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rhC1INH: a new drug for the treatment of attacks in hereditary angioedema caused by C1-inhibitor deficiency

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Recombinant human C1 esterase inhibitor (rhC1INH) (Ruconest®, Pharming) is a new drug developed for the relief of symptoms occurring in patients with angioedema due to C1-inhibitor deficiency. Pertinent results have already been published elsewhere; this article summarizes the progress made since then. Similar to the purified C1-inhibitor derived from human plasma, the therapeutic efficacy of rhC1INH results from its ability to block the actions of enzymes belonging to the overactivated bradykinin-forming pathway, at multiple locations. During clinical trials into the management of acute edema, a total of 190 subjects received recombinant C1-inhibitor by intravenous infusion on 714 occasions altogether. Dose-ranging efficacy studies established 50 U/kg as the recommended dose, and demonstrated the effectiveness of this agent in all localizations of hereditary angioedema attacks. Studies into the safety of rhC1INH based on 300 administrations to healthy subjects or hereditary angioedema patients followed-up for 90 days have not detected the formation of autoantibodies against rhC1INH or IgE antibodies directed against rabbit proteins, even after repeated administration on multiple occasions. These findings met favorable appraisal by the EMA, which granted European marketing authorization for rhC1INH. Pharming is expected to file a biological licence with the US FDA by the end of 2010 to obtain marketing approval in the USA. The launch of rhC1INH onto the pharmaceutical market may represent an important progress in the management of hereditary angioedema patients.

Keywords: complement • hereditary angioedema • recombinant human C1-inhibitor • rhC1INH • rhucin • therapy

The analysis of results from the clinical studies conducted with human recombinant C1 esterase inhibitor (rhC1INH) (Ruconest®) has yielded a number of conclusions, which have contributed positively to the marketing authorization of the product [1]. The following discussion provides a summary of these new findings.

Hereditary angioedema & C1-inhibitor deficiency

Hereditary angioedema (HAE) is a rare disorder characterized by paroxysms of subcutaneous and submucosal edema. Types I and II of HAE (HAE-C1INH) are autosomal dominant disorders caused by a defect of the gene for C1-inhibitor (C1INH) [2]. In type I (85% of cases), the antigenic level and functional activity of the C1INH protein are low in the circulation. Type II is characterized by the synthesis of functionally reduced C1INH protein, the antigenic plasma level of which may be normal or occasionally elevated. Notwithstanding this, C1INH activity is lower than normal. More than 200 different mutations of the *C1INH* gene have been described to date [3,101]. A third form of HAE of unknown etiology has also been described; this form predominantly afflicts females with a normal complement profile [4]. Missense mutation of the *F12* gene and additional defects can be detected in a proportion of cases; however, the pathomechanism of this condition has yet to be accurately elucidated. The clinical manifestations of HAE type III are identical to those observed in HAE caused by C1INH deficiency. Production of F XII is dependent on estrogen, which is probably the main reason of dominancy of females in HAE type III. Acquired C1INH deficiency results in angioedema formation. Acquired angioedema caused by C1INH deficiency is characterized by

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hyperactivation of the classical pathway of human complement and angioedema symptoms mediated by bradykinin released by inappropriate activation of the contact kinin system. Aquired angioedema recurs in association with various conditions and particularly with different forms of lymphoproliferative disorders. Neutralizing autoantibodies to C1INH are present in the majority of patients [5]. The clinical manifestations of HAE and acquired C1INH deficiency are identical.

Pathomechanism

C1-inhibitor deficiency is associated with activation of contact systems and the release of vasoactive substances, which enhance vascular permeability. Extravasation of fluid from the intravascular compartment causes edema. Although bradykinin is known to play a major role in the pathophysiology of HAE, histamine is not involved in the process [6]; additional (inflammation) factors that are not completely known are also likely to be important, as well as in bradykinin-mediated angioedema [7]. The initial event is autoactivation of coagulation factor XII, which is bound to the surface of the endothelial cells lining the vascular lumen. Factor XIIa then activates prekallikrein and thereby results in the release of kallikrein. Blood plasma kallikrein is an effective activator of factor XII, producing the active enzymes XIIa and XIIf. Furthermore, kallikrein is responsible for the release of bradykinin from high-molecular-weight kininogen. The key regulator of the bradykinin release is C1INH, as it inhibits the activity of factors XIIa and XIIf, as well as kallikrein. Moreover, C1INH is involved in the regulation of proteases belonging to additional enzyme systems including C1r, C1s, MASP-1, MASP-2 (complement), plasmin (fibrinolysis) and factor XIa (coagulation). Excessive activation of these enzyme systems may also have a role in edema formation [8].

Prevalence, incidence & mortality

According to international data, the estimated prevalence of HAE is one in 10,000 to one in 50,000. Attacks first occur around the time of entering school age. Half of afflicted patients experience the first attack during the first decade of life, while an additional third experience the first attack during the second decade. Extremely high mortality (estimated at 30%) is explained by the lack of early diagnosis and inappropriate therapy; however, these data have not been studied in enough detail. The predominant cause of death is suffocation consequent to laryngeal edema [9].

Diagnosis

The diagnosis of HAE can be established through evaluation of the medical history and clinical manifestations of patients, as well as by conducting complement studies. Identifying a mutation of the *C1INH* gene may supply additional confirmation in uncertain cases.

History

The occurrence of edematous symptoms in additional members of the patient's family can aid diagnosis. In approximately 75% of HAE cases, *C1INH* gene mutation carriers are found among the first-degree relatives. Family members of the remaining 25% of patients are not afflicted by this abnormality; in these cases, disease is probably caused by *de novo* mutations [10].

