

A phase I study of recombinant human C1 inhibitor in asymptomatic patients with hereditary angioedema

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Background: Hereditary angioedema (HAE) is a congenital disorder with recurrent attacks of localized swelling of submucosal tissue, subcutaneous tissue, or both caused by a deficiency of the plasma protein C1 inhibitor (C1 esterase inhibitor [C1INH]).

Objective: We sought to evaluate the effects of recombinant human C1INH (rhC1INH) isolated from the milk of transgenic rabbits in 12 asymptomatic patients with HAE.

Methods: rhC1INH was intravenously administered at doses of 6.25 to 100 U/kg on 2 occasions.

Results: rhC1INH appeared safe and was well tolerated. The course of functional C1INH in plasma showed a full initial recovery (dose-normalized maximum concentration of about 0.02 U/mL/U/kg) and a dose-dependent clearance of rhC1INH. After infusion of rhC1INH at 100 U/kg, a clearance of approximately 13 mL/min, a half-life of approximately 3 hours, and a volume of distribution of approximately 3 L were observed. Infusion at this dose led to functional C1INH levels in plasma of at least twice the normal level for about 2 hours and greater than 0.4 U/mL for about 9 hours. rhC1INH displayed dose-dependent biologic activity by increasing the C4 level, which was about 2-fold at 12 hours after rhC1INH at 100 U/kg, and decreasing levels of cleaved C4.

Conclusion: The observed safety profile and biologic activity of rhC1INH warrants further clinical studies to assess its efficacy in treating HAE attacks. (*J Allergy Clin Immunol* 2005;116:876-83.)

Key words: Hereditary angioedema, C1 inhibitor, C4, complement, pharmacodynamics, pharmacokinetics, human

Abbreviations used

C1INH: C1 esterase inhibitor

C4b/c: C4b, C4bi, or C4c

C_{max}: Maximum concentration

C_{min}: Minimum concentration

ECG: Electrocardiography

HAE: Hereditary angioedema

PD: Pharmacodynamics

PK: Pharmacokinetics

rhC1INH: Recombinant human C1 inhibitor

T_{max}: Time of maximum concentration

T_{min}: Time of minimum concentration

The clinical syndrome of hereditary angioedema (HAE) and acquired angioedema results from a deficiency of functional C1 esterase inhibitor (C1INH),^{1,2} which regulates the activation of the classical and lectin pathway of complement, as well as the contact system. Episodes of angioedema in subcutaneous tissue, submucosal tissue, or both in patients with HAE have been attributed to the release of the vasoactive peptides bradykinin and C2-derived kinin caused by recurrent (uncontrolled) activation of these systems.^{3,4} The median plasma level of functional C1INH in patients with HAE is about 20% of the level found in healthy individuals (ie, less than the level of 40% of normal [0.4 U/mL]),⁵ which is considered a critical level to control excessive activation of the classical route of complement.⁶ Indeed, levels of C4, C2, or both, which are substrates of activated C1, are typically low in asymptomatic patients with HAE.

Angioedema attacks are debilitating and might limit patients in their normal daily life.¹ Abdominal attacks often cause severe pain resembling abdominal emergencies, and attacks occurring in the larynx might lead to asphyxiation. Therefore many patients use oral maintenance therapy with attenuated androgens^{7,8} or antifibrinolytic agents.⁹ This reduces the frequency and severity of attacks but is not or is only partially effective in the treatment of attacks.⁹ Moreover, both types of maintenance treatment are associated with serious side effects.⁸⁻¹¹ As an alternative, prophylactic treatment with C1INH has been proposed.^{1,12}

Severe angioedema attacks can be successfully treated with intravenous infusion of plasma-derived C1INH.^{9,11-17} However, availability of this drug is limited, and it

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TABLE I. Administration schedule of rhC1INH

Group	Dose (U/kg), study period 1	Washout	Dose (U/kg), study period 2
A	6.25		25.0
B	12.5		50.0
C	25.0		100
D	50.0		100

carries a small risk of transmission of human pathogenic viruses.^{1,17}

Production of recombinant human C1INH (rhC1INH) with transgenic rabbits was started to optimize the treatment options in patients with HAE. A stable pharmaceutical product containing highly purified rhC1INH with less than 20 ppm contaminants was prepared. rhC1INH is highly similar to human plasma-derived C1INH, except for differences in glycosylation.¹⁸ Preclinical studies indicated that rhC1INH at high doses was safe.

