Ann Allergy Asthma Immunol 117 (2016) 508–513

Contents lists available at ScienceDirect

Assessment of inhibitory antibodies in patients with hereditary angioedema treated with plasma-derived C1 inhibitor

Henriette Farkas, PhD [*]; Lilian Varga, PhD [*]; Dumitru Moldovan, PhD [†]; Krystyna Obtulowicz, PhD [‡]; Todor Shirov, PhD [§]; Thomas Machnig, MD [||]; Henrike Feuersenger, PhD [||]; Jonathan Edelman, MD [¶]; Debora Williams-Herman, MD [¶]; Mikhail Rojavin, MD [¶]

[*] *Semmelweis University, Budapest, Hungary*
[†] *University of Medicine and Pharmacy Tîrgu-Mureş, Romania*
[‡] *Jagiellonian University, Krakow, Poland*
[§] *MHAT "TsaritsaYoanna - ISUL", Sofia, Bulgaria*
[||] *CSL Behring, Marburg, Germany*
[¶] *CSL Behring, King of Prussia, Pennsylvania*

ARTICLE INFO

Article history:
Received for publication June 8, 2016.
Received in revised form August 10, 2016.
Accepted for publication August 23, 2016.

ABSTRACT

Background: Limited data are available regarding C1 inhibitor (C1-INH) administration and anti–C1-INH antibodies.
Objective: To assess the incidence of antibody formation during treatment with pasteurized, nanofiltered plasma-derived C1-INH (pnfC1-INH) in patients with hereditary angioedema with C1-INH deficiency (C1-INH-HAE) and the comparative efficacy of pnfC1-INH in patients with and without antibodies.
Methods: In this multicenter, open-label study, patients with C1-INH-HAE (≥12 years of age) were given 20 IU/kg of pnfC1-INH per HAE attack that required treatment and followed up for 9 months. Blood samples were taken at baseline (day of first attack) and months 3, 6, and 9 and analyzed for inhibitory anti–C1-INH antibody (iC1-INH-Ab) and noninhibitory anti–C1-INH antibodies (niC1-INH-Abs).
Results: The study included 46 patients (69.6% female; mean age, 38.9 years; all white) who received 221 on-site pnfC1-INH infusions; most patients received 6 or fewer infusions. No patient tested positive (titer ≥1:50) for iC1-INH-Ab at any time during the study. Thirteen patients (28.2%) had detectable niC1-INH-Abs in 1 or more samples. Nine patients (19.6%) had detectable niC1-INH-Abs at baseline; 3 of these had no detectable antibodies after baseline. Of 10 patients (21.7%) with 1 or more detectable result for niC1-INH-Abs after baseline, 6 had detectable niC1-INH-Abs at baseline. Mean times to symptom relief onset and complete symptom resolution per patient were similar for those with or without anti–niC1-INH-Abs.
Conclusion: Administration of pnfC1-INH was not associated with iC1-INH-Ab formation in this population. Noninhibitory antibodies were detected in some patients but fluctuated during the study independently of pnfC1-INH administration and appeared to have no effect on pnfC1-INH efficacy.
Trial Registration: clinicaltrials.gov Identifier: NCT01467947.

Introduction

Hereditary angioedema (HAE) is a rare autosomal dominant disorder. Three forms of HAE have been described, 2 of which are characterized by a deficiency of functional C1-INH (C1-INH-HAE). Type I accounts for approximately 85% of cases and is characterized by impaired synthesis of C1-INH, resulting in a quantitative decrease of C1-INH antigenic blood concentrations. Type II HAE is associated with production of dysfunctional C1-INH but presents with normal or high levels of the protein. A third phenotypic variant, HAE with normal C1-INH (sometimes referred to as type III), is sometimes associated with a genetic factor XII mutation; however, in most cases, the cause remains unknown.[1–4]

Reprints: Mikhail Rojavin, MD, CSL Behring, 1020 First Ave, King of Prussia, PA 19406; E-mail: mikhail.rojavin@cslbehring.com.
Disclosures: Dr Farkas reported receiving honoraria fees from CSL Behring, Shire, and Swedish Orphan Biovitrum. Dr Moldovan reported receiving consultation fees from Pharming Technologies and Swedish Orphan Biovitrum. Dr Shirov reported receiving honoraria fees for lecturing from UCB Bulgaria. Dr Machnig reported being an employee and shareholder of CSL Behring. Drs Feuersenger, Edelman, and Rojavin reported being employees at CSL Behring. Dr Williams-Herman reported being an employee at CSL Behring during study conduct. No other disclosures were reported.
Funding Sources: This study was funded by CSL Behring, Marburg, Germany. The authors retained full control over data interpretation and manuscript content.