Clinical manifestations

Time of onset, incidence, location and severity of clinical manifestations all exhibit inter-individual variation. In these respects, substantial differences can exist even within the same family [11]. An abundance of factors can play a role in inducing attacks (including mechanical trauma, menstruation, pregnancy, infections, oral contraceptives and angiotensin-converting enzyme inhibitors); however, the precise pathomechanism still remains unknown [12–14]. Edema most commonly occurs on the extremities – it is not accompanied by erythema or pruritus, and it resolves over a couple of days [15]. In 40% of cases, erythema marginatum (appearance of a reddish, map-like pattern on the skin) precedes the attack as a prodromal sign [16]. However, there is considerable variability of the prodromal manifestations that may occur before or during HAE attacks and it is not proven whether they can predict edema attacks [17]. Edema can develop on the trunk, genitals and face (Figure 1) and also in the mucosa of the upper airways or the intestines. Attacks involving the intestinal mucosa can mimic the clinical manifestations of the 'acute abdomen'. Not all abdominal pains in HAE patients are caused by angioedema, but the differential diagnostic is difficult and, accordingly, HAE patients are often subjected to unwarranted surgery. Abdominal attacks are the second most frequent manifestations of the disease; their symptoms include colicky abdominal pain, nausea, vomiting, as well as profuse watery diarrhea occuring after the resolution of the attack [15,18,19]. Edema involving the mucosa of the upper airway is the most dangerous form of this disorder. The manifestations of this form of attack progress rapidly and, without treatment, can lead to suffocation within a short time. These features are responsible for the high mortality of the disease [9]. Infrequently, cerebral edema can develop with neurological abnormalities, whereas pulmonary edema is uncharacteristic, presumably because bradykinin – with a major role in edema formation – is promptly inactivated in the lung [20].

Complement testing

Diagnosis is established by conducting complement studies. In type I HAE, these reveal reduced antigenic and functional C1INH levels in the serum. In type II, by contrast, normal or even elevated antigenic C1INH level is ascertained, along with a reduced functional C1INH level. Substantial reductions of complement C4 levels are observed in HAE. Genetic studies are important in prenatal diagnostics as well as for confirming obscure cases. In 5% of cases, no mutation of the *C1INH* gene could be demonstrated [3]. Nevertheless, a valid and substantiated diagnosis of HAE can be established without molecular biology studies, by complement measurements only [12].

Management

The management of patients with HAE is dependent on the severity of the disease. Intervention is indispensable in all cases of laryngeal edema, as airway obstruction, requiring emergency

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endotracheal intubation or tracheostomy, may evolve in the lack of appropriate therapy [21]. Treatment is also warranted in severe and moderately severe abdominal attacks, whereas mild cases may improve with symptomatic measures. In addition, administration of replacement therapy in subcutaneous edema may be important in improving the patient's quality of life. Subcutaneous edema evolving on the neck or face may progress to involve tongue, pharynx or larynx, or any combination of these three, and this necessitates intervention. The management of HAE consists of the therapy of edematous attacks, as well as the prophylaxis of their recurrence. The relevant international guidelines and review articles a provide more detailed assistance in choosing appropriate medicinal products and determining their dosage [22–27].

Treatment of acute edema

Life-threatening edematous attacks involving the upper airway or the intestinal mucosa (with manifestations resembling those of an acute abdominal catastrophe) require prompt and appropriate intervention. Remedies conventionally used to relieve angioedema – including glucocorticoids, antihistamines and epinephrine – are ineffective in this condition. Currently, the following three options of pharmacotherapy exist. Supplementation of deficient C1INH is feasible with purified, human plasma-derived concentrate [28–30] or C1INH manufactured by recombinant technology [31]. Treatment with ecallantide, a recombinant kallikrein inhibitor suppressing the release of bradykinin is appropriate for relieving acute edema in patients with HAE [32]. The third option is therapy with icatibant, a bradykinin receptor 2 antagonist that renders bradykinin ineffective [33,34]. Exceptionally, fresh frozen plasma may be administered if the aforementioned drugs are not available [35].

Attack prophylaxis

The elimination of provoking factors is an effective means for primary prevention; however, it is only feasible if the provoking factors are known. Long-term prophylaxis is justified in patients with a history of severe, life-threatening edematous attacks, as well as if the latter recur frequently (monthly or weekly). Several forms of preventive drug therapy are currently available. Antifibrinolytic agents and anabolic androgens are most commonly used for long-term prophylaxis [36–38], although their mode of action is still not precisely known. The lowest effective dose of anabolic androgens is very important, because the risk of adverse events increases with the daily dose and the duration of treatment [39]. Administering C1INH concentrate for long-term prophylaxis is also currently appropriate [40–42]. Short-term prophylaxis is recommended for patients undergoing surgical or diagnostic interventions contemplated on the head or neck. In this case, attenuated androgens or antifibrinolytic agents could be administered in increased doses before the intervention, as well as for 4 to 5 days thereafter. However, the most effective prophylaxis is C1INH concentrate replacement, administered preferably 1 h, and no more than 6 h, before the intervention [28,43–46]. Alternatively, combined use of preventive drugs can be expedient [47]. Education, counselling, and follow-up of patients are indispensable adjuncts to appropriately chosen pharmacotherapy.

