

CSL Behring product (Berinert P) has just completed phase III clinical trials and the company has announced that both of these preparations have been in use in Europe for well over 20 years, and it is almost certain that the data will prove this product to be effective. As mentioned, both the CSL Behring product, manufactured in Germany, and the Lev product, manufactured in The Netherlands, use plasma collected in the United States and are extensively tested for viruses as required by the FDA. It is of interest that both of these products are far more pure biochemically than the Immuno Pharmaceuticals product that we used a decade ago for our double-blind trials.

The clinical signs of HAE can be markedly influenced by psychological factors, and double-blind study of the use of these agents is of great importance.⁸ For example, we have been told by several of the companies conducting clinical trials that just enrolling patients in a study and assuring them that they will have access to effective products to end attacks has markedly decreased their incidence of attacks, making the studies of course more difficult to complete. The first double-blind study of C1 inhibitor purified from plasma was conducted by Waytes et al³⁰ and reported in 1996. The product tested was a vapor-heated C1 inhibitor prepared

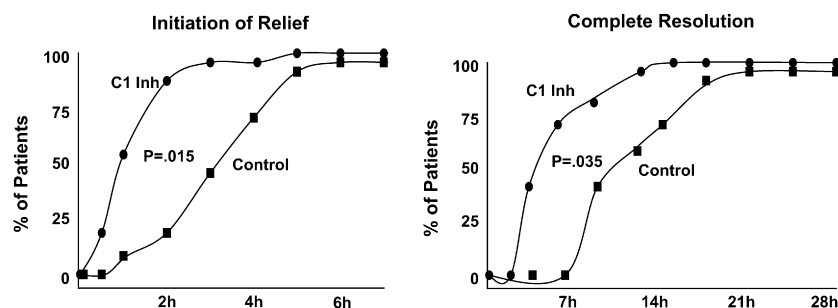


FIG 4. The clinical response of patients to home infusion of C1 inhibitor (C1 Inh). The comparison group shown is a group of historical controls. Adapted from Levi. *J Allergy Clin Immunol*.

from plasma by Immuno Pharmaceuticals, Austria, a product also available in Europe. It was quite impure in terms of percent C1 inhibitor/total protein but was biologically effective. The study was performed in 2 locations. The inpatient portion of the study was performed by Dr Thomas Waytes and Dr Michael M. Frank at the National Institutes of Health. We hospitalized patients with severe disease, randomized their treatment courses, and gave them C1 inhibitor placebo intravenously every 3 days in a double-blind fashion. Their clinical symptomatology was evaluated, and blood was taken for C4 and C1 inhibitor levels. The outpatient portion of the study was performed at Boston Children's Hospital by the late Dr Fred S. Rosen and colleagues. In Boston, patients having acute angioedema attacks were administered C1 inhibitor or placebo in a double-blind fashion and evaluated for the next 4 to 6 hours in an outpatient setting. Patients were asked to estimate whether and when their clinical symptoms began to resolve. Some of our reported findings were used in the development of a clinical trial by Baxter Pharmaceuticals that used the same C1 inhibitor preparations some years later.

Fig 3 shows the results of the inpatient portion of the C1 inhibitor study. As seen in Fig 3, A, infusion of C1 inhibitor led to a rapid rise in blood C1 inhibitor levels and a rapid fall thereafter, reflecting the short half-life of C1 inhibitor in the circulation. Infusion of placebo did not change the level of circulating C1 inhibitor. As in the 1980 study (Fig 3, B), the C4 gradually rose in patients treated with C1 inhibitor to the point that, at about 3 days, the level was above the lower limit of normal for C4. Again, in placebo-treated patients, the level did not change. Symptom scores determined by an observer with no knowledge of treatment were far lower in the patients during infusion of C1 inhibitor. In the outpatient portion of the study, patients were asked to evaluate attacks and determine or estimate when they thought their attacks were improving (Table II). As noted, essentially all of the patients thought they were improving by 4 hours. The only exception was a few patients whose attacks were associated with angioedema of the extremities. In this case, it appears that the absorption of the edema fluid from peripheral sites is slower than absorption from the gastrointestinal mucosa. Many of the patients thought their attacks were starting to resolve at 30 minutes, and this certainly was not true of the patients receiving placebo. Overall, we interpreted this double-blind study to indicate that C1 inhibitor was very effective in terminating attacks of HAE. Unfortunately, the Immuno Pharmaceuticals was sold, and the drug was never licensed for use in America. Because of a series of misadventures, no C1 inhibitor product has been licensed for use in America. This has led in part to the tremendous interest in developing new agents currently.