http://dx.doi.org/10.1016/j.anai.2016.08.025

C1-INH concentrate is routinely recommended as a therapeutic option in HAE management.[3,5,6] The plasma-derived, highly purified, pasteurized, nanofiltered C1-INH concentrate (pnfC1-INH [Berinert], CSL Behring, King of Prussia, PA) is approved in the United States for the treatment of acute facial, abdominal, and laryngeal HAE attacks in adolescents and adults and has been available since 2009. pnfC1-INH has been available in a pasteurized, nanofiltered formulation in Europe since 2010 and was preceded by a pasteurized predecessor version first marketed in 1985; it is currently licensed in the European Union for the treatment of acute HAE attacks and for short-term prophylaxis in children and adults. In one study, pnfC1-INH was found to have the highest purity profile of human plasma-derived C1-INH products.[7]

Therapeutic proteins may present a risk of inducing an unwanted antibody (Ab) response, including the development of Abs to the protein itself.[8–10] Such antidrug Abs can be transiently expressed and of no clinical consequence (noninhibitory) or may potentially result in reduced efficacy if they block the activity of the therapeutic protein (inhibitory or neutralizing). Auto-Abs to C1-INH (C1-INH-Abs) are a hallmark of acquired C1-INH deficiency[11–13] and may alter clinical responsiveness to exogenous C1-INH administration because of rapid catabolism.[14] C1-INH-Abs have been observed in some patients with type I and type II C1-INH-HAE but do not necessarily arise as a consequence of previous administration of C1-INH concentrate; furthermore, C1-INH-Abs that occur in patients with C1-INH-HAE do not appear to be inhibitory or neutralizing.[15,16] Data from a large open-label extension study suggest that pnfC1-INH treatment of patients with C1-INH-HAE was not associated with the development of inhibitory C1-INH-Abs.[17] However, further research is needed to confirm these findings.

The current study was designed to assess whether use of pnfC1-INH in patients with C1-INH-HAE type I or type II is associated with an increased incidence of C1-INH-Abs that inhibit C1-INH function. An exploratory objective was to evaluate the efficacy of pnfC1-INH in individuals with and without C1-INH-Abs.

Methods

Study Design

This prospective, international, multicenter, nonrandomized, open-label study (NCT01467947) was conducted at 4 study centers in Poland, Romania, Bulgaria, and Hungary between November 23, 2011, and October 17, 2014. Study procedures were conducted in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines, and the protocol was approved by a central independent ethics committee for each participating center. All participants or a legally acceptable representative provided written informed consent.

Patients with C1-INH-HAE were treated per routine medical practice and followed up for 9 months after the first study-recorded HAE attack treated with pnfC1-INH (Fig 1). In a previous phase 3 trial in which patients were tested for Ab status approximately every 3 months,[17] 19 of 57 patients (33%) tested positive at least once for anti–C1-INH-Abs (all noninhibitory), including 8 who tested positive at screening. Therefore, the current study design with 4 sampling time points and 3-month intervals between sampling time points was expected to provide an adequate duration of observation to characterize the dynamics of C1-INH-Ab formation.

The study enrolled male and female participants 12 years or older with a diagnosis of C1-INH-HAE type I or II and who were assessed by the investigator as being likely to require treatment with pnfC1-INH during the 9-month study period. Exclusion criteria included the use of C1-INH products other than pnfC1-INH within 30 days of the study or planned during the study, immunization within 30 days of study entry, autoimmune conditions requiring immunosuppressant therapy during the study, and previous participation in a study of pnfC1-INH for which C1-INH-Ab results had been submitted to the US Food and Drug Administration. Concomitant therapy with any other approved or experimental human or recombinant C1-INH was prohibited throughout the study. The following concomitant therapies were prohibited during the acute phase of treatment (from 24 hours before start of attack until resolution of attack): any approved or experimental drug that targeted the biological mechanisms of action of C1-INH; fresh frozen plasma; attenuated androgens, tranexamic acid, or aminocaproic acid (for individuals not previously treated); or increased doses of these drugs (for individuals already receiving such treatment).

Patients received 20 IU/kg of pnfC1-INH (Berinert) at the study site for each HAE attack warranting such treatment. There was no time limit between screening and the first HAE attack. Once the first recorded study attack occurred, the patient was followed up for the subsequent 9 months. Any doses of pnfC1-INH given outside the study site were not included in the study analysis.