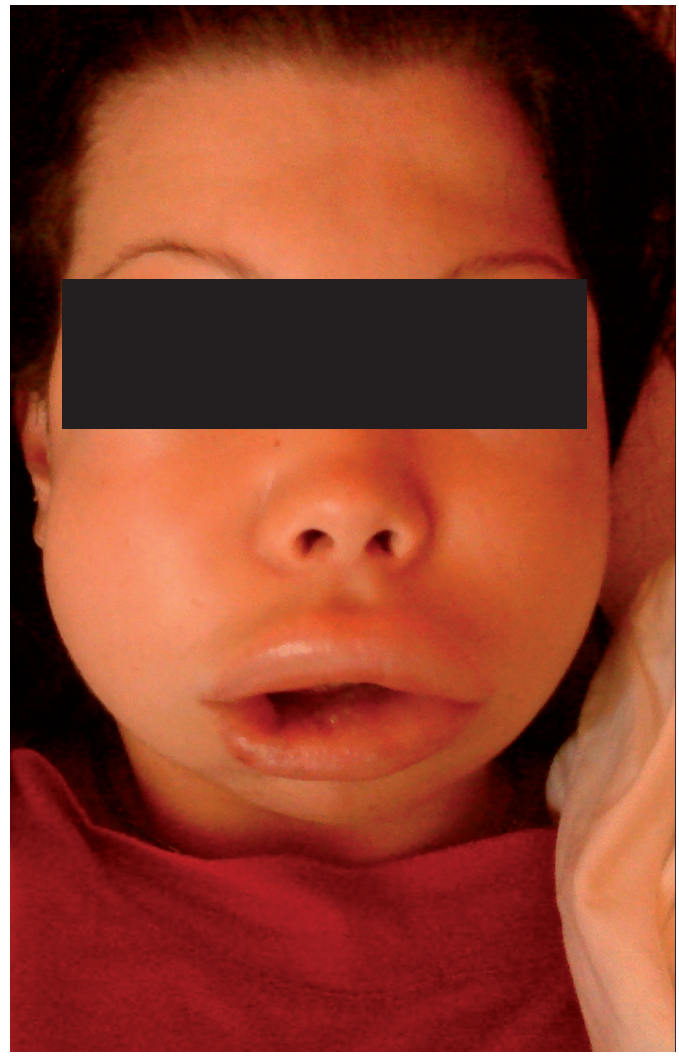

Figure 1. Angioedema of the face caused by hereditary C1-inhibitor deficiency.

Market overview

The effectiveness of agents administered for attack prophylaxis, that is, of antifibrinolytics (tranexamic acid and ε-aminocaproic acid) and anabolic androgens (methyltestosterone, danazole, stanosolol and oxandrolone), has been confirmed by many studies during the past 50 years. Nevertheless, the use of these drugs is limited by the risk of abundant side effects. Although a proportion of the latter can be partially avoided by administering the lowest effective dose and their timely recognition is afforded by periodic laboratory screens (complete blood count, liver enzyme activity, creatine kinase and urinalysis) or abdominal ultrasound imaging, adverse drug reactions can lead to discontinuation in certain cases. Anabolic androgens are not recommended for pregnant women but, if warranted, may be administered to children with close monitoring. Androgen side effects include decreased growth rate, virilization and behavioral disorders during childhood, whereas in adolescence, menstruation irregularities and elevation of serum transaminase levels may occur. Adverse reactions can be avoided by administering the lowest effective dose [48,49].

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With regards to acute attacks, the treatment of choice has been C1INH concentrate derived from human plasma (pdC1INH). Currently, three products are commercially available. Berinert P® (Berinert® in the USA) produced by CSL Behring is currently approved in 28 countries worldwide. In the USA, it has been approved by the US FDA for facial and abdominal – but not peripheral – edema in adolescents and adults [102]. The second preparation is Cetor®, which is manufactured by the Dutch not-for-profit Sanquin Blood Supply Foundation, and has been used for the emergency therapy of HAE for more than 30 years. The third C1INH concentrate product is Cinryze® (Viropharma) – this is a version of the original product developed by Sanquin, but purified further by nanofiltration. Cinryze is available in the USA only and the FDA has approved its use for the prevention of edematous attacks in adolescents and adults [103]. The bradykinin receptor antagonist icatibant is available in several European countries. Although the evaluation of this agent has been initiated by Jerini AG, the clinical program was completed and the product is marketed currently by Shire under the brand name Firazyr®. It has been approved by the EMA for the management of acute edematous attacks (regardless of their localization) in adults – however, Firazyr is not approved in the USA [104]. Ecallantide is a recombinant kallikrein inhibitor (DX88, Kalbitor®) developed by Dyax Corporation. This is the sole product that has been recently approved by the FDA for all localizations of acute edematous HAE attacks occurring in patients over 16 years of age [105]. Both icatibant and ecallantide are administered subcutaneously by healthcare professionals. Recombinant C1INH product is developed by Pharming Group NV and was approved by the EMA in 2010. It is available in a few countries and was launched by Swedish Orphan Biovitrum (SOBI) under the brand name Ruconest [106].