There is a considerable literature on the effectiveness of open-label C1 inhibitor in treating attacks of HAE. In most cases, the reports have used historical controls. Nevertheless, these reports are very useful. For example, in 2005 and 2006, Bork et al^{28,31} reported estimates of the time to relief of abdominal pain in a large group of patients treated with the plasma purified C1 inhibitor prepared by CSL Behring (Berinert P). Many of the patients started to find relief of symptoms within 30 minutes. Because data in these reports do not come from blind studies, some of the patients were queried afterward about their time to relief. Nevertheless, Bork et al^{28,31} found that in all patients and particularly in the patients with the most severe disease, administration of C1 inhibitor shortened or improved the duration of attacks. In keeping with these results, within the past few weeks the company has reported in a press announcement that their phase III double-blind clinical trial has shown a statistically significant improvement in time to resolution of attacks. Levi et al,³² studying the Sanquin C1 inhibitor preparation, have reported on the use of self-administered C1 inhibitor, as have Longhurst et al.³³ As shown in Fig 4, self-administration at home of C1 inhibitor causes a rapid initiation of symptom relief and early complete resolution of attacks. Again, the control group in this study is not patients given placebo at the time of attack but recollection of patients of their previous attacks; therefore, these numbers may not be entirely accurate. Levi et al³² and Longhurst et al³³ also report that self-administration of C1 inhibitor on a regular basis can decrease attack frequencies, in some cases to 0, and that C1 inhibitor only had to be infused once a week. As mentioned, we infused patients with C1 inhibitor every 3 days in our limited National Institutes of Health study, but the blood level fell back to baseline in this period, and it will be interesting to see whether an infusion as infrequent as once a week will control attacks in a larger group of patients. This is very important, because one of the major problems that patients have is traveling to a hospital emergency department for treatment with a stay that may extend for hours. In studies of plasma-derived C1 inhibitor, the dose of C1 inhibitor used to terminate attacks has been in the range of about 20 to 25 U/kg, with 1 U representing the amount of activity in 1 mL plasma.

RECOMBINANT C1 INHIBITOR

A third C1 inhibitor product for infusion under development is prepared by the Dutch company Pharming. This is a recombinant protein not prepared from human plasma. Here, the human gene for C1 inhibitor has been cloned and introduced into animals, in this case rabbits, under the gene regulatory control of a bovine α

S1-casein promoter.³⁴ Under these conditions, the human protein is secreted in the milk of the lactating rabbit, and the milk can be collected and the human protein isolated. Thus far, we have relatively little published information about the use of this material in HAE, but details of the pharmacokinetics have been reported.³⁵ It has been reported that patients do respond to therapy with this material. Because the rabbit-produced C1 inhibitor is glycosylated differently than the normal human protein, it has a much shorter half-life in the recipient than the native protein.³⁶ The half-life is dose-related. The half-life at 100 U/kg, the dose used to treat acute attacks, is only about 3 hours.³⁵ Use of the product has been shown to lead to the rapid disappearance of C4 fragments from the patient's serum. Because the effect of C1 inhibitor is to prevent the cleavage of C4 and C2 by activated C1, and because this cleavage continues in the absence of C1 inhibitor, the presence of C4 fragments in the circulation is an indication of uncontrolled activation of C1. The disappearance of these fragments suggests that the infused C1 inhibitor was effective in inhibiting C1. Pharming states that the product reliably terminates attacks as noted in phase I and phase II studies, and the company has recently announced that the material has proved to be efficacious in European trials.