Assessments

Clinical and demographic characteristics were recorded at baseline. Each patient maintained a diary to record changes in concomitant therapy, adverse events (AEs) occurring between office visits, the time to onset of HAE symptom relief (TtRel) after pnfC1-INH administration, and the time to complete resolution of all HAE attack symptoms (TtRes). Patients were contacted by

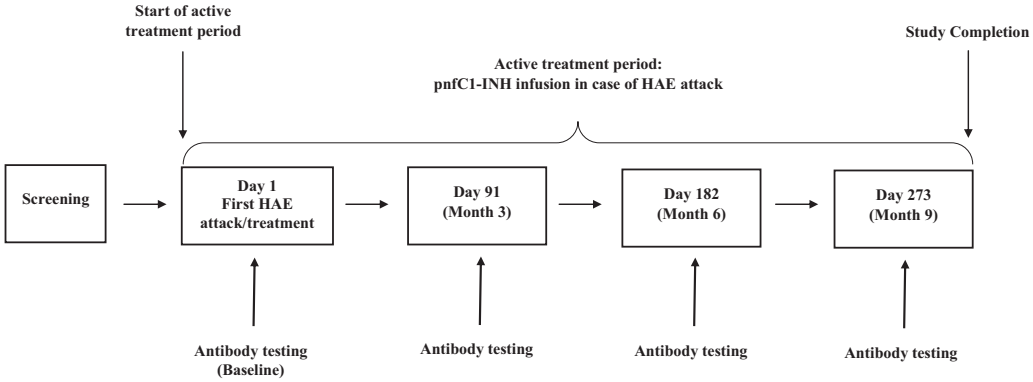

Figure 1. Study design. HAE, hereditary angioedema; pnfC1-INH, pasteurized, nanofiltered plasma-derived C1 inhibitor.

telephone approximately 7 days after each administration of pnfC1-INH to review the subject diary and to inquire about AEs, concomitant medications, and resolution of HAE symptoms.

C1-INH-Abs

Blood samples (5 mL) for the assessment of C1-INH-Abs were collected on day 1 (baseline; day of first treated study attack) and at 3, 6, and 9 months and were analyzed by a central laboratory for inhibitory C1-INH-Abs (iC1-INH-Abs) and noninhibitory C1-INH-Abs (niC1-INH-Abs). Samples were stored between −20°C and −70°C as citrate plasma. The assay for detecting anti–C1-INH-Abs was based on the principle of a direct binding enzyme-linked immunosorbent assay (ELISA) (screening and confirmatory assays, described below).

Anti–C1-INH-Ab screening assay

Purified pnfC1-INH was coated onto microplates and probed by subject samples, and anti–C1-INH-Abs were detected using a second enzyme-conjugated Ab complex recognizing human IgM, IgG, and IgA. Positivity for anti–C1-INH-Abs was based on a titer of 1:50 or greater. The presence of specific C1-INH-Abs was confirmed using anti–C1-INH isotyping assays, and titrations of samples were assayed for quantification. To minimize the probability of false-negative results, the detection limit would allow the determination of false-positive results with a probability of approximately 5%. This cutoff was determined by testing of samples from at least 100 healthy individuals.

Anti–C1-INH isotyping assays (confirmatory assay)

The presence of specific C1-INH-Abs was to be confirmed in anti–C1-INH-Ab isotyping assays. In addition, titrations of the samples were to be assayed for quantification. The assay procedures were similar to the screening assay. In contrast, for detection of isotype M, G, and A, 3 different anti-human horseradish peroxidase–labeled conjugated polyclonal Abs were used. Serial sample dilutions were analyzed, with the titer determined as the last sample dilution before the cut-off.

Samples that tested above the cutoff by screening ELISA were subjected to anti–C1-INH-Ab inhibitory assay for assessing the neutralizing capacity of the respective Abs. Equal volumes of standard human plasma were to be mixed with the subject sample and incubated at 37°C for a standard period. The residual C1-INH activity was to be measured and compared with control samples.

Safety

Physical examination and laboratory safety parameters (hematology and blood chemistry; urinalysis) were performed on day 1 (baseline), month 3, and month 9 (or study completion). Vital signs were evaluated on day 1 and at each HAE attack treatment visit. AEs and changes in concomitant therapy were recorded throughout the study in the patient diary. The investigator graded the intensity of each AE as follows: mild (symptoms were easily tolerated and did not interfere with daily activities), moderate (symptoms caused enough discomfort to cause some interference with daily activities), and severe (symptoms were incapacitating, with inability to work or perform usual activities).

