

Novel Therapies for Hereditary Angioedema

Bruce L. Zuraw, MD

*University of California San Diego, 9500 Gilman Drive, Mailcode 0732,
La Jolla, CA 92093-0732, USA*

Despite remarkable progress in understanding the molecular basis of C1 inhibitor (C1INH) deficiency (see article by Wagenaar-Bos and Hack elsewhere in this issue) and the mechanism of swelling in hereditary angioedema (HAE) (see article by Davis elsewhere in this issue), the pharmacologic options available for the treatment of HAE in the United States have remained virtually unchanged over the past 40 years [1,2]. Prophylactic use of alpha-alkylated androgens or anti-fibrinolytic agents are generally successful in decreasing the number and severity of angioedema attacks; however, these medications are associated with significant adverse effects and do not successfully address the treatment of acute attacks. Taken together, these problems indicate a significant unmet need for improved treatment options of HAE in the United States.

Fortunately, a variety of novel therapies for HAE are currently undergoing clinical evaluation for use in HAE under Food and Drug Administration (FDA) orphan drug designation. The rationale for the development and use of each of these novel molecules is based on the understanding of the molecular mechanisms that underlie HAE, suggesting that they will be more effective and safer than currently available treatments. This article will review the status of these emerging therapies, and provide a framework for considering their use in patients with HAE. These molecules define two separate approaches to the treatment of HAE: replacing the missing C1INH function; or inhibiting the kinin pathway that is responsible for the increased vascular permeability responsible for angioedema. [Table 1](#) summarizes the key benefits and drawbacks of each of the molecules undergoing clinical trial for use in HAE.

This work was supported in part by Grants AI36220 and RR00833 from the National Institutes of Health.

E-mail address: bzuraw@ucsd.edu

Table 1
Comparison of advantages and disadvantages of new drugs

Drug	Advantages	Disadvantages
Plasma-derived C1INH	Gold standard; extensive clinical experience; inhibits classical complement pathway and plasmin as well as bradykinin generation; long plasma half-life	Intravenous administration; potential viral/infectious contamination
rhC1INH	Same inhibitory spectrum as plasma-derived C1INH; no human viral/infectious risk; ability to be scaled up to easily produce as much product as needed	Intravenous administration; short half-life; risk of allergic reaction to contaminating rabbit protein; potential for xenotransmission of infectious agent
DX-88	More potent plasma kallikrein inhibitor than C1INH; no infectious risk; subcutaneous administration	May be antigenic; risk of anaphylactic reactions or development of neutralizing antibodies; short half-life; occasional nonresponder or rebound attack
Icatibant	Small peptide that is nonimmunogenic; no infectious risk; subcutaneous administration	Local discomfort at site of injection; short half-life; occasional nonresponder or rebound attack

Abbreviations: C1INH, C1 inhibitor; rh, recombinant human.

Replacement of C1 inhibitor

Rationale

In 1963, Donaldson and Evans demonstrated that the pathophysiologic basis of HAE was a deficiency of C1INH [3], a discovery that was soon recognized to also explain the lack of kallikrein inhibitory activity in HAE patient plasma observed the year before by Landerman et al [4]. While the precise mediator of the swelling remained an issue of some controversy for many years, it became immediately apparent that even partial replacement therapy to increase plasma C1INH levels could be an effective strategy to treat attacks of HAE. Early studies by Spath et al [5] demonstrated that plasma C1INH levels below 38% of normal were associated with evidence of enhanced complement activation. Anabolic androgens provided significant protection against attacks of angioedema when used at levels that increased plasma C1INH activity only marginally [6], thus suggesting that it was not necessary to normalize C1INH levels to achieve a good response. The success achieved by treatment of acute attacks of HAE with fresh frozen plasma (FFP) was also a harbinger of the potential efficacy of C1INH replacement therapy [7]. The clinical use of FFP, however, is complicated by questions regarding its safety with respect to viral or other infectious

risk as well as concern by some investigators that under certain circumstances it can lead to rapid worsening of the angioedema. Taken together, these observations strongly supported the concept that C1INH replacement therapy can be used to treat attacks of angioedema.

While the major mediator of hereditary angioedema is almost certainly bradykinin (see article by Davis elsewhere in this issue), some investigators believe that other mediators may contribute to the vascular permeability defect. The efficacy of anti-fibronolytic drugs in prophylaxis has suggested to some that plasmin may also be important in triggering attacks. A theoretic advantage of C1INH therapy for acute attacks HAE compared with therapy directed against bradykinin generation or action is thus the ability of C1INH to inhibit complement proteases and plasmin in addition to contact system proteases.

Existing clinical experience with C1 inhibitor replacement therapy

Efficacy of plasma-derived C1 inhibitor

The efficacy of plasma C1INH as replacement therapy for acute attacks of HAE was confirmed more than 25 years ago [8–10]. Multiple other studies over the past 25 years have led to C1INH concentrates becoming the preferred modality of treatment for acute attacks of HAE in countries where it is available [11–14]. C1INH infusion results in an immediate increase in C1INH plasma levels, rapid improvement in symptoms (typically within 30 to 60 minutes), and a delayed increase in C4 levels (reflecting that this represents newly synthesized C4) [15–17]. The efficacy of C1INH concentrates appears to be equal for all types of HAE attacks, including laryngeal attacks where it can be lifesaving. Noteworthy in this regard is a report detailing the use of C1INH concentrate (Behriner-P, ZLB Behring) for the treatment of 193 episodes of laryngeal angioedema in HAE patients without a single failure [12]. A history of 517 attacks of laryngeal angioedema was obtained in 42 patients from a cohort of 95 HAE patients. Of these, 193 episodes in 18 patients were treated with C1INH (500 to 1000 plasma units; 1 plasma unit is defined as being equal to the amount of C1INH in 1 mL of normal human plasma or approximately 270 $\mu\text{g/mL}$). Beginning of improvement in the laryngeal angioedema was noted in an average of 42.2 ± 19.9 minutes in the C1INH treated patients, and none of the treated patients required intubation. Resolution of the swelling symptoms occurred in an average of 15.3 ± 9.3 hours in the C1INH treated group compared with 100.8 ± 26.2 in the historical control group not treated with C1INH. Cicardi and Zingale [18] reported that C1INH concentrates were successful in the treatment for 629 of 630 acute attacks in their HAE population. They also point out that a beneficial response to C1INH infusion can be used as a reliable marker for the decision to avoid surgery and other expensive or invasive testing in patients with HAE who present with signs and symptoms of a possible “surgical abdomen.”