KININ PATHWAY INHIBITION

Two products have been developed that directly affect the bradykinin pathway, thought to be directly responsible for angioedema formation. Ecallantide, manufactured by Dyax, is a reversible inhibitor of the enzyme plasma kallikrein.^{37,38} It is a 60-amino acid peptide derived from a Kunitz domain structural backbone with 7 unique amino acids that is synthesized in the yeast *Pichia pastoris*. The kallikrein enzymatic binding site has an on rate for this inhibitor of $2 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ and an off rate of $2 \times 10^5 \text{ s}^{-1}$. Thus it binds very rapidly and is released more slowly. This material can be administered intravenously or subcutaneously. It is usually kept frozen before administration. Ecallantide is quite specific and has a very high apparent KI of 4×10^{11} . The C1 inhibitor has a KI of only 1.7×10^4 for plasma kallikrein. Ecallantide does not strongly inhibit activated C1r or C1s, the 2 enzymatically active subunits of C1; they are inhibited more strongly by C1 inhibitor. It has a relatively low affinity for coagulation factors XIIa and XIa. C1 inhibitor has a relatively low affinity for these latter products as well. When Ecallantide was given to mice in Davis' mouse model of C1 inhibitor deficiency and the animals were challenged with mustard oil, the Ecallantide in a dose-dependent fashion decreased the extravasation into the tissues of albumin with bound Evans blue, suggesting that the drug prevented bradykinin-induced angioedema.³⁹ In an open-label study called EDEMA 0, 9 subjects were given Ecallantide intravenously and had relief of angioedema symptoms.²⁵ EDEMA 1 was a randomized double-blind trial studying increasing doses of Ecallantide given intravenously to define an effective therapeutic dose of the drug. In a group of 49 patients, the drug proved to be effective, with 72.5% of patients having attacks responding to the drug, whereas 25% of patients on placebo responded.⁴⁰ In EDEMA 2, a phase II dose-finding study, it was noted that 92% of patients responded to 5 mg/M², 86% to 10 mg/M², and 100% to 20 mg/M². The drug was found to be effective when administered as a subcutaneous agent, and EDEMA 3 was developed as a phase III trial, examining the usefulness of the agent in a total of 71 patients when given in 2 sites with a total

of 30 mg drug or placebo. The results of this study are currently available in abstract form,⁴¹ and the study reports the agent highly effective at terminating attacks compared with placebo. EDEMA 4, an additional double-blind trial, is currently underway. As mentioned, Ecallantide is a large peptide prepared in a yeast. One patient is reported to have had an anaphylactic response to the subcutaneous injection of the agent and several other patients to have had mild hypersensitivity.⁴²

The final product is Icatibant. Icatibant is a bradykinin type 2 receptor antagonist.⁴³ The bradykinin type 2 receptor is a member of the 7-transmembrane-spanning receptor superfamily. This 10-amino acid synthetic peptide is not inhibitory for the bradykinin I receptor, which optimally recognizes des-Arg-bradykinin. It is a potent, specific, reversible, competitive 10-amino acid peptide antagonist of this receptor with an inhibitory concentration₅₀ of 1.4 nmol/L. Five of the amino acids in the drug's sequence are not natural amino acids, and the drug resists hydrolysis in tissues. Icatibant is 96% bioavailable after subcutaneous injection, and patients are being studied with abdominal, cutaneous, and laryngeal edema attacks. In case reports, it is reported to be effective in treatment of HAE.⁴⁴ Two trials have been completed, FAST-1 and FAST-2. The trial in America (FAST-1) studied drug against placebo. The FAST-2 trial in Europe studied drug against a control agent, tranexamic acid, a drug not thought to be effective in acute attacks. The trial in Europe showed a statistically significant difference between the drug and tranexamic acid, and the trial in America missed statistical significance. In both double-blind studies, the severity data from all types of attacks were given a numerical rating and the data pooled. In the American trial, the response of abdominal pain to drug was consistent with the findings in Europe, but patients receiving placebo responded more quickly than expected. Because the protocol developed by Jerini pooled numerical data from multiple types of attacks, the data analysis may have led to difficulties with adequate comparisons. Like Ecallantide and like purified C1 inhibitor, Icatibant prevents capillary leak in the Davis C1 inhibitor deficient mouse model.⁴⁵ Like the other agents, this agent is likely to be effective in patients.

At this point, there is relatively little published about any of these new clinical trials, but press releases from Lev, Pharming, Dyax, and Jerini are encouraging. The CSL product has been on the market for decades, and there are many publications suggesting that it is effective. Nevertheless, it is clear that all of the products in development have theoretical disadvantages and advantages. The plasma products are likely to be effective because they represent replacement of a missing protein. This protein under physiologic conditions prevents attacks. Plasma protein infusion always carries the risk of infection. In this case, the risk is relatively minor, because according to the manufacturers, no infection has been observed with the current products, and they have been used for more than 20 years. Both start with the blood of healthy donors and go through potent virus reduction purification. A second disadvantage of these products is that they are currently being given intravenously rather than subcutaneously, as are some of the other products. They are more difficult to administer at home. Because all patients are heterozygotes and make normal protein, significant allergy to the administered product is unlikely. It is likely that subcutaneous administration of these products will be examined.