Statistical Analysis

Determination of sample size

On the basis of previous clinical experience,[17] the prevalence of patients being Ab positive (Ab+) at time of enrollment in the study was expected to be approximately 15%. The percentage of patients who were Ab negative at study start and Ab+ at study end was also expected to be 15%, resulting in an expected total percentage of Ab+ patients of 30% at the end of study. A limit of 20% was set as the relevant upper threshold for the incidence proportion of patients with iC1-INH-Abs. With a conservative assumption of 4.5% of patients having iC1-INH-Abs, then a sample size of at least 40 patients was determined to provide approximately 80% power for an incidence less than 20%, with a 1-sided α of 2.5%.

General considerations

Summary statistics (mean [SD], median [range]) were presented for continuous variables, and 2-sided 95% confidence intervals (CIs) were presented when appropriate. In addition, summary statistics were presented for categorical variables (group frequencies and percentages). All data were analyzed using SAS statistical software, version 9.2 (SAS Institute Inc).

Study end points

The primary end point was iC1-INH-Abs. The number and percentage of patients categorized as positive or negative for iC1-INH-Abs for each visit and the incidence of patients with at least 1 positive postbaseline result were summarized. The percentage and incidence proportion of patients with at least 1 positive postbaseline result were to be presented with the corresponding 95% CIs based on the Wilson score. The secondary end point was C1-INH-Abs (inhibitory or noninhibitory). The number and percentage of patients categorized as positive or negative for any C1-INH Abs, whether inhibitory or noninhibitory, for each visit and the incidence of patients with at least 1 positive postbaseline result were determined by visit and overall.

Efficacy variables

The TtRel and TtRes per attack and per patient were summarized descriptively overall and for patients with and without C1-INH-Abs at any point during the study.

Safety variables

The number and percentage of patients experiencing treatment-emergent AEs and discontinuations attributable to AEs were summarized. Treatment-emergent AEs were summarized by relationship to study medication and by severity. Serious AEs were included in overall tallies and also described separately. Laboratory parameters and vital signs were summarized by visit. Abnormal physical examination results were recorded as AEs if noted at a visit after the first attack (pnfC1-INH administration).

Results

Study Participants

Sixty patients were screened and met the study inclusion/exclusion criteria; of these, 14 did not experience an HAE attack requiring pnfC1-INH treatment before the study ended. Because they did not receive the study drug, these 14 individuals were excluded from all analyses. The remaining 46 patients had at least 1 pnfC1-INH–treated attack during the study and comprised the full analysis population and the safety set. All 46 patients completed the entire 9 months of study participation.

Patients were predominantly female (69.6%) and white (100.0%) and ranged in age from 14 to 78 years (mean [SD], 38.9 [14.4] years). Most patients had C1-INH-HAE type I (82.6%). Mean (SD) body mass index (calculated as the weight in kilograms divided by the square of height in meters) was 25.0 (4.8). For all patients, the mean (SD) time since HAE diagnosis was 11.0 (7.6) years. The mean number of prior HAE attacks in the 6 months before the start of the active treatment period was 4.3 attacks, with the greatest percentage of patients (41.3%) reporting 3 attacks in the last 6 months. The most commonly reported HAE attacks during the 6 months before study entry were peripheral (87.0% of patients) and abdominal (60.9% of patients), with laryngeal and facial attacks reported less frequently (10.9% and 23.9% of patients, respectively).

Table 1
Results of C1-INH-Ab Testing

Test	Patients, n (%) (N = 46)
iC1-INH-Abs[a]	
Negative at all study time points	46 (100)
Positive at ≥1 time point during the study	0
niC1-INH-Abs	
Negative at all study time points	33 (71.7)
Positive at ≥1 time point during the study	13 (28.3)

Abbreviations: C1-INH-Ab, C1 inhibitor antibody; iC1-INH-Abs, inhibitory C1-INH antibodies; niC1-INH-Abs, noninhibitory C1-INH antibodies.
[a]Primary end point.

HAE Attacks

A total of 221 HAE attacks in 46 patients were treated with pnfC1-INH at the study site. An additional 101 HAE attacks were treated outside the study site with a variety of medications, including 22 infusions of pnfC1-INH administered to 8 patients; data from these attacks are not reported here. Most patients were treated for 1 (30.4% of patients) or 2 (21.7%) attacks at the study site, whereas 23.9% of patients were treated for more than 6 HAE attacks (range, 7–21 attacks). The most common anatomical locations of attacks were peripheral and abdominal attacks, reported by 56.5% and 52.2% of participants, respectively. Most patients (87.0%) reported at least 1 moderate intensity attack, and 58.7% of patients reported at least 1 severe attack. Most attacks (n = 133 [60.2%]) were moderate in severity.

C1-INH Antibodies

None of the 46 patients tested positive for iC1-INH-Abs at any time during the study (95% CI for Wilson score for incidence, 0.000–0.077) (Table 1). The upper limit of the CI was less than 20%, and the incidence of iC1-INH-Abs in patients with HAE treated with pnfC1-INH can be assumed to be less than 20%.