Introduction to the drug

Human C1-inhibitor belongs to the superfamily of the serine protease inhibitors in plasma. Proteases inactivated by C1INH include C1r, C1s, MASP-1 and MASP-2 of the complement system; factor XII and plasma kallikrein of the contact system; factor XI and thrombin of the coagulation system; and plasmin and tissue plasminogen activator of the fibrinolytic system [50]. C1INH is encoded by a single gene located on chromosome 11; it consists of eight exons and seven introns. The entire genomic sequence is known and encodes a protein of 500 amino acids, including a 22 amino acid signal sequence [51]. Plasma C1INH is a single-chain plasma glycoprotein of approximately 71 kDa (478 amino acids), and is heavily glycosylated; up to 26% of its molecular mass consists of carbohydrate [52]. Previously, C1INH was successfully expressed in COS cells [53,54], in *Escherichia coli* [55] and by the baculovirus expression vector [56], but either the expression level was low or most of the protein was denatured [12]. Ruconest, which was developed by Pharming Group NV (Leiden, The Netherlands), is a recombinant human C1INH obtained from the milk of transgenic rabbits. The cloned human *C1INH* gene was inserted into a mammary gland-specific expression vector under the control of an S1 casein promoter. The construct was introduced into fertilized New Zealand white rabbit oocytes by microinjection and a line expressing high levels of secreted rhC1INH was generated, yielding 12 mg/ml [57]. Rabbits have a short generation time and can produce sufficient quantities of rhC1INH. Only healthy animals are used for milking. Several purification procedures, such as skimming, cation- and anion-exchange chromatography and filtration precede the lyophilization step and result in a >99% pure protein substance (5–15 parts per million of non-protein-related impurities) [12].

Chemistry

Human recombinant C1 esterase inhibitor or conestat alfa ($C_{2355}H_{3745}N_{613}O_{728}S_{17}$) is a human plasma protease C1-inhibitor, glycoform α, N,O-glycosylated recombinant protein expressed in the mammary gland of transgenic rabbits. It is identical to human plasma-derived C1INH at the amino acid level and demonstrates the same inhibitory profile as pdC1INH [107]. Analyses of the primary structure, higher order structure and the molecular mass of rhC1INH have demonstrated that rhC1INH is consistently produced as an intact C1INH molecule. The molecular mass of the single-chain recombinant protein was approximately 67,000 kDa. This difference is imputable to differences in glycosylation (approximately 22% for rhC1INH and approximately 26% for plasma-derived C1INH) [52,107]. By comparing N- and O-glycosylation patterns of plasma-derived hC1INH and of rhC1INH expressed in the milk of transgenic rabbits, it was concluded that total processing in native serum hC1INH is more complete than in rhC1INH. Taken together, when compared with native serum hC1INH (in terms of sialylation), the glycans of rhC1INH are undersialylated [58]. However, the inhibitory capacity of rhC1INH towards target proteases was comparable with that of plasma-derived C1INH [12]. As N-linked glycosylation is important for the folding of proteins, the influence of lactation parameters was studied on N-glycosylation of rhC1INH. The major N-glycan structures on rhC1INH from rabbit milk were similar to those on native hC1INH, with only slight inter-individual variances. The results demonstrated that sialylation levels and total rhC1INH-bound sialic acid content decreased with the progress of lactation [59].

Pharmacodynamics

As C1INH is the only proven, potent inhibitor of the initial activation of the classical complement pathway, restoration of the functioning of C1INH abates the consumption of C4. Therefore, the pharmacodynamics of rhC1INH were assessed by measuring C4 concentration and split products in asymptomatic patients with HAE. To avoid high inter-individual differences at baseline, the concentrations of C4 were normalized by expressing them as the percentage change from baseline for each individual. rhC1INH exhibited dose-dependent biological activity with increasing C4 level, which was approximately twofold at 12 h after the administration of rhC1INH 100 U/kg. The 50-U/kg dose was established as the lowest dose to exert a favorable pharmacodynamic effect on the C4 levels of HAE patients. Accordingly, the concentration of C4b/c (cleaved C4) decreased immediately, achieved a nadir at

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approximately 40 min and demonstrated an inverse relationship with functional C1INH level [60]. Pharmacodynamic parameters were also evaluated during angioedematous attacks. Figure 2 illustrates the changes of plasma C4 levels in 14 HAE patients who received 100 U/kg (bodyweight) rhC1INH as emergency treatment for an acute attack. C4 level peaked at 24 h and remained above the baseline level on the long term, even on day 22 after dosing [61].

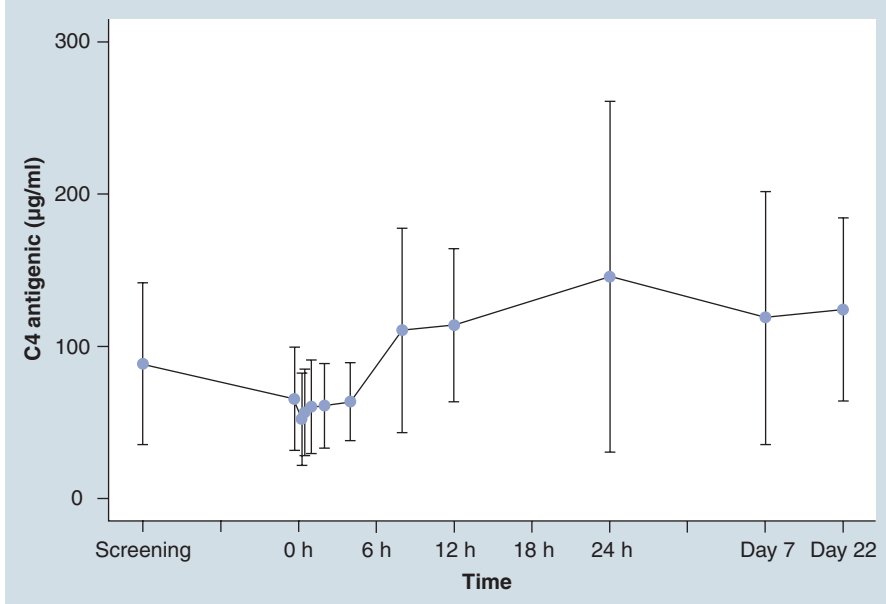

Figure 2. Course of C4 levels in 14 hereditary angioedema patients suffering from an acute angioedema attack following intravenous administration of human recombinant C1 esterase inhibitor at 100 U/kg bodyweight.
Error bars represent standard deviation.