In the case of recombinant C1 inhibitor, glycosylation differs from normal C1 inhibitor, and the half-life of the product is short.

Given the difference in glycosylation, there is some risk of hypersensitivity, although none has been reported, and certainly there is some risk of allergy from contaminating rabbit protein, although the C1 inhibitor itself is highly purified. There are 2 advantages of this product. First, it is not prepared from pooled human plasma; therefore, it is unlikely to contain infectious human pathogens. Second, because it is made in animals, theoretically the supply is limitless.

The kallikrein inhibitor (Ecallantide) and the bradykinin receptor antagonist (Icatibant) are both foreign peptides. In both cases, there is some risk of allergy with repeated administration, and their half-life of action is short. With both, there is a risk of rebound, and the drug dosage may have to be repeated. Icatibant has been associated with minor cutaneous reactions at the site of injection. We know little about the long-term effect of bradykinin inhibition in human beings. Because in most patients these attacks do not occur extremely frequently, and because it is not proposed to use these agents chronically, this is not likely to be a long-term problem. Cardiac difficulties have been seen in animals with chronic administration of high-dose bradykinin receptor antagonists. To our knowledge, this has never been seen in patients administered drug. As mentioned, both products are given subcutaneously, both are relatively inexpensive to make compared with the serum product, and there is no risk of infection. As with the repeated administration of any foreign peptide, there is a risk of sensitization. Ecallantide is a 60-amino acid peptide made by biosynthesis in a yeast, *P. pastoris*, and purified after biosynthesis. Icatibant is a 10-amino acid peptide prepared by direct biosynthesis. One would expect some greater risk of sensitization with repeated use of the larger peptide.

Self-administration of the plasma products has been reported already and it is expected that there will be pressure to develop means of administration that do not involve a prolonged stay in the emergency department of local hospitals. Similarly, there have been prophylactic use trials that are reported to be successful, and it is to be expected that patients who have frequent attacks will opt for prophylaxis. The short-acting agents are not useful for this indication. On the other hand, agents that can be used subcutaneously are easy to use at home. Patients for the most part have a prodrome that indicates to them they are going to have attacks, and there are data to suggest that early treatment of attacks leads to more rapid resolution. Therefore, all of these agents may be expected to be used early to abort attacks.

The plasma products would be expected to be safe in childhood and in pregnancy, because these patients have the product of 1 normal gene in their circulation at baseline, and their use in childhood and pregnancy without complications has been reported in limited numbers of patients. They should also be useful in patients experiencing side effects from androgen therapy and in patients who do not respond to androgens. They should be effective in prophylaxis of surgery and dental procedures. There is too little experience with the 3 other products to recommend their use in pregnancy, but they will probably come to be tried in childhood to treat the rare severe attack. They should be effective in patients who do not respond to androgens and in patients with androgen toxicity and should be effective if a surgical or dental patient has an acute attack.

It should be clear from this discussion that the outlook for new patient therapy appears to be excellent at this time. These new therapies will lead to a totally new approach to treatment of HAE. Many patients currently receiving chronic impeded androgen

therapy will elect to discontinue chronic therapy and treat each acute attack as it occurs. This will certainly be true if there is a convenient drug they can easily use at home. Those more rare patients with many attacks per month may choose to receive chronic prophylaxis with purified C1 inhibitor. Either approach should minimize long-term toxicity and allow appropriate treatment of children and pregnant women.