Bork and colleagues [19] have also published a retrospective analysis of 131,110 angioedema attacks in 221 patients with HAE. Interestingly, the etiology of unusual symptoms (headache, urinary or renal retention/pain, muscle and joint swelling/pain, chest tightness/pressure, and painful swallowing) in this cohort was suggested to be angioedema by the ability of C1INH infusions to induce resolution of symptoms [19].

Four placebo-controlled double blind studies of C1INH for acute attacks of HAE have been performed. In 1996, Waytes and colleagues [20] published the results of two double-blind placebo-controlled studies comparing plasma-derived C1INH (25 plasma units/kg; Immuno AG) to placebo. The first was a crossover study involving prophylactic treatment of six severely affected HAE patients who received study drug every 3 days. Subjects were admitted for 17 days on two occasions (separated by at least 3 weeks), receiving five treatments during each admission. During the admission where they received C1INH, subjects increased their plasma C1INH functional levels, normalized their C4 titers, and had significantly less swelling than they did during the admission where they received placebo. The second study assessed the time to improvement following study drug in 22 patients with acute attacks of HAE. The beginning of relief occurred significantly faster in C1INH treated patients than in placebo-treated patients (55 versus 563 minutes). A third placebo-controlled randomized trial of Immuno C1INH by Kunschak and colleagues [17] also demonstrated efficacy of the C1INH compared with placebo in acute HAE attacks. Pharmacokinetic analysis of the Immuno C1INH in this study revealed an apparent half-life of 37.87 ± 19.75 hours during HAE attacks. Finally, a pivotal phase III trial of this drug, conducted by Baxter Health Care, surprisingly failed to show any improvement of the C1INH compared with placebo in acute HAE attacks. The failure of this study has been generally attributed to problems in the study design (60-minute crossover). Subsequent to this study, the Baxter C1INH product (identical to the Immuno C1INH) was withdrawn from the market worldwide.

Although C1INH is not available in the United States, current practice in Europe and Canada is to treat significant acute attacks of HAE with 500 to 1000 plasma units of C1INH by intravenous infusion [21]. The median functional half-life of infused C1INH has been estimated to be 4.48 days in healthy subjects, 46.5 hours in subjects with mild HAE, and 31.75 hours in subjects with severe HAE [21]. While not available as a licensed drug in the United States, a number of US patients import C1INH (Behrinert-P or Ceter) under the FDA personal importation program [22].

In addition to its use for acute attacks of HAE, C1 inhibitor concentrates have also been successfully used for both short-term and long-term prophylactic treatment of HAE. Long-term prophylaxis with C1INH may be particularly useful for patients with very severe disease and possibly during pregnancy in women who experience an increased frequency of attacks. Bork and Witzke [23] reported the successful use of C1INH concentrate

for long-term prophylaxis in a patient with HAE with frequent attacks that could not be well controlled with androgens or anti-fibrinolytic drugs. Subsequently, other investigators have reported the successful use of long-term prophylactic C1INH (two to three times per week) for patients with severe HAE [21]. Long-term prophylaxis with C1INH has also been successfully used in pregnancy [24]. For short-term prophylaxis, purified C1INH should be at least as effective as fresh frozen plasma but with improved safety parameters. A number of reports have also demonstrated the efficacy of short-term prophylaxis with C1INH before dental work or surgery [25–28].

C1INH infusions have also been shown to be effective in the treatment of acute attacks of angioedema in patients with acquired C1INH deficiency. Even patients with acquired C1INH deficiency and anti-C1INH autoantibodies respond to C1INH infusion, although the dose may need to be increased in this setting [29]. As expected, however, C1INH infusion was shown to be ineffective in patients with type III HAE [30].

Because C1INH replacement requires intravenous administration of the drug, patients have needed to go to emergency rooms or physicians' offices to get the infusions. Because of the delay and inconvenience inherent in this approach to treatment, a number of investigators have allowed patients to self-administer C1INH at home after receiving instruction in proper technique. Self-administration of C1INH concentrate in patients with frequent or severe attacks of the angioedema for on-demand treatment of severe attacks (31 subjects, including 28 patients with HAE and 3 patients with acquired C1INH deficiency) or for prophylaxis (12 subjects, including 10 patients with HAE and 2 patients with acquired C1INH deficiency) was recently reported by Levi and colleagues [31]. Self-administration of C1INH was shown to be both effective and safe for both therapeutic and prophylactic use. Furthermore, the interval between onset of symptoms and initiation of therapy was significantly decreased in patients receiving self-administration (1.4 ± 1.0 hours) compared with conventional treatment (3.4 ± 2.1 hours). The earlier initiation of therapy was associated with a markedly reduced time to complete resolution of symptoms in patients during self-administration (5.9 ± 2.2 hours) compared with conventional treatment (13.8 ± 2.9 hours).

Safety of plasma-derived C1 inhibitor

C1INH concentrates are typically prepared from human plasma (except for the new recombinant transgenic C1INH; see the section "Recombinant transgenic human C1INH"); therefore, viral safety is a critical and ongoing issue that must be addressed prospectively and then continuously monitored. Before the incorporation of viral inactivation steps into the manufacturing process, cases of hepatitis C virus (HCV) transmission from C1INH concentrates were reported; however, this problem has been successfully addressed in the current group of concentrates by combining careful screening of plasma donors, a variety of viral inactivation steps during manufacturing, and polymerase chain reaction (PCR) monitoring for

viral contamination. Cicardi and Zingale [18] report that more than 80% of patients receiving the earliest C1INH concentrates became infected with HCV (without any instance of documented HIV infection). In contrast, they report that use of a steam-heated C1INH (Immuno) in several hundred patients resulted in only a single case in which HCV conversion could not be excluded as being due to the concentrate. Waytes and colleagues [20] found no instances of seroconversion after 106 infusions of steam-heated C1INH (Immuno). A review of the use of pasteurized C1INH (Berinert P) between 1985 and 2003 found no documented cases of viral transmission [21].