Pharmacokinetics & metabolism

The pharmacokinetic parameters of rhC1INH were characterized in a Phase I study conducted on 12 asymptomatic patients with HAE. Patients were given different doses (ranging from 6.25 to 100 U/kg) of rhC1INH. After administration of the study medication, the maximum concentration of C1INH above baseline (C_{max}) and the duration of the period during which functional C1INH concentration exceeded 0.4 U/ml were determined. C_{max} exhibited a dose-dependent increase (0.32–2.46 U/ml). Dose-normalized C_{max} was constant (p = 0.48), but clearance (71.4–12.7 ml/min) and elimination half-life (28–172 min) were dependent on dose. The Michaelis–Menten constant was estimated at 0.57 U/ml; maximum enzymatic activity (V_{max}) was approximately 45 U/min, and the volume of distribution was approximately 3.3 l [60]. In a Phase II clinical trial, 13 severe angioedema attacks were treated with 100 U/kg doses of rhC1INH. C_{max} was significantly higher than that observed in asymptomatic patients treated with the same dose of rhC1INH (3.8 ± 0.8 U/ml vs 2.5 ± 0.2 U/ml) [62]. Simulation studies using a population pharmacokinetic model based on actual data from 214 administrations of rhC1INH in 120 patients revealed that doses of 50 U/kg restored functional C1INH in plasma up to or above the lower level of normal (0.7 U/ml) in virtually all patients. A fixed dose of 2100 U restored functional C1INH in the normal range in approximately 75% of the patients [63]. The elimination half-life of rhC1INH was approximately 3 h, much shorter than the half-life of plasma-derived C1INH products [107,108].

Clinical efficacy

Based on pharmacokinetic and pharmacodynamic data from the Phase I clinical trial, 100 U/kg was chosen as the initial studied dose of rhC1INH [60]. Two exploratory open-label, clinical studies were conducted in 14 HAE patients who were treated with rhC1INH for 21 acute angioedema attacks. Seven out of 14 patients were treated for one acute angioedema attack, with the remaining patients treated for two acute angioedema attacks. The symptoms of the attacks were assessed both by the physician and by the patients using a questionnaire with visual analogue scales (VAS; 10 cm/scale). Primary outcome measures included time to the onset of relief (defined as a decrease of ≥20 mm in the VAS scores for pain or swelling, compared with baseline) and time to minimal symptoms (defined as pain or swelling scoring ≤20 mm on the VAS). Median time to the onset of symptom relief was <60 min for all symptom assessment methods, whereas median time to minimal symptoms was <8 h for the VAS assessment methods [62,107].

Two separate randomized, double-blind, placebo-controlled clinical trials were conducted in Europe and North America. The European randomized, double-blind, placebo-controlled Phase III study was conducted with 32 patients. Median time to the onset of symptom relief was 1 h, versus 8 h in the placebo group, whereas median time to minimal symptoms was 6.1 versus 20.2 h, respectively. All instances of treatment with rhC1INH were successful, with no relapses [57]. A second randomized, double-blind, placebo-controlled study with rhC1INH was conducted at several sites in the USA and Canada. In total, 38 acute HAE attacks (laryngeal, facial, abdominal, urogenital and peripheral) were treated in 38 patients, randomized to either of two doses of rhC1INH (100 or 50 U/kg) or to placebo. For the primary end point, intent-to-treat analysis demonstrated that patients who had received the 100-U/kg dose of rhC1INH reported onset of symptom relief at approximately 68 min (median), and those who had received placebo reported relief of symptoms at approximately 258 min. The time to minimal clinical symptoms (i.e., the secondary end point), reported by patients in the 100-U/kg, 50-U/kg and placebo groups was approximately 245, 247 and 1098 min, respectively. The primary and secondary end point results obtained with both doses of rhC1INH were clinically meaningful and statistically significant relative to placebo with p-values of <0.01 (Figure 3) [1,31,63].

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After the completion of randomized studies, HAE patients were invited to participate in open-label study extensions (OLEs) for the treatment of subsequent acute angioedema attacks in all anatomical localizations. The purpose of the OLE was to obtain further evidence about the safety and efficacy of rhC1INH, particularly when given repeatedly for subsequent HAE attacks. Overall, 105 attacks were treated in 60 patients with a range of rhC1INH doses (100 U/kg or 50 U/kg bodyweight or an initial fixed dose with 2100 U, independent of the bodyweight). No clinically significant adverse events were observed in these HAE patients. In line with the efficacy findings from the two randomized controlled studies, median time to the onset of relief following rhC1INH administration was 1 h, and median time to minimal symptoms was 4 h in both the North American and European OLEs. Consistent findings of efficacy were observed across all anatomical locations of HAE attacks, including laryngeal attacks. Although all doses of rhC1INH assessed in the OLEs were found to be effective, there were indications that the 2100 U dose may be less effective. No reduction in efficacy was observed when rhC1INH was used repeatedly to treat subsequent angioedema attacks [64]. rhC1INH has proven consistently safe and effective in patients receiving up to 26 treatments, with no evidence of decreased response to the study drug. Review of efficacy data performed with multiple doses demonstrated improving efficacy with increasing doses. The percentage of attacks with response within 4 h was 41% in patients treated with placebo, 85% in patients treated with an initial fixed dose of 2100 U (one vial, estimated 18–40 U/kg bodyweight), as well as 92 and 93% in patients treated with 50 and 100 U/kg rhC1INH, respectively. Optimal efficacy is afforded by 50 U/kg, as no further improvement is achieved with a dose of 100 U/kg. Therapeutic failure was reported in 11 and 10% of attacks treated with 50 and 100 U/kg, respectively, compared with 17% for the single vial strategy [63]. The lowest dose identified with optimal efficacy for the treatment of acute attacks of angioedema is 50 U/kg bodyweight. These data support the concept that restoration of functional C1INH to above the lower limit of normal (0.7 U/ml) maximizes the therapeutic response [61].