Thirteen patients (28.3%) tested positive for niC1-INH-Abs at least once at any time during the study (at baseline or after baseline). Thirty-three patients (71.7%) did not test positive for niC1-INH-Abs at any time point during the study. The percentage of participants who tested positive for niC1-INH-Abs at any one study visit, including baseline, ranged from 13.3% to 19.6% of participants (Table 2). Most positive titers (20 [70.0%] of 29) were 1:50, whereas a few titers were between 1:150 and 1:300 (Table 2).

Table 2
Antibody Titers by Study Visit for Patients With at Least 1 Positive niC1-INH-Ab Result by Baseline Ab Status

Patient	Antibody titers by study visit (total study N = 46)			
	Day 1 (baseline)	Month 3	Month 6	Month 9
No detectable niC1-INH-Abs at baseline				
A	…	…	1:50	1:50
B	…	1:50	…	…
C	…	…	…	1:50
D	…	1:50	…	…
Detectable niC1-INH-Abs at Baseline				
E	1:50	1:50	…	…
F	1:50	…	…	…
G	1:150	1:150	1:150	1:150
H	1:50	1:50	1:50	1:50
I	1:50	…	1:50	1:50
J	1:50	1:150	1:150	1:50
K	1:50	…	…	…
L	1:50	…	…	…
M	1:50	1:150	1:300	1:200
No. (%) of patients with Abs	9 (19.6)	7 (15.2)	6 (13.3)	7 (15.2)

Abbreviations: Ab, antibody; niC1-INH-Abs, noninhibitory C1 inhibitor antibodies.

Ten patients (21.7%) had at least 1 detectable result for niC1-INH-Abs after baseline, for an incidence of 0.087 (95% CI for Wilson score for incidence, 0.034–0.203). Of these patients, 6 had detectable Abs on day 1 (baseline), whereas 4 had at least 1 detectable result for Abs after baseline with none detected at day 1 (baseline).

Among the 4 patients who were antibody negative at baseline and had positive niC1-INH-Ab titers at 1 or more subsequent study visits, the number of pnfC1-INH infusions during the study ranged from 1 to 3 (Table 2). Three patients had positive niC1-INH-Abs titers at baseline but nondetectable titers at all other study visits; these patients had 1, 5, and 9 pnfC1-INH infusions, respectively, during the study.

Efficacy

The mean TtRel per attack and TtRel per patient were similar for the 13 patients (35 attacks) who tested positive for niC1-INH-Abs and the 33 patients (186 attacks) who had no C1-INH-Abs at any assessment during the study, including baseline (Table 3). The mean TtRes per attack was approximately 10 hours shorter for the 13 patients (35 attacks) who tested positive at least once for niC1-INH-Abs compared with the mean TtRes for the 33 patients (186 attacks) who had no detectable C1-INH-Abs at any assessment during the study (Table 3). The mean TtRes per patient was similar between the 2 groups.

Safety

A total of 52 AEs were reported in 15 patients (32.6%) who received 1 dose or more of pnfC1-INH at the study site. None of the AEs were considered related to pnfC1-INH or its administration. The most commonly reported AEs were headache (26 events reported in 6 patients), hypotension (3 events reported in 3 patients), upper respiratory tract infection (3 events reported in 2 patients), and oropharyngeal pain (2 events reported in 2 patients); all other AEs were reported in 1 patient each. Two of the 3 AEs of hypotension occurred during abdominal HAE attacks, and 1 occurred during a peripheral HAE attack. Most AEs were of moderate or severe intensity.

A total of 2 serious AEs (SAEs), spontaneous abortion and an acute abdominal HAE attack of severe intensity, occurred in a total of 2 patients (4.3%). Both of these SAEs were considered unrelated to pnfC1-INH or the study procedure. The patient who experienced the spontaneous abortion received no further pnfC1-INH treatment (per protocol guidelines in the event of pregnancy occurring during the study) but continued with all other study procedures. No thromboembolic AEs or deaths were reported during the study.