Rare instances of anaphylactoid reactions to C1INH infusions have been reported [32]. Waytes and colleagues [20] screened for evidence of autoantibodies to C1 inhibitor that could have resulted from exposure to the concentrates in sera from all patients in their prophylaxis study and 13 patients in their treatment study. Notably, all samples were negative for anti-C1INH autoantibodies. Recently, elevated levels of IgM anti-C1INH autoantibodies were reported in patients with HAE compared with healthy controls; however, the titer of antibody did not bear any relationship to history of exposure to C1INH infusions [33].

Drugs in current clinical trials

Based on the demonstrated efficacy of C1INH concentrate therapy in HAE (virtually 100% response) together with the excellent safety profile of concentrates made using the newer manufacturing methods, licensing of C1INH replacement therapy in the United States is anticipated following a successful phase III study. There are three studies currently under way in the United States with the intent of leading to licensure of C1INH products. Each of these studies is briefly described below.

Plasma-derived C1 inhibitor concentrates

Lev Pharmaceuticals is conducting a phase III study of a nanofiltered pasteurized C1INH concentrate (C1INH-nf). This product is manufactured using a similar process as the current Ceter C1INH product (Sanguin). Ceter C1INH is widely used in The Netherlands, and has been shown to be extremely safe. The only difference between the C1INH-nf and Ceter products is the addition of a double nanofiltration step at the end of the purification for the C1INH-nf. Nanofiltration provides an extra margin of safety by excluding viral particles based on size rather than a particular physicochemical property of the virus. Nanofiltration is a robust and reliable viral reduction technique that predictably removes more than 4 to 6 logs of a wide range of viruses, including both enveloped and nonenveloped viruses [34]. There is also some data indicating that nanofiltration may remove prions [34]. The Lev C1 inhibitor in hereditary angioedema nanofiltration generation evaluating efficacy (CHANGE) study is composed of two separate randomized double-blind placebo-controlled studies of C1INH-nf.

The first is studying the efficacy and safety of C1INH-nf for the treatment of acute attacks of HAE. The second is a study of the efficacy of C1INH-nf for long-term prophylactic treatment of severe HAE.

ZLB Behring is conducting a phase II/III study of Behrinert P for the treatment of acute attacks of HAE. As reviewed above, Behrinert P has a long track record of safety and efficacy. The current study is a placebo-controlled, dose-finding, 3-arm, double-blind clinical trial in subjects with HAE during acute episodes of angioedema. The three treatment groups will receive one of the following: Behrinert P 10 U/kg, Behrinert P 20 U/kg, or placebo.

Recombinant transgenic human C1 inhibitor

Pharming Technologies BV (Leiden, The Netherlands) engineered rabbits that express a human C1INH transgene under the control of a mammary gland-specific promoter. The resulting transgenic recombinant human C1INH protein (rhC1INH) is secreted into the breast milk of female rabbits. Purification of the rhC1INH from the breast milk yields large amounts of human C1INH, which is fully functional but exhibits subtle differences in posttranslational glycosylation compared with plasma-derived human C1INH [35]. The rhC1INH was administered to 12 asymptomatic patients with HAE at doses ranging from 6.25 to 100 U/kg [36]. No side effects were observed, and the intravenous administration of this drug was associated with a rapid increase in functional plasma C1INH activity and a corresponding fall in C4 activation, followed by a slower increase in C4 levels. The half-life of the protein was dose dependent and was longest at the highest dose used (100 U/kg) where it was estimated to be 3 hours. The significantly faster clearance of rhC1INH from the plasma space compared with plasma-derived C1INH is presumably caused by the glycosylation differences in the transgenic protein.

A number of HAE patients have now been successfully treated for acute attacks of angioedema with the rhC1INH in an open-label phase II study, and a report of two successfully treated patients has been published [37]. Because the rhC1INH is purified from rabbit milk, there is potential concern regarding contamination of the C1INH concentrate with trace amounts of rabbit protein that could cause allergic reactions. The efficient purification process used by Pharming is suggested to minimize this risk, and a final assessment awaits further clinical experience. Based on the promising safety and efficacy data from the phase I and II studies, Pharming has now initiated a phase II/III study of its rhC1INH in the United States.

The potential advantages of rhC1INH include the ability to scale up production as needed and the freedom from potential contamination with viruses from human plasma. Whether the shorter plasma half-life of the rhC1INH has any clinical implications for treatment of acute attacks remains to be clearly demonstrated; however, the half-life of the rhC1INH

would likely make this product less desirable than plasma-derived C1INH for prophylactic treatment.

Therapeutic uses of C1 inhibitor for conditions other than angioedema

C1INH is a broadly anti-inflammatory molecule [38], and it will likely be evaluated for its therapeutic potential in a variety of other situations. While the use of C1INH concentrates for nonangioedema indications is beyond the scope of this article, it has been reviewed in several recent articles [13,38–44].

Inhibition of bradykinin-mediated enhanced vascular permeability

Rationale

C1INH inhibits a number of different proteases, including the classical complement proteases C1r and C1s, the contact system proteases plasma kallikrein and factor XIIa (activated Hageman factor), the fibrinolytic protease plasmin, the coagulation factor XIa, and mannan-binding lectin (MBL)-associated serine proteases (MASPs). Most importantly, C1INH is the primary inhibitor of the complement proteases C1r and C1s and the contact system proteases plasma kallikrein and factor XIIa. During attacks of angioedema, a variety of abnormalities are seen in these proteolytic pathways. Activation of the early components of the classic complement pathway is a hallmark of HAE, and this is increased during episodes of angioedema. Similarly, contact system activation is seen during attacks of angioedema [45,46].

The mediator of angioedema in HAE was controversial for many years, with investigators favoring either C2-kinin (a product of complement plus plasmin activation) [47,48] or bradykinin (a product of contact system activation) [4,45,46,49–51]. As discussed in the article by Davis elsewhere in this issue, there is now very strong evidence that bradykinin is the major if not sole mediator of swelling in HAE. Based on the emerging consensus that bradykinin causes the swelling of HAE, therapeutic strategies to inhibit bradykinin generation or bradykinin action have been considered. Fig. 1 shows a schematic of the plasma contact system and demonstrates potential therapeutic modalities that may be useful to decrease bradykinin-mediated increases of vascular permeability. The first, C1INH replacement, has been reviewed above. The others, inhibition of plasma kallikrein activity and antagonism of the effect of bradykinin at its receptor, are reviewed below. The apparent efficacy of both of these drugs in treating acute attacks of HAE (see the following sections) strongly supports the previous scientific presumptions that bradykinin is the key mediator of angioedema in this disease.