Treatment of peripheral attacks

The clinical value of administering rhC1INH to HAE patients suffering from an acute peripheral angioedema attack was also evaluated. In the clinical trials, 36 of the randomized patients experienced peripheral edema; 22 of these received rhC1INH and 14 were treated with placebo. rhC1INH shortened the duration of pain, dysfunction and swelling of peripheral angioedema attacks in patients with HAE [65]. Treatment with rhC1INH was as effective for mitigating edema of the extremities as in any other localization. Analyzing all the attacks experienced by the whole study population, 65 out of 93 (70%) HAE patients had an angioedema attack of peripheral location(s). The majority of peripheral angioedema attacks in HAE occur at multiple locations (55%). Symptom VAS scores for the peripheral location(s) were available from 64 patients. A total of 18 patients (28%) indicated moderate and 35 (55%) severe pain for the peripheral location. Eight (13%) patients indicated moderate and 51 (80%) severe dysfunction associated with the peripheral location. The symptom VAS score was the highest for swelling in 24 patients (38%), for pain in ten patients (16%) and for dysfunction in 30 patients (47%). Only three (5%) peripheral locations had swelling with minimal or no pain and dysfunction. The medical need for treatment is underestimated in HAE patients suffering from an acute peripheral angioedema attack [65].

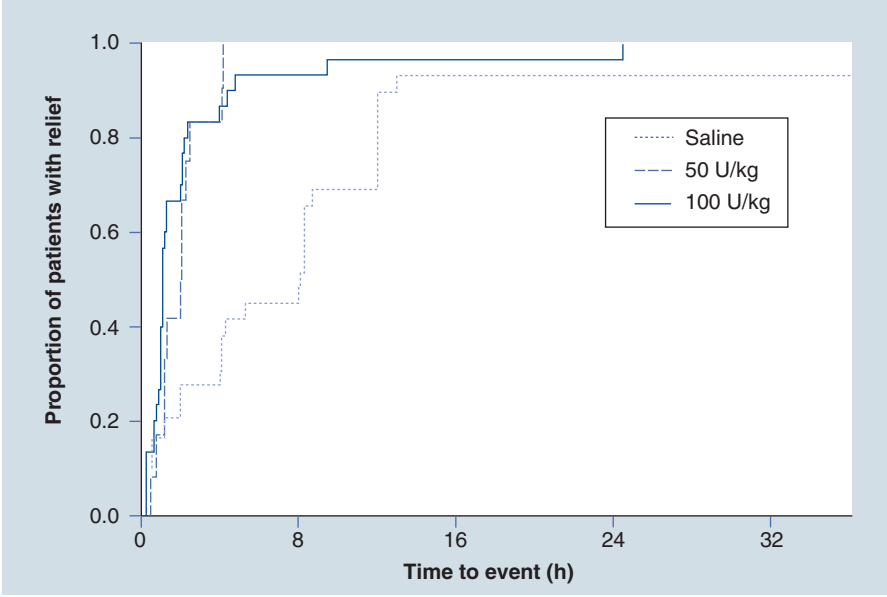

Figure 3. Time to beginning of relief in acute angioedema attacks treated with human recombinant C1 esterase inhibitor 100 U/kg, 50 U/kg or placebo.
Adapted with permission from [31].

Prophylaxis with rhC1INH

An exploratory open-label Phase II study (the OPERA study) on the safety and prophylactic effect of rhC1INH in patients with HAE terminated recently. In this study, 25 asymptomatic HAE patients with a history of frequent attacks received once-weekly administrations of 50 U/kg of rhC1INH for 8 weeks. Patients reported a median of 60 HAE attacks (range: 39–467) over the past 2 years, corresponding to an average of 0.6 attacks per week (range: 0.4–4.5). The breakthrough attack rate observed during the study was much lower with a median of two attacks over the 8-week period, corresponding to an average of 0.25 attacks per week (range: 0–1.5). Weekly administration of rhC1INH 50 U/kg was generally safe and well tolerated. These results suggest that rhC1INH, in addition to the approved indication in Europe for the acute treatment of

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angioedema attacks, could also offer significant value for long-term prophylaxis in patients with frequent attacks [109].

Safety & tolerability

The clinical experience supporting the safety of rhC1INH consists of 300 administrations (83 administrations to healthy subjects or asymptomatic HAE patients and 217 administrations to 119 HAE patients). Adverse reactions were usually mild-to-moderate in severity. The incidence of adverse reactions were similar for all dose groups and did not increase upon repeated administrations [107]. Laboratory tests for monitoring safety included hematological, biochemical and coagulation parameters, viral serology and antibody assays (anti-C1INH and antibodies against any impurities in rabbit milk). Assays for IgE antibody and analysis of C1INH activity-neutralizing antibodies were undertaken [31,60,62].