REFERENCES

- Spaulding WB. Methyltestosterone therapy for hereditary episodic edema (hereditary angioneurotic edema). *Ann Intern Med* 1960;53:739-45.
- Nilsson IM, Andersson L, Bjorkman SE. Epsilon-aminocaproic acid (E-ACA) as a therapeutic agent based on 5 year's clinical experience. *Acta Med Scand Suppl* 1966;448:1-46.
- Lundh B, Laurell AB, Wetterqvist H, White T, Granerus G. A case of hereditary angioneurotic edema, successfully treated with epsilon-aminocaproic acid: studies on C1 esterase inhibitor, C1 activation, plasminogen level and histamine metabolism. *Clin Exp Immunol* 1968;7:733-45.
- Champion RH, Lachmann PJ. Hereditary angio-oedema treated with E-aminocaproic acid. *Br J Dermatol* 1969;10:763-5.
- Frank M, Gelfand JA, Alling DW, Sherins RJ. Epsilon aminocaproic acid for hereditary angioedema. *N Engl J Med* 1977;21:1235-6.
- Sheffer AL, Austen KF, Rosen FS. Tranexamic acid therapy in hereditary angioneurotic edema. *N Engl J Med* 1972;9:452-4.
- Blohm G. Treatment of hereditary angioneurotic oedema with tranexamic acid: a random double-blind cross-over study. *Acta Med Scand* 1972;4:293-8.
- Frank MM, Gelfand JA, Atkinson JP. Hereditary angioedema: the clinical syndrome and its management. *Ann Intern Med* 1976;5:580-93.
- Gelfand JA, Sherins RJ, Alling DW, Frank MM. Treatment of hereditary angioedema with danazol: reversal of clinical and biochemical abnormalities. *N Engl J Med* 1976;26:1444-8.
- Davis PJ, Davis FB, Charache P. Long-term therapy of hereditary angioedema (HAE): preventive management with fluoxymesterone and oxymetholone in severely affected males and females. *Johns Hopkins Med J* 1974;6:391-8.
- Rosse WF, Logue GL, Silberman HR, Frank MM. The effect of synthetic androgens in hereditary angioneurotic edema: alteration of C1 inhibitor and C4 levels. *Trans Assoc Am Physicians* 1976;LXXXIX:122-32.
- Sheffer AL, Fearon DT, Austen KF. Clinical and biochemical effects of stanozolol therapy for hereditary angioedema. *J Allergy Clin Immunol* 1981;3:181-7.
- Donaldson VH, Evans RR. A biochemical abnormality in hereditary angioneurotic edema: absence of serum inhibitor of C' 1-esterase. *Am J Med* 1963;35:37-44.
- Farkas H, Harmat G, Fust G, Varga L, Visy B. Clinical management of hereditary angio-oedema in children. *Pediatr Allergy Immunol* 2002;3:153-61.
- Schwartz RP. Ambiguous genitalia in a term infant due to exposure to danazol in utero. *Am J Dis Child* 1982;136:474.
- Zurlo JJ, Frank MM. The long-term safety of danazol in women with hereditary angioedema. *Fertil Steril* 1990;1:64-72.
- Ziegenfuss J, Carabasi R. Androgens and hepatocellular carcinoma. *Lancet* 1973;7797:262.
- Bork K, Schneiders V. Danazol-induced hepatocellular adenoma in patients with hereditary angio-oedema. *J Hepatol* 2002;5:707-9.
- Crampon D, Barnoud R, Durand M, Ponard D, Jacquot C, Sotto JJ, et al. Danazol therapy: an unusual aetiology of hepatocellular carcinoma. *J Hepatol* 1998;6:1035-6.
- Pickering RJ, Gewurz H, Good RA. Complement inactivation by serum from patients with acute and hypocomplementemic chronic glomerulonephritis. *J Lab Clin Med* 1968;2:298-307.
- Davis AE 3rd. The pathophysiology of hereditary angioedema. *Clin Immunol* 2005;1:3-9.
- Alsenz J, Bork K, Loos M. Autoantibody-mediated acquired deficiency of C1 inhibitor. *N Engl J Med* 1987;22:1360-6.
- Gadek JE, Hosea SW, Gelfand JA, Santaella M, Wickerhauser M, Triantaphyllopoulos DC, et al. Replacement therapy in hereditary angioedema: successful treatment of acute episodes of angioedema with partly purified C1 inhibitor. *N Engl J Med* 1980;10:542-6.
- Agostoni A, Cicardi M, Bergamaschini L, Boccassini G, Tucci A. (1980) C1-inhibitor concentrate for treatment of hereditary angioedema. *N Engl J Med* 1980;9:526-7.
- Zuraw BL. Novel therapies for hereditary angioedema. *Immunol Allergy Clin North Am* 2006;4:691-708.
- Bork K. Hereditäres angioneurotisches Odem. Klinik sowie erweiterte diagnostische und therapeutische Möglichkeiten. *Dtsch Med Wochenschr* 1979;11:405-9.