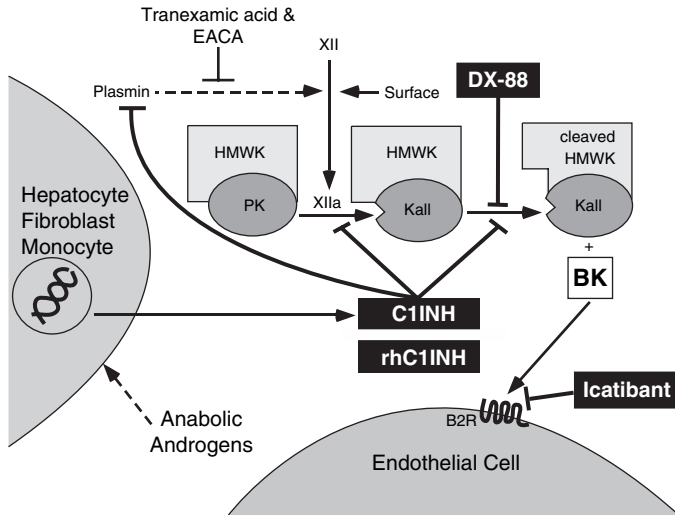


Fig. 1. Mechanisms of the action of the novel therapeutic drugs for the treatment of HAE based on their impact on bradykinin generation and effect. Plasma prekallikrein (PK) circulates in complex with high-molecular-weight kininogen (HMWK). Upon activation of the contact system, coagulation factor XII and prekallikrein are proteolytically activated to XIIa and kallikrein (Kall), respectively. Kallikrein then cleaves high-molecular-weight kininogen, releasing bradykinin (BK), the major active peptide involved in attacks. Bradykinin binds to bradykinin type 2 receptors (B2R) on endothelial cells, thereby increasing vascular permeability, which causes the edema. Anabolic androgens increase plasma levels of normal C1INH, although it is unclear whether this actually involves increased gene expression. Plasmin has been reported to release BK from HK, and the antifibrinolytics (EACA and tranexamic acid) may act by inhibiting activation of factor XII by plasmin. C1INH and rhC1INH inhibit both XIIa and kallikrein (as well as C1r, C1s, and plasmin). DX-88 specifically inhibits kallikrein, and Icatibant blocks binding of bradykinin to B2R on endothelial cells.

Plasma kallikrein inhibition

As discussed in the preceding section and reviewed in the accompanying chapter by Dr. Davis, activation of plasma kallikrein and generation of bradykinin appear to be the major cause of the increased vascular permeability in HAE. Inhibition of plasma kallikrein, which generates bradykinin from high molecular weight kininogen substrate, is thus a logical strategy to treat attacks of HAE. The first plasma kallikrein inhibitor, other than C1INH, to be used for the treatment of HAE was aprotinin (Trasylol), a broad spectrum serpin that has more potent inhibitory activity against trypsin and plasmin than it does against kallikrein. Aprotinin is primarily used in cardiac surgery to prevent excessive blood loss and inflammation. While aprotinin was shown to be effective for the treatment of acute attacks of HAE [10,52], it is a bovine protein (derived from cow lung) and its administration has been associated with severe anaphylactic reactions, rarely on first exposure, but particularly on repeated exposure [53,54]. The use of aprotinin for

HAE, which is characterized by repeated episodes of angioedema, is thus obviously contraindicated.

More recently, Dyax Inc identified a highly potent and specific inhibitor of plasma kallikrein by screening a phage display library consisting of rationally designed variants of the first Kunitz domain of human lipoprotein-associated coagulation inhibitor (LACI, also known as tissue factor pathway inhibitor) [55]. The resulting molecule, DX-88 (ecallantide), is a 60-amino acid protein with a molecular weight of 7054 Daltons that is produced as a recombinant protein in the yeast, *Pichia pastoris*. DX-88 is a reversible and highly potent plasma kallikrein inhibitor (apparent K_i for human plasma kallikrein of 44 pM compared with 13 nM for C1INH) that has a rapid on-rate ($k_{on} 2 \times 10^6 \text{ m}^{-1}\text{s}^{-1}$) and a slow off-rate ($k_{off} 2 \times 10^5 \text{ m}^{-1}\text{s}^{-1}$). Unlike aprotinin, DX-88 is also a highly specific plasma kallikrein inhibitor with an apparent K_i of at least 10 nM for 10 other human serine proteases. In three separate phase I protocols, DX-88 was demonstrated to be well tolerated with consistent pharmacokinetics. At a dose of 20 mg given intravenously, DX-88 had a half-life of approximately 72 minutes. DX-88 was subsequently reformulated as a subcutaneous rather than an intravenous drug. As anticipated, the subcutaneous formulation showed a longer half-life (2 hours) than the intravenous formulation.

The efficacy of DX-88 for the treatment of C1INH deficiency has been demonstrated in several different models. When given to C1INH null mice, DX-88 almost completely abrogated the chronic vascular permeability defect observed in these animals [56]. DX-88 has been studied in three different phase II protocols. The first (EDEMA0) was an open-label phase study involving nine HAE subjects in which intravenous DX-88 treatment resulted in improvement of symptoms in a time frame similar to that seen for C1INH concentrates. The second phase II study (EDEMA1) was a placebo-controlled randomized double-blind escalating single-dose protocol assessing the efficacy of ascending doses (5, 10, 20, and 40 mg/m^2) of intravenous DX-88 in 48 HAE subjects. This study confirmed that DX-88 had a statistically significant beneficial effect for the treatment of acute attacks of HAE. DX-88-treated subjects were significantly more likely to report improvement within 4 hours of dosing (72.5% responders; median time to improvement 70 minutes) than were placebo-treated subjects (25%; median time to improvement 246 minutes). The final phase II study (EDEMA2) was an open-label multiple treatment extension protocol. An interim analysis of EDEMA2 involving 180 attacks in 63 subjects demonstrated the following response rates: 92% at 5 mg/m^2 intravenously (IV) ($n = 24$), 86% at 10 mg/m^2 IV ($n = 135$), and 100% at 20 mg/m^2 IV ($n = 15$) and 30 mg subcutaneously ($n = 6$). Median time to improvement was 30 minutes. A phase III study (EDEMA3), using 30 mg of the subcutaneous DX-88 for the treatment of acute attacks of HAE, is currently ongoing in the United States.