No clinically significant treatment-emergent changes were noted for hematology, blood chemistry, or urinalysis assessments. No treatment-emergent changes in vital signs were considered

Table 3
Association Between niC1-INH-Abs and Efficacy Outcomes

	With niC1-INH-Abs[a]	Without niC1-INH-Abs[b]
No. of patients	13	33
No. of attacks	35	186
Time to onset of relief, mean (SD) [95% CI], h		
Per attack	1.51 (4.07) [0.11–2.91]	1.40 (0.80) [1.29–1.52]
Per participant	1.17 (1.43) [0.31–2.04]	1.31 (0.60) [1.10–1.52]
Time to symptom resolution, mean (SD) [95% CI], h		
Per attack	22.86 (21.06) [15.62–30.09]	33.59 (58.47) [25.13–42.05]
Per participant	31.02 (24.08) [16.46–45.57]	29.59 (18.59) [23.00–36.18]

Abbreviations: CI, confidence interval; niC1-INH-Abs, noninhibitory C1 inhibitor antibodies.
[a]At any study visit.
[b]At all study visits.

clinically significant, with the exception of the 3 AEs of hypotension reported for 3 patients in whom decreased blood pressures were transient and temporally associated with HAE attacks. All clinically significant physical examination findings were consistent with signs or symptoms of HAE attacks and were limited to edema, pain, or tenderness at the sites of HAE attack.

Discussion

The presence of iC1-INH-Abs has been described in the context of acquired angioedema in which circulating auto-Abs cause the inactivation and catabolism of C1-INH.[11–13] Administration of exogenous proteins has the potential to induce formation of inhibitory and noninhibitory Abs,[9,10] and this phenomenon continues to be a subject of interest in HAE patients treated with C1-INH products. In the current study, pnfC1-INH did not induce formation of iC1-INH-Abs in patients with C1-INH-HAE. This observation is consistent with the findings of the International Multicentre Prospective Angioedema C1-INH Trial (IMPACT) 2 study of pnfC1-INH in patients with C1-INH-HAE.[17] In that prospective study conducted at 15 centers in North America, 57 patients with C1-INH-HAE were administered pnfC1-INH for a median study duration of 24 months for treatment of HAE attacks. Samples were drawn every 3 months to test for C1-INH-Abs. No iC1-INH-Abs were detected in any patient in the IMPACT 2 study. In addition, long-term treatment with pnfC1-INH was not associated with significant changes in anti–C1-INH IgG, IgA, or IgM titers or with evidence of abnormally high titers characteristic of patients with acquired C1-INH deficiency.[18] A lack of inhibitory antibody development has also been noted in association with C1-INH products other than pnfC1-INH, including both human plasma-derived[19–21] and recombinant human C1-INH (rhC1-INH).[9,22]

In the current study, 13 patients (28.2%) had detectable levels of niC1-INH-Abs at 1 or more assessments during the study, including 9 patients (19.6%) who had detectable niC1-INH-Abs at baseline. Similarly, in the IMPACT 2 study, 33% of patients were noted to have detectable niC1-INH-Abs at least once.[17] The presence of niC1-INH-Abs in the current study did not appear to influence the efficacy of pnfC1-INH as assessed by time to onset of symptom relief or time to complete resolution of HAE symptoms, as evaluated per attack or per patient. Regardless of the presence of niC1-INH-Abs, onset of relief typically occurred within approximately 1.5 hours, and complete resolution of HAE symptoms was generally achieved within 35 hours. These results are consistent with those reported in the IMPACT 2 study, which found that median times to onset of symptom relief and complete resolution of HAE symptoms were comparable when analyzed according to the presence or absence of niC1-INH-Abs.[23]

Data from the current study add to an accumulating body of evidence suggesting that C1-INH-Abs are detectable in approximately one-third of C1-INH-HAE patients treated with C1-INH. Findings suggest that the antibodies are not inhibitory, are often transient, and do not lessen the clinical response to C1-INH administration. A prospective study of 95 patients with type I or type II C1-INH-HAE followed up for a 4-year period found that C1-INH-Abs were detected at a similar frequency between patients treated with C1-INH concentrate and treatment-naive patients.[15] In C1-INH concentrate-naive patients, a strong positive correlation was observed between titers of IgM type C1-INH-Abs and disease severity, with 5 of 6 patients with frequently occurring high-titer IgM C1-INH-Abs having disease of the highest severity. IgM type C1-INH-Ab titers were also strongly correlated with attack frequency. The authors suggested that this correlation is likely attributable to high-titer IgM-type C1-INH-Abs developing as a consequence of activation of the classic complement pathway and other plasma enzyme systems during acute attacks. A subsequent study by some of the same authors, which included 130 patients with type I or type II C1-INH-HAE followed up for up to 11 years, confirmed that IgM C1-INH-Abs are commonly observed in HAE, with 31% of patients followed up for 9 to 11 years having IgM C1-INH-Abs in more than 3 serum samples.[15] Presence of IgMC1-INH-Abs was not related to previous C1-INH concentrate treatment.