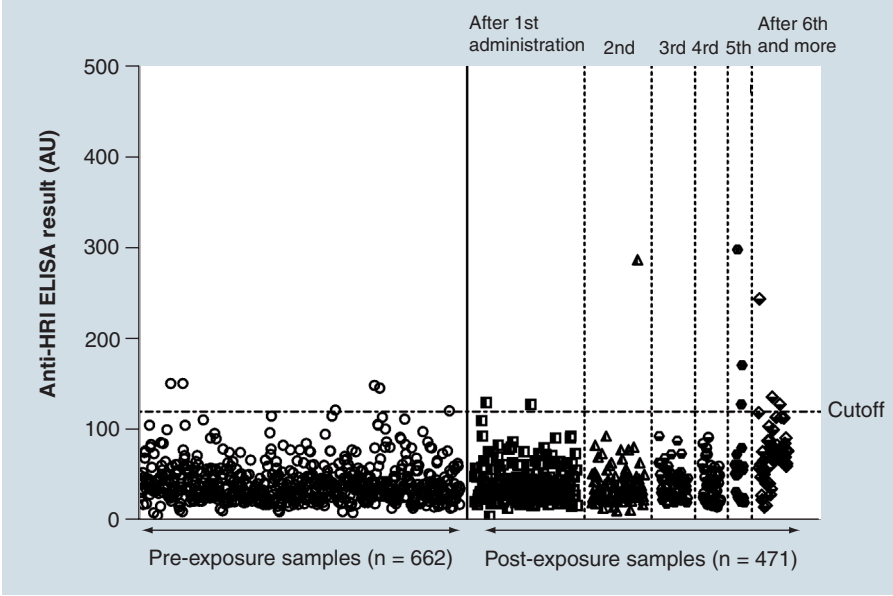

Figure 4. Assessment of antibodies to residual rabbit proteins (host-related impurities) in symptomatic hereditary angioedema patients after repeat treatment with human recombinant C1 esterase inhibitor. The horizontal line represents the cutoff level for the ELISA.
HRI: Host-related impurities.

Evaluation of the immunosafety

Plasma samples from 139 HAE patients who had undergone 322 treatments with rhC1INH for an acute angioedema attack were tested in these clinical studies. A total of 14 HAE patients had received rhC1INH for the treatment of at least five acute angioedema attacks (maximum 20 in a single patient). The frequency of antibody-positive results in pretreatment versus post-treatment samples was comparable, suggesting that the results above cutoff are indicative were of false-positive responses. Similar results were obtained with anti-pdC1INH ELISAs [66]. For confirmatory purposes, positive samples were tested for the presence of neutralizing antibodies and were found to be negative. With regard to possible antibody formation against residual rabbit proteins (host-related impurities [HRI]), the frequency of positive anti-HRI results for pre- and postexposure samples is depicted in Figure 4. This observation suggests the sporadic induction of anti-HRI antibodies after repeated treatment. Following initial and/or repeated treatment with rhC1INH, no treatment-emergent, positive antibody responses to C1INH and no neutralizing antibodies were found. No persistent anti-HRI responses were ascertained in rhC1INH-treated HAE patients. Sporadic, positive anti-HRI results were not associated with any adverse clinical finding [67].

In addition, pre-exposure samples were tested for pre-existing IgE against 14 different animal-derived allergens. Testing for the induction of IgE was undertaken using plasma samples obtained after the last exposure to rhC1INH. A total of 137 healthy volunteers and HAE patients were tested for the presence of IgE antibodies (ImmunoCap, Phadia) against four rabbit allergens and ten other animal allergens including cow milk. In total, 24 out of 137 (17.5%) subjects had at least one positive IgE antibody test result at baseline, including five subjects with a positive test against rabbit epithelium (dander) and/or rabbit urine. Three of the five subjects positive for IgE against rabbit dander or urine allergens reported allergic-type symptoms post-rhC1INH exposure. One was a healthy volunteer with the highest rabbit dander IgE level (39 kU/l), who developed an anaphylactic reaction following the initial exposure (this is the sole allergic reaction reported to date following exposure to rhC1INH). Two positive rabbit IgE subjects reported allergic-type symptoms 3 days post-rhC1INH exposure, but these were not considered to be related to treatment. In addition, three out of 24 subjects had pre-existing IgE against cow milk. None of these three patients developed allergic-type symptoms upon exposure to rhC1INH. Post-rhC1INH, none of the 24 subjects with pre-existing, positive IgE findings had any increase in IgE levels to rabbit or cow milk allergens. One of 113 subjects with prior negative IgE findings for rabbit and cow milk allergens subsequently developed IgE against cow milk allergens just above the test cutoff level, but not against rabbit (milk) allergens. Another patient developed IgE just above the test cutoff level against rabbit meat, but no IgE against any of the other allergens tested. Neither of these two subjects developed allergic-type symptoms upon first or repeated exposure to rhC1INH. The only clinically relevant allergic reaction reported to rhC1INH occurred in a healthy volunteer. This subject had the highest level of pre-existing IgE against rabbit dander among all the subjects tested. Pretreatment testing to exclude IgE against rabbit dander is advised before the therapeutic use of rhC1INH. No clinically relevant IgE antibody responses were found after rhC1INH exposure [64,68]. A reassuring immunosafety profile has been found upon repeat treatment of acute angioedema attacks in HAE patients with rhC1INH [64].