DX-88 is also being studied in cardiac surgery where early data indicate that it is able to decrease blood loss. DX-88 has also recently shown beneficial activity in preventing cerebral ischemia-reperfusion injury in a murine model [57].

More than 450 doses of DX-88 have been successfully given to more than 180 HAE subjects, and the drug appears to be generally well tolerated. Nevertheless, a few concerns have arisen. Unlike C1INH, there appear to be rare attacks that may not respond to DX-88; and several subjects have experienced “rebound” angioedema, ie, a return of angioedema symptoms a number of hours after apparent response to the drug. Interestingly, the “rebound” angioedema appears to be primarily associated with lower doses of DX-88 and thus may not be a substantial issue as experience with the 30-mg subcutaneous dose increases.

Prolongation of the aPTT is commonly seen, representing a pharmacologic effect of the drug without any enhanced risk of bleeding. There have been eight reports of mild anaphylactic-like reactions to first-dose exposure to DX-88, and at least some of these may have been related to the speed of infusion. Tryptase levels following these reactions have been universally negative. Patients who experienced acute dosing reactions all showed improvement in their HAE symptoms, and several of them received subsequent treatment with DX-88 without adverse reaction or loss of efficacy. Two of the subjects who experienced acute dosing reactions were found by Dyax to have antibodies to the study drug: one had non-IgE antibodies to DX-88 and the other had anti-*Pichia pastoris* IgE antibodies. Both of these subjects underwent DX-88 rechallenge without problems. There is also one report of a first-dose anaphylactic-like reaction that was associated with serum antibodies to a low molecular component of the drug (IgE, IgG, IgM, and IgA) detected by immunoblotting [58]. Using a validated ELISA, Dyax was unable to confirm antibodies against either DX-88 or *P pastoris* in this subject [59], and studies are ongoing to resolve this issue. Eleven subjects have developed detectable anti-DX-88 antibodies, seven to contaminating *P pastoris* proteins, three to the DX-88 itself, and one to both. Two of the subjects with anti-DX-88 protein antibodies have continued to receive DX-88 open-label treatment without adverse effect or loss of efficacy. Furthermore, changes in the manufacturing process have been reported to have reduced the contamination with *P pastoris* proteins by 2 orders of magnitude.

Bradykinin receptor antagonist

DX-88 inhibits plasma kallikrein, the protease that cleaves high molecular weight kininogen (HMWK) and releases bradykinin. Blocking the biologic actions of bradykinin at the level of its receptor (the bradykinin B2 receptor) on endothelial cells represents an alternative therapeutic strategy. A variety of potent small bradykinin B2 receptor antagonists have been synthesized [60,61]. Icatibant (Hoe 140; d-Arg[Hyp3,Thi5, d-Tic7, Oic8]- bradykinin) is

a synthetic decapeptide second-generation bradykinin B2 receptor antagonist that displays high affinity, little residual agonist activity, and an impressive resistance to peptidases (due to the use of non-natural amino acids in its backbone) [62]. Based on its demonstrated ability to antagonize bradykinin effects at the B2 receptor, the use of B2 receptor antagonists for the treatment of HAE has been under discussion for a number of years.

Treatment of C1INH null mice with Hoe-140 corrected the permeability defect seen in these animals [56]. Hoe-140, furthermore, has been safely administered to more than 700 human subjects for a variety of indications. Based on all of these considerations, Icatibant was identified by Jerini AG as a promising therapeutic drug for the treatment of HAE attacks.

An initial Phase I study of intravenous Icatibant showed that it was safe, highly bioavailable, and had excellent vascular distribution. Subsequently, Jerini developed a subcutaneous formulation of Icatibant that had similar safety and pharmacokinetic parameters. Interestingly, the subcutaneous formulation of Icatibant is stable at room temperature for at least 1 year.

Phase II studies performed in Europe by Jerini suggested that Icatibant was effective in abrogating acute angioedema attacks in eight patients with HAE. An initial open-label phase II study showed efficacy in eight attacks of HAE. A follow-up phase II study assessed the efficacy of five different doses of intravenous or subcutaneous Icatibant in patients with HAE experiencing attacks of angioedema (four subjects per group). Results of this study are expressed as the time to beginning of relief/time to complete resolution of symptoms, and were as follows: 0.4 mg/kg intravenously over 2 hours, 90 min/435 min; 0.4 mg/kg intravenously over 30 minutes, 85 min/1050 min; 0.8 mg/kg intravenously over 30 minutes, 67 min/833 min; 30 mg subcutaneously, 35 min/770 min; 45 mg subcutaneously, 27 min/1202 min. In contrast, the times to beginning of relief and time to complete resolution for historical matched untreated attacks were 34 hours and 3.25 days, respectively.

More than 300 subcutaneous open-label treatments with Icatibant have been administered for abdominal, cutaneous, and laryngeal HAE attacks. The vast majority of subjects treated with open-label Icatibant responded completely and did not require additional therapy. In one series, 9% of subjects receiving open-label drug required additional treatments. Interestingly, 20 subjects received open-label Icatibant for laryngeal angioedema, and all reported rapid relief with no need for re-treatment.

Jerini initiated two phase III studies (FAST-1 and FAST-2) for the treatment of acute HAE attacks using the subcutaneous formulation of Icatibant. One study compared Icatibant to placebo in the United States, Argentina, Australia, and Canada. The other study compared Icatibant to tranexamic acid in Europe and Israel. Preliminary analyses of the results revealed that subjects receiving Icatibant in the FAST-1 study reported improvement in a median time of 2.5 hours compared to 4.6 hours in placebo treated subjects ($p = 0.131$). In the FAST-2 study, subjects receiving

Icatibant experienced onset of relief in 2.0 hours compared to 12.0 hours in subjects receiving tranexemic acid ($p < 0.001$). Both studies are now continuing in an open-label extension phase. Icatibant is also being pursued as a potential therapeutic modality for a variety of inflammatory diseases including asthma, liver disease, and severe burns [63,64].