An integrated analysis of 5 clinical trials (2 of which were open-label extension studies) evaluated the immunogenicity of rhC1-INH in 155 symptomatic HAE patients who received a total of 424 administrations of rhC1-INH.[9] The proportions of anti–C1-INH-Ab screening test results that were above the cutoff level were similarly low for preexposure (1.5%) and postexposure (1.3%) plasma samples. No neutralizing antibodies were detected, and there was no correlation between antibody findings and efficacy or AEs associated with rhC1-INH treatment. In a recent open-label extension study of rhC1-INH, no neutralizing antibodies were found after repeated treatment.[22] Five patients had anti–rhC1-INH antibodies of the IgG isotype, but these were not apparently clinically relevant because similar efficacy was observed for these patients in the presence and absence of positive antibody results (ie, during attacks when antibody levels were elevated vs unelevated).

The antigenic potential of plasma-sourced therapeutic agents appears to differ between HAE and hemophilia. Inhibitory antibodies to factor VIII (FVIII) are a clinically relevant phenomenon in approximately 25% of patients with severe hemophilia[24] but have not been observed in HAE with C1-INH therapy. Although the reasons for this phenomenon are not yet clear, certain theoretical explanations can be entertained. In some patients with hemophilia, there is a severe deficiency mutation within the FVIII gene.[24] As a result, there is a complete absence of protein production, and the exogenous protein may present as a neoantigen. In contrast, patients with C1-INH-HAE have at least some intrinsic C1-INH, and the protein is familiar to the immune system.[25] Furthermore, differences in immunogenicity to C1-INH compared with FVIII may be related to the fact that inhibitory Abs react with the reactive center loop of the C1-INH, which is a very conservative region, whereas the C1 and C2 domains in FVIII evolutionally appeared later.[26,27] It is reported that hemophilia patients with missense mutations in the C1 and C2 domains of FVIII have a 3-fold higher risk of developing FVIII inhibitors than patients with missense mutations in other domains.[28] Finally, most patients with HAE are exposed to exogenous C1-INH on a sporadic and sometimes infrequent basis, whereas patients with hemophilia A are more likely to receive regular and more frequent administration of FVIII.

Interpretation of the study results is limited by the absence of confirmed pnfC1-INH–naive patients at baseline and the lack of a control group. All study participants were white; the racial distribution likely reflects the predominant demographics of the Eastern European countries in which this study was conducted. The study duration of 9 months could be considered a possible limitation, given the possibility that the frequency and nature of antibody development might differ with longer treatment exposure.

Results of this open-label, uncontrolled study suggest that administration of a highly purified human plasma-derived C1-INH is not associated with the development of inhibitory antibodies in C1-INH–deficient patients (type I or type II C1-INH-HAE), likely reflects immunotolerance to the human C1-INH protein.

Acknowledgments

Writing assistance was provided by Adrienne Drinkwater, PhD, of Churchill Communications (Maplewood, New Jersey), funded by CSL Behring. The authors thank all the patients who participated in this trial, and also Sylvia Herget and Doris Lang for their contribution to this trial.

References

[1] Zuraw BL, Bork K, Binkley KE, et al. Hereditary angioedema with normal C1 inhibitor function: consensus of an international expert panel. *Allergy Asthma Proc*. 2012;33:S145–S156.

[2] Bernstein JA. HAE update: epidemiology and burden of disease. *Allergy Asthma Proc*. 2013;34:3–6.

[3] Cicardi M, Aberer W, Banerji A, et al. Classification, diagnosis, and approach to treatment for angioedema: consensus report from the Hereditary Angioedema International Working Group. *Allergy*. 2014;69:602–616.

[4] Bork K. Hereditary angioedema with normal C1 inhibitor. *Immunol Allergy Clin North Am*. 2013;33:457–470.

[5] Betschel S, Badiou J, Binkley K, et al. Canadian hereditary angioedema guideline. *Allergy Asthma Clin Immunol*. 2014;10:50.

[6] Zuraw BL, Banerji A, Bernstein JA, et al. US hereditary angioedema association medical advisory board 2013 recommendations for the management of hereditary angioedema due to C1 inhibitor deficiency. *J Allergy Clin Immunol Pract*. 2013;1:458–467.

[7] Feussner A, Kalina U, Hofmann P, Machnig T, Henkel G. Biochemical comparison of four commercially available C1 esterase inhibitor concentrates for treatment of hereditary angioedema. *Transfusion*. 2014;54:2566–2573.

[8] Donaldson VH, Bissler JJ, Welch TR, Burton MF, Davis AE 3rd. Antibody to C1-inhibitor in a patient receiving C1-inhibitor infusions for treatment of hereditary angioneuroticedema with systemic lupus erythematosus reacts with a normal allotype of residue 458 of C1-inhibitor. *J Lab Clin Med*. 1996;128:438–443.