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Dosage & administration

Human recombinant C1 esterase inhibitor is being marketed under the brand name Ruconest in Europe, and is under review in the USA. Ruconest is a freeze-dried protein, free of preservatives. One unit of Ruconest corresponds to the mean quantity of C1INH present in 1 ml of normal fresh plasma. It is recommended not to store above 25°C and advisable to store in the original package in order to protect from light. Before Ruconest can be administered, it needs to be dissolved in water for injections. Once reconstituted, the product should be used immediately. Conestat alfa contains 2100 units per vial, corresponding to 2100 units per 14 ml after reconstitution, or a concentration of 150 units/ml. Ruconest is developed for intravenous use only. Ruconest is given by slow injection into a vein over approximately 5 min. The dose depends on the patient's bodyweight. The recommended dose for the routine treatment of acute attacks is 50 U/kg bodyweight with a maximum of 4200 U (two vials) for patients with bodyweight ≥84 kg. One injection is usually enough to treat an attack, but a second injection may be given if the patient does not sufficiently improve after the first. A patient should not be given more than two injections within any 24-h period. The medicine should only be administered by a healthcare professional. Patients who have not received Ruconest before should be tested to ascertain whether they have pre-existing antibodies against rabbit dander (shed skin and hair) in their blood – they should only be given Ruconest if their tests are negative. The most common side effect with Ruconest (observed in between one and ten patients in 100) is headache. Ruconest should not be used in people who may be hypersensitive (allergic) to conestat alfa or any of the other ingredients. It must not be used in patients with known or suspected allergy to rabbits [107].

Regulatory status

The European Commission granted marketing authorization valid for the European Economic Area for Ruconest on 28 October 2010. The marketing authorization is valid for 5 years and renewable. The EPAR for Ruconest is available from the EMEA website [107].

Conclusion

Phase I, II and III clinical studies have demonstrated that rhC1INH (Ruconest) is an effective and safe drug for the treatment of all types of HAE attacks. Administered in a dose of 50 U/kg bodyweight, clinical symptoms resolved rapidly and no clinically significant adverse events, clinically relevant abnormalities or relapses were observed. Moreover, the efficacy of the drug did not decrease when rhC1INH was used repeatedly to treat subsequent angioedema attacks. Immunology data also supported the immunosafety of Ruconest. Taking these findings into consideration aggregately, the Committee for Medicinal Products for Human Use (CHMP) decided in 2010 that Ruconest should be given marketing authorization.

Expert commentary

Until recently, the management of HAE patients had not changed for decades. The advent of innovative medicinal products expanded the range of therapeutic agents with preparations that substantially changed the management of HAE patients. Using these new drugs physicians can manage the individual needs of patients. Experience obtained with plasma-derived C1INH preparations has been invariably excellent over many years; these drugs have proven useful not only for the emergency treatment of attacks, but also for short-term prophylaxis. rhC1INH has all the biological properties attributed to native C1INH, that is, it acts on all four cascade systems. As shown by the results of clinical studies, rhC1INH was effective in all localizations of HAE attacks. Unlimited production of this product may eventually contribute to wider availability, and this would make for improvement of the patients' quality of life through the effective management of heretofore-untreated edemas (e.g., those involving the extremities). pdC1INH concentrate is also appropriate for pediatric patients and pregnant women. Considering its safety profile and efficacy, it is reasonable to surmise that rhC1INH will be appropriate for the management of these patient groups. The number of available donors does not limit the production scale of rhC1INH and its therapeutic use is devoid of the impediments associated with blood products (e.g., transmission of infection and religious prohibition of use). Furthermore, the quality of blood products may be influenced by the starting material – this pitfall is also eliminated by recombinant technology. Intravenous administration of the product may prove occasionally difficult. Patients with allergy to rabbits are contraindicated. Sensitization to rhC1INH has not been observed to date.

At present, this drug is not suitable for home administration, owing to the requirement for administration by a healthcare professional.

As C1INH is a regulatory protein of the complement cascade, it is a promising therapeutic solution for disorders associated with complement activation. Research into this exciting field has already started [7,69,70].

Five-year view

Development and clinical testing of a number of innovative drugs for the treatment of HAE attacks have been completed. The next step is to use these medicinal products in clinical practice, as well as to accumulate and analyze relevant clinical experience. The expanding range of therapeutic alternatives makes much more specific and individualized decisions possible in choosing the most appropriate treatment, because these agents are superior to their predecessors, both with regard to the technology of their production, as well as their mechanism of action and mode of administration. Even nowadays, emergency therapy is still unavailable to patients in many countries. It is to be hoped that the next 5 years will open up a new, better era for HAE patients by making state-of-the-art drugs available to them in an increasing number of countries. In addition, novel agents are expected to supersede pre-existing therapeutic options with inferior effectiveness and safety profile – this would improve patients' quality of life substantially.

Further clinical studies are to be expected to demonstrate that these novel agents are also effective and safe in children, to expand the indications for their use, and to launch training programs for self-administration.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Key issues

- Ruconest® (Rhucin) is the only recombinant C1 esterase inhibitor (C1INH) preparation.
- Its mode of action is identical to that of C1INH concentrate derived from human plasma.
- As regards to the efficacy of C1INH, dose is more important than half-life.
- Optimum therapeutic efficacy was found when Ruconest was delivered at doses of 50 U/kg and above.
- Ruconest was effective in all types of hereditary angioedema (HAE) attacks.
- Repeated use did not reduce the effectiveness of Ruconest.
- No rebound effect was ascertained during its use.
- Ruconest is safe and no clinically significant adverse reactions were observed during the treatment of HAE attacks.
- Ruconest is a new alternative in the management of HAE.
- The EMA authorized the marketing of Ruconest as a medicinal product.

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