27. Bork K, Barnstedt SE. Treatment of 193 episodes of laryngeal edema with C1 inhibitor concentrate in patients with hereditary angioedema. *Arch Intern Med* 2001;5:714-8.
28. Bork K, Meng G, Staubach P, Hardt J. Treatment with C1 inhibitor concentrate in abdominal pain attacks of patients with hereditary angioedema. *Transfusion* 2005; 11:1774-84.
29. Cicardi M, Zingale L. How do we treat patients with hereditary angioedema. *Transfus Apher Sci* 2003;3:221-7.
30. Waytes AT, Rosen FS, Frank MM. Treatment of hereditary angioedema with a vapor-heated C1 inhibitor concentrate. *N Engl J Med* 1996;334:1630-4.
31. Bork K, Staubach P, Eckardt AJ, Hardt J. Symptoms, course, and complications of abdominal attacks in hereditary angioedema due to C1 inhibitor deficiency. *Am J Gastroenterol* 2006;3:619-27.
32. Levi M, Choi G, Picavet C, Hack CE. Self-administration of C1-inhibitor concentrate in patients with hereditary or acquired angioedema caused by C1-inhibitor deficiency. *J Allergy Clin Immunol* 2006;4:904-8.
33. Longhurst HJ, Carr S, Khair K. C1-inhibitor concentrate home therapy for hereditary angioedema: a viable, effective treatment option. *Clin Exp Immunol* 2007;1:11-7.
34. Reuser AJ, Van Den Hout H, Bijvoet AG, Kroos MA, Verbeet MP, Van Der Ploeg AT. Enzyme therapy for Pompe disease: from science to industrial enterprise. *Eur J Pediatr* 2002;(Suppl):S106-11.
35. van Doorn MB, Burggraaf J, van Dam T, Eerenberg A, Levi M, Hack CE, et al. A phase I study of recombinant human C1 inhibitor in asymptomatic patients with hereditary angioedema. *J Allergy Clin Immunol* 2005;4:876-83.
36. Koles K, van Berkel PH, Pieper FR, Nuijens JH, Mannesse ML, Vliegenthart JF, et al. N- and O-glycans of recombinant human C1 inhibitor expressed in the milk of transgenic rabbits. *Glycobiology* 2004;1:51-64.
37. Williams A, Baird LG. DX-88 and HAE: a developmental perspective. *Transfus Apher Sci* 2003;3:255-8.
38. Levy JH, O'Donnell PS. The therapeutic potential of a kallikrein inhibitor for treating hereditary angioedema. *Exp Opin Investig Drugs* 2006;15:1077-90.
39. Han Lee ED, Pappalardo E, Scafidi J, Davis AE 3rd. Approaches toward reversal of increased vascular permeability in C1 inhibitor deficient mice. *Immunol Lett* 2003; 2-3:155-60.
40. Schneider L, Lumry W, Vegh A, Williams AH, Schmalbach T. Critical role of kallikrein in hereditary angioedema pathogenesis: a clinical trial of Ecallantide, a novel kallikrein inhibitor. *J Allergy Clin Immunol* 2007;2:416-22.
41. Levy R, McNeil D, Li H, Sheffer A, Beck T, Roberts J, et al. Results of a phase 3 double-blind, placebo-controlled trial, EDEMA 3: a study of subcutaneous DX88 (Ecallantide) in patients with hereditary angioedema [abstract]. Arlington Heights (IL): The American College of Allergy, Asthma and Immunology; 2007.
42. Caballero T, Lopez-Serrano C. Anaphylactic reaction and antibodies to DX-88 (kallikrein inhibitor) in a patient with hereditary angioedema. *J Allergy Clin Immunol* 2006;2:476-7; discussion 477.
43. Bas M, Bier H, Greve J, Kojda G, Hoffmann TK. Novel pharmacotherapy of acute hereditary angioedema with bradykinin B2-receptor antagonist icatibant. *Allergy* 2006;12:1490-2.
44. Bork K, Frank J, Grundt B, Schlattmann P, Nussberger J, Kreuz W. Treatment of acute edema attacks in hereditary angioedema with a bradykinin receptor-2 antagonist (Icatibant). *J Allergy Clin Immunol* 2007;6:1497-503.
45. Han ED, MacFarlane RC, Mulligan AN, Scafidi J, Davis AE 3rd. Increased vascular permeability in C1 inhibitor-deficient mice mediated by the bradykinin type 2 receptor. *J Clin Invest* 2002;8:1057-63.