[9] Hack CE, Mannesse M, Baboeram A, Oortwijn B, Relan A. Immunogenicity assessment of recombinant human c1-inhibitor: an integrated analysis of clinical studies. *BioDrugs*. 2012;26:303–313.

[10] Schellekens H. The immunogenicity of therapeutic proteins. *Discov Med*. 2010;9:560–564.

[11] Jackson J, Sim RB, Whelan A, Feighery C. An IgG autoantibody which inactivates C1-inhibitor. *Nature*. 1986;323:722–724.

[12] Alsenz J, Bork K, Loos M. Autoantibody-mediated acquired deficiency of C1 inhibitor. *N Engl J Med*. 1987;316:1360–1366.

[13] Jackson J, Sim RB, Whaley K, Feighery C. Autoantibody facilitated cleavage of C1-inhibitor in autoimmune angioedema. *J Clin Invest*. 1989;83:698–707.

[14] Cicardi M, Zingale LC, Pappalardo E, Folcioni A, Agostoni A. Autoantibodies and lymphoproliferative diseases in acquired C1-inhibitor deficiencies. *Medicine (Baltimore)*. 2003;82:274–281.

[15] Varga L, Széplaki G, Visy B, et al. C1-inhibitor (C1-INH) autoantibodies in hereditary angioedema: strong correlation with the severity of disease inC1-INH concentrate naïve patients. *Mol Immunol*. 2007;44:1454–1460.

[16] Varga L, Füst G, Csuka D, Farkas H. Treatment with C1-inhibitor concentrate does not induce IgM type anti-C1-inhibitor antibodies in patients with hereditary angioedema. *Mol Immunol*. 2011;48:572–576.

[17] Craig TJ, Bewtra AK, Bahne SL, et al. C1 esterase inhibitor concentrate in 1085 hereditary angioedema attacks: final results of the I.M.P.A.C.T. 2 study. *Allergy*. 2011;66:1604–1611.

[18] Farkas H, Csuka D, Zotter Z, et al. Treatment of attacks with plasma-derived C1-inhibitor concentrate in pediatric hereditary angioedema patients. *J Allergy Clin Immunol*. 2013;131:909–911.

[19] Farkas H, Jakab L, Temesszentandrási G, et al. Hereditary angioedema: a decade of human C1-inhibitor concentrate therapy. *J Allergy Clin Immunol*. 2007;120:941–947.

[20] Farkas H, Varga L. Human plasma-derived, nanofiltered, c1-inhibitor concentrate (Cinryze®), a novel therapeutic alternative for the management of hereditary angioedema resulting from c1-inhibitor deficiency. *Biol Ther*. 2012;2:2.

[21] Hofstra JJ, KleineBudde I, van Twuyver E, et al. Treatment of hereditary angioedema with nanofiltered C1-esterase inhibitor concentrate (Cetor®): multi-center phase II and III studies to assess pharmacokinetics, clinical efficacy and safety. *Clin Immunol*. 2012;142:280–290.

[22] Li HH, Moldovan D, Bernstein JA, et al. Recombinant human-C1 inhibitor is effective and safe for repeat hereditary angioedema attacks. *J Allergy Clin Immunol Pract*. 2015;3:417–423.

[23] Craig TJ, Pürsün EA, Bork K, et al. WAO guideline for the management of hereditary angioedema. *World Allergy Organ J*. 2012;5:182–199.

[24] Reipert BM, van Helden PM, Schwarz HP, Hausl C. Mechanisms of action of immune tolerance induction against factor VIII in patients with congenital haemophilia A and factor VIII inhibitors. *Br J Haematol*. 2006;136:12–25.

[25] Moldovan D, Bernstein JA, Cicardi M. Recombinant replacement therapy for hereditary angioedema due to C1 inhibitor deficiency. *Immunotherapy*. 2015;7:739–752.

[26] Kumar A, Bhandari A, Sarde SJ, Goswami C. Molecular phylogeny of C1 inhibitor depicts two immunoglobulin-like domains fusion in fishes and rayfinned fishes specific intron insertion after separation from zebrafish. *Biochem Biophys Res Commun*. 2014;450:219–226.

[27] Davidson CJ, Hirt RP, Lal K, et al. Molecular evolution of the vertebrate blood coagulation network. *Thromb Haemost*. 2003;89:420–428.

[28] Oldenburg J, Schroder J, Brackmann HH, Muller-Reible C, Schwaab R, Tuddenham E. Environmental and genetic factors influencing inhibitor development. *Semin Hematol*. 2004;41:82–88.