Icatibant has been tested in various formulations in a variety of diseases and has been shown to be safe and well tolerated in more than 1100 subjects to date. The current 30-mg subcutaneous dose has been administered in over 300 treatments, also with good tolerability, although some subjects complain of local pain and redness at the injection site. These local reactions are self-limited and have not required any specific treatment.

Future considerations regarding the treatment of hereditary angioedema

Current treatment options for patients with HAE are severely limited, and associated with significant side effects. Successful completion and licensing of some or all of the discussed drugs will provide new opportunities for treating patients with HAE that should help resolve this unmet need. To begin to address how HAE treatment might be optimally managed, several guidelines can be defined that may help in choosing the optimal treatment strategy. First, it is crucial that patients with HAE be treated in a manner that maximizes their health while minimizing potential side effects. Second, the treatment of HAE patients should be individualized. And third, treatment decisions should take into account the disruption of normal life caused by attacks of angioedema. [Table 2](#) summarizes how these new drugs might be used to treat HAE.

Some of the implications of maximizing the health of patients with HAE while minimizing potential side effects are obvious. Thus, it is clear that effective therapy for severe or life-threatening attacks of angioedema must be readily available to patients with HAE. Similarly, use of high doses of anabolic androgens or anti-fibrinolytic drugs are predictably associated with potentially severe side effects, and thus should be avoided. Perhaps less obviously, it will also be crucial to improve the recognition and diagnosis of HAE among caregivers as many patients with HAE are believed to remain undiagnosed. In addition, the particular morbidity associated with the use of anabolic androgens in women raises the possibility that these drugs (that have been the main treatment modality for HAE) may become relatively contraindicated in women.

The likelihood of having multiple effective medications for the treatment of HAE offers the possibility of individualizing therapeutic strategy. Patients may require different therapeutic approaches based on a number of parameters, including differences in attack frequency or severity, differences in response to individual therapeutic agents due to pharmacogenetics, and differences in the ability of patients to tolerate acute attacks

Table 2
Utilization of new drugs

Scenario	Indication	Best drugs	Comment
Acute attack, treated at home	Preferred method of treating attacks to minimize interval between symptom onset and definitive treatment	DX-88 and Icatibant may be preferred because of ease of self-administration	There is strong precedent for patients/families learning to self-administer intravenously, which would allow plasma-derived C1INH and rhC1INH to also be ideal drugs for this use
Acute attack, treated in medical facility	Patients who present in medical facility with an attack either primarily or after failure of home therapy	Plasma-derived C1INH	C1INH replacement is likely to remain the gold standard treatment when one wants to definitively treat an attack without risk of rebound. If clinical studies show that rhC1INH is as effective as plasma-derived C1INH without nonresponders or rebound attacks, then this drug may become the preferred treatment in this scenario.
Short-term prophylaxis	Before extensive dental work, invasive procedures, or surgery	Plasma-derived C1INH	C1INH replacement has been shown to be effective in this situation. Additional experience with the other drugs may expand the recommended drug list.
Long-term prophylaxis	Patients with very severe or frequent attacks who cannot be controlled with or cannot tolerate low-dose anabolic androgens, especially pregnant patients, women, and children	Plasma-derived C1INH	C1INH replacement is likely to remain the only recommended treatment for long-term prophylaxis.

because of age, pregnancy, coexisting medical conditions, or access to medical care. Individualization of therapy will involve the specific medication used as well as the schedule of usage (for acute attacks versus prophylactic).

Finally, optimal treatment must attempt to limit the disruption of normal life that occurs in many patients with HAE. School or work absence is a major issue for patients, and preventing such absences is an important therapeutic goal. Since the natural history of HAE attacks evolves typically over approximately 3 days, many patients suffer substantial absences from school or work. Such absences are not confined to severe attacks, as HAE attacks involving the dominant hand or bottom of the feet can also make it impossible to work or go to school. In addition, there are compelling data suggesting that the time to complete resolution of angioedema symptoms is strongly influenced by the interval between onset of symptoms and institution of effective therapy [31]. Thus, the availability of self-administration (whether subcutaneously or intravenously) of an effective therapeutic agent early in an attack will likely be a key issue in optimal care of patients. Self-administration can obviate the need for patients to go to hospitals, emergency rooms, or even physician offices during an acute attack and thus dramatically shorten this important interval.

A significant fraction of patients with HAE in the United States are either not adequately controlled on currently available therapy or require doses of medications that expose them to the risk of serious side effects. All HAE patients, moreover, are at risk of having severe episodes of angioedema (potentially including life-threatening attacks involving their airway), for which there is currently no specific treatment available in the United States. Based on significant advances in understanding of HAE pathogenesis, novel therapies for HAE have been developed that promise much more specific and effective therapy than what has previously been available. The emergence of drugs that specifically address the pathophysiology of HAE thus has important implications for the management of these patients. Replacement therapy with purified plasma-derived or recombinant C1INH or treatment with a recombinant plasma kallikrein inhibitor or bradykinin B2 receptor antagonist each show the potential to provide reliable control of acute attacks of angioedema. In addition, C1INH replacement therapy provides new possibilities for both short-term and long-term prophylaxis. The availability of these new therapies could also obviate many of the most difficult problems encountered in treating patients with HAE, particularly for children and pregnant women. To take full advantage of these opportunities, however, clinicians and health care systems will need to radically change their existing algorithms for the care of HAE patients. Ultimately, the availability of these drugs coupled with patient-centric treatment plans should allow patients to reap the benefits of improved health with fewer side effects.

References

- [1] Nilsson IM, Andersson L, Bjorkman SE. Epsilon-aminocaproic acid (E-ACA) as a therapeutic agent based on 5 years' clinical experience. *Acta Med Scand Suppl* 1966;448:1–46.
- [2] Spaulding WB. Methyltestosterone therapy for hereditary episodic edema (hereditary angioneurotic edema). *Ann Intern Med* 1960;53:739–45.
- [3] Donaldson VH, Evans RR. A biochemical abnormality in hereditary angioneurotic edema: absence of serum inhibitor of C¹I- esterase. *Am J Med* 1963;35:37–44.
- [4] Landerman NS, Webster ME, Becker EL, et al. Hereditary angioneurotic edema. II. Deficiency of inhibitor for serum globulin permeability factor and/or plasma kallikrein. *J Allergy* 1962;33:330–41.
- [5] Spath PJ, Wuthrich B, Butler R. Quantification of C1-inhibitor functional activities by immunodiffusion assay in plasma of patients with hereditary angioedema—evidence of a functionally critical level of C1-inhibitor concentration. *Complement* 1984;1:147–59.
- [6] Sheffer AL, Fearon DT, Austen KF. Hereditary angioedema: a decade of management with stanazolol. *J Allergy Clin Immunol* 1987;80:855–60.
- [7] Pickering RJ, Good RA, Kelly JR, et al. Replacement therapy in hereditary angioedema. Successful treatment of two patients with fresh frozen plasma. *Lancet* 1969;1:326–30.
- [8] Agostoni A, Bergamaschini L, Martignoni G, et al. Treatment of acute attacks of hereditary angioedema with C1-Inhibitor concentrate. *Ann Allergy* 1980;44:299–301.
- [9] Gadek JE, Hosea SW, Gelfand JA, et al. Replacement therapy in hereditary angioedema. Successful treatment of acute episodes of angioedema with partly purified C1 inhibitor. *N Engl J Med* 1980;302:542–6.
- [10] Marasini B, Cicardi M, Martignoni GC, et al. Treatment of hereditary angioedema. *Klin Wochenschr* 1978;56:819–23.
- [11] Bergamaschini L, Cicardi M, Tucci A, et al. C1 INH concentrate in the therapy of hereditary angioedema. *Allergy* 1983;38:81–4.
- [12] Bork K, Barnstedt SE. Treatment of 193 episodes of laryngeal edema with C1 inhibitor concentrate in patients with hereditary angioedema. *Arch Intern Med* 2001;161:714–8.
- [13] Kirschfink M, Mollnes TE. C1-inhibitor: an anti-inflammatory reagent with therapeutic potential. *Expert Opin Pharmacother* 2001;2:1073–83.
- [14] Logan RA, Greaves MW. Hereditary angio-oedema: treatment with C1 esterase inhibitor concentrate. *J R Soc Med* 1984;77:1046–8.
- [15] Agostoni A, Cicardi M. Hereditary and acquired C1-inhibitor deficiency: biological and clinical characteristics in 235 patients. *Medicine (Baltimore)* 1992;71:206–15.
- [16] Bork K, Hardt J, Schicketanz KH, et al. Clinical studies of sudden upper airway obstruction in patients with hereditary angioedema due to C1 esterase inhibitor deficiency. *Arch Intern Med* 2003;163:1229–35.
- [17] Kunschak M, Engl W, Maritsch F, et al. A randomized, controlled trial to study the efficacy and safety of C1 inhibitor concentrate in treating hereditary angioedema. *Transfusion* 1998;38:540–9.
- [18] Cicardi M, Zingale L. How do we treat patients with hereditary angioedema? *Transfus Apher Sci* 2003;29:221–7.
- [19] Bork K, Meng G, Staubach P, et al. Hereditary angioedema: new findings concerning symptoms, affected organs, and course. *Am J Med* 2006;119:267–74.
- [20] 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.
- [21] De Serres J, Groner A, Lindner J. Safety and efficacy of pasteurized C1 inhibitor concentrate (Berinert P) in hereditary angioedema: a review.jean.de.serres@aventis.com. *Transfus Apheresis Sci* 2003;29:247–54.
- [22] US HAE Association. C1-inhibitor (C1INH) personal importation. Available at: <http://www.hereditaryangioedema.com/import.html>. Accessed October 20, 2006.

- [23] Bork K, Witzke G. Long-term prophylaxis with C1-inhibitor (C1 INH) concentrate in patients with recurrent angioedema caused by hereditary and acquired C1-inhibitor deficiency. *J Allergy Clin Immunol* 1989;83:677–82.
- [24] Altman AD, McLaughlin J, Schellenberg R, et al. Hereditary angioedema managed with low-dose danazol and C1 esterase inhibitor concentrate: a case report. *J Obstet Gynaecol Can* 2006;28:27–31.
- [25] Lehmann A, Lang J, Boldt J, et al. Successful off-pump coronary artery bypass graft surgery in a patient with hereditary angioedema. *J Cardiothorac Vasc Anesth* 2002; 16:473–6.
- [26] Leimgruber A, Jaques WA, Spaeth PJ. Hereditary angioedema: uncomplicated maxillofacial surgery using short-term C1 inhibitor replacement therapy. *Int Arch Allergy Immunol* 1993; 101:107–12.
- [27] Maves KK, Weiler JM. Tonsillectomy in a patient with hereditary angioedema after prophylaxis with C1 inhibitor concentrate. *Ann Allergy* 1994;73:435–8.
- [28] Mohr M, Pollok-Kopp B, Gotze O, et al. [The use of a C1-inhibitor concentrate for short-term preoperative prophylaxis in two patients with hereditary angioedema.]. *Anaesthesist* 1996;45:626–30 [in German].
- [29] Alsenz J, Lambris JD, Bork K, et al. Acquired C1 inhibitor (C1-INH) deficiency type II. Replacement therapy with C1-INH and analysis of patients' C1-INH and anti-C1-INH autoantibodies. *JCI* 1989;83:1794–9.
- [30] Bork K, Barnstedt SE, Koch P, et al. Hereditary angioedema with normal C1-inhibitor activity in women. *Lancet* 2000;356:213–7.
- [31] Levi M, Choi G, Picavet C, et al. Self-administration of C1-inhibitor concentrate in patients with hereditary or acquired angioedema caused by C1-inhibitor deficiency. *J Allergy Clin Immunol* 2006;117:904–8.
- [32] Bergamaschini L, Cicardi M. Recent advances in the use of C1 inhibitor as a therapeutic agent. *Mol Immunol* 2003;40:155–8.
- [33] Varga L, Szeplaki G, Visy B, et al. C1-inhibitor (C1-INH) autoantibodies in hereditary angioedema Strong correlation with the severity of disease in C1-INH concentrate naive patients. *Mol Immunol* 2007;44:1454–60.
- [34] Burnouf T, Radosevich M. Nanofiltration of plasma-derived biopharmaceutical products. *Haemophilia* 2003;9:24–37.
- [35] Koles K, van Berkel PH, Pieper FR, et al. N- and O-glycans of recombinant human C1 inhibitor expressed in the milk of transgenic rabbits. *Glycobiology* 2004;14:51–64.
- [36] van Doorn MB, Burggraaf J, van Dam T, et al. A phase I study of recombinant human C1 inhibitor in asymptomatic patients with hereditary angioedema. *J Allergy Clin Immunol* 2005;116:876–83.
- [37] Porebski G, Bilo B, Obtulowicz K, et al. [Recombinant human C1-inhibitor is effective in the treatment of acute attacks of hereditary angioedema—case report]. *Przegl Lek* 2005;62: 317–20 [in Polish].
- [38] Caliezi C, Wuillemin WA, Zeerleder S, et al. C1-Esterase inhibitor: an anti-inflammatory agent and its potential use in the treatment of diseases other than hereditary angioedema. *Pharmacol Rev* 2000;52:91–112.
- [39] Cai S, Davis AE 3rd. Complement regulatory protein C1 inhibitor binds to selectins and interferes with endothelial-leukocyte adhesion. *J Immunol* 2003;171:4786–91.
- [40] Cicardi M, Zingale L, Zanichelli A, et al. C1 inhibitor: molecular and clinical aspects. *Springer Semin Immunopathol* 2005;27:286–98.
- [41] Horstick G. C1-esterase inhibitor in ischemia and reperfusion. *Immunobiology* 2002;205: 552–62.
- [42] Kirschfink M. C1-inhibitor and transplantation. *Immunobiology* 2002;205:534–41.
- [43] Liu D, Cai S, Gu X, et al. C1 inhibitor prevents endotoxin shock via a direct interaction with lipopolysaccharide. *J Immunol* 2003;171:2594–601.

- [44] Storini C, Rossi E, Marrella V, et al. C1-inhibitor protects against brain ischemia-reperfusion injury via inhibition of cell recruitment and inflammation. *Neurobiol Dis* 2005;19:10–7.
- [45] Schapira M, Silver LD, Scott CF, et al. Prekallikrein activation and high-molecular-weight kininogen consumption in hereditary angioedema. *N Engl J Med* 1983;308:1050–4.
- [46] Zuraw BL, Curd JG. Demonstration of modified inactive first component of complement (C1) inhibitor in the plasmas of C1 inhibitor-deficient patients. *JCI* 1986;78:567–75.
- [47] Donaldson VH, Ratnoff OD, Da Silva WD, et al. Permeability-increasing activity in hereditary angioneurotic edema plasma. II. Mechanism of formation and partial characterization. *JCI* 1969;48:642–53.
- [48] Donaldson VH, Rosen FS, Bing DH. Role of the second component of complement (C2) and plasmin in kinin release in hereditary angioneurotic edema (H.A.N.E.) plasma. *Trans Assoc Am Physicians* 1977;40:174–83.
- [49] Curd JG, Prograis LJ Jr, Cochran CG. Detection of active kallikrein in induced blister fluids of hereditary angioedema patients. *J Exp Med* 1980;152:742–7.
- [50] Curd JG, Yelvington M, Burrige N, et al. Generation of bradykinin during incubation of hereditary angioedema plasma. *Mol Immunol* 1983;19:1365.
- [51] Fields T, Ghebrehwet B, Kaplan AP. Kinin formation in hereditary angioedema plasma: evidence against kinin derivation from C2 and in support of “spontaneous” formation of bradykinin. *J Allergy Clin Immunol* 1983;72:54–60.
- [52] Juhlin L, Michaelsson G. Use of a kallikrein inhibitor in the treatment of urticaria and hereditary angioneurotic edema. *Acta Derm Venereol* 1969;49:37–44.
- [53] Bauer J, Futterman S, Dreiling DA. Anaphylactic shock secondary to initial Trasylol administration. *Am J Gastroenterol* 1971;56:542–4.
- [54] Proud G, Chamberlain J. Anaphylactic reaction to aprotinin [letter]. *Lancet* 1976;2:48–9.
- [55] Williams A, Baird LG. DX-88 and HAE: a developmental perspective. *Transfus Apheresis Sci* 2003;29:255–8.
- [56] Han ED, MacFarlane RC, Mulligan AN, et al. Increased vascular permeability in C1 inhibitor-deficient mice mediated by the bradykinin type 2 receptor. *J Clin Invest* 2002;109:1057–63.
- [57] Storini C, Bergamaschini L, Gesuete R, et al. Selective inhibition of plasma kallikrein protects brain from reperfusion injury. *J Pharmacol Exp Ther* 2006;318:849–54.
- [58] 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;117:476–7.
- [59] Beck TR, Baird LG. Reply: anaphylactic reaction and antibodies to DX-88 (kallikrein inhibitor) in a patient with hereditary angioedema. *J Allergy Clin Immunol* 2006;117:477.
- [60] Stewart JM, Gera L, Hanson W, et al. A new generation of bradykinin antagonists. *Immunopharmacology* 1996;33:51–60.
- [61] Stewart JM, Vavrek RJ. Kinin antagonists: design and activities. *J Cardiovasc Pharmacol* 1990;15:S69–74.
- [62] Hock FJ, Wirth K, Albus U, et al. Hoe 140 a new potent and long acting bradykinin-antagonist: in vitro studies. *Br J Pharmacol* 1991;102:769–73.
- [63] Akbary AM, Wirth KJ, Schölkens BA. Efficacy and tolerability of Icatibant (Hoe 140) in patients with moderately severe chronic bronchial asthma. *Immunopharmacology* 1996;33:238–42.
- [64] Moreau ME, Garbacki N, Molinaro G, et al. The kallikrein-kinin system: current and future pharmacological targets. *J Pharmacol Sci* 2005;99:6–38.