This article describes the first administration of rhC1INH in human subjects. The study was performed in symptom-free patients with HAE and investigated the safety and tolerability, as well as the pharmacokinetics and biologic effects, of rhC1INH.

METHODS

The study protocol for this open-label dose-escalation study was approved by the Medical Ethical Committee of the Leiden University Medical Centre and performed according to the principles of the International Conference on Harmonisation and Good Clinical Practice and the Helsinki Declaration. Twelve subjects with a typical medical history of HAE with functional C1INH and C4 levels of less than 40% of normal and less than 300 µg/mL, respectively, who were symptom free for a period of at least 2 weeks before the administration of rhC1INH were included in the study. Patients receiving maintenance therapy continued their medication during the study.

Exclusion criteria included a history of administration of pharmaceuticals derived from rabbits; a history of anaphylaxis or severe allergies to food, proteins, and/or drugs; a history of allergic reactions to C1INH concentrates or rabbit protein; the presence of anti-C1INH antibodies or acquired angioedema; and pregnancy, breast-feeding, or current intention to become pregnant.

The patients were divided into 4 groups of 3 patients each (Table I), and each patient was given 2 doses of rhC1INH (15-minute intravenous infusion), with a washout period of at least 5 weeks. Continuation of dosing and dose escalation was decided after each dose had been administered to a group of 3 patients.

Study medication

An overview of the properties of rhC1INH in comparison with plasma-derived C1INH is provided in Agostoni et al.¹⁸ The various comparative analyses showed a lower degree of glycosylation in rhC1INH: about 21% and 28% of the molecular mass of rhC1INH and plasma-derived C1INH (approximately 67 and 73 kD on MALDI-TOF mass spectroscopy), respectively, is due to carbohydrate structures. N-linked glycan profiling indicated that most of the N-linked complex structures of rhC1INH were monosialylated.¹⁹ Differential glycosylation does not affect the activity of rhC1INH *in vitro* because the inhibitory potency of rhC1INH toward target proteases C1s, kallikrein, Factor XIa, and Factor XIIa, as well as that

against plasmin and thrombin, was comparable with that of plasma-derived C1INH (detailed experimental procedures will be published separately). rhC1INH is consistently produced as an active and intact molecule with purity exceeding 99.98% and containing no detectable amounts of cleaved rhC1INH, endogenous rabbit C1INH, and rabbit milk proteins.

Blood sampling and measurements

During each study period, blood samples for functional C1INH, antigenic C1INH, C4, and C4b, C4bi, or C4c (C4b/c) assays were drawn at screening (at approximately 30 days, 14 days, or both before the administration of rhC1INH), at 25 minutes and 5 minutes before dosing, at 16 and 30 minutes from the start of administration, and at 1, 2, 4, 8, 12, 16, 24, 48, and 96 hours and 22 days after dosing. Blood samples for anti-C1INH antibody testing were taken at screening and before dosing (–25 minutes) and at days 7, 22, and 90 after drug administration.

Tolerability assessment consisted of frequent measurements of vital signs, 12-lead electrocardiographic (ECG) recording, blood and urine sampling for routine laboratory tests, and adverse event assessments.

Sample handling and assays

Blood samples for C1INH, C4, and anti-C1INH antibody assays were kept at room temperature and centrifuged at 2000g for 10 minutes within 30 minutes of collection. Plasma samples were stored at –70°C until analysis. Plasma levels of functional C1INH were determined with a chromogenic assay (Berichrome assay; Dade-Behring, Marburg, Germany) by reference to pooled normal plasma. One unit of C1INH activity (functional C1INH) was defined as the C1 esterase inhibiting activity present in 1 mL of pooled normal plasma. Normal values of functional C1INH in healthy volunteers varied between 0.76 and 1.33 U/mL. One milligram of rhC1INH corresponds with 6 U of functional C1INH. C1INH antigen and antigenic C4 plasma concentrations were determined by using nephelometry with commercially available polyclonal antisera. Normal values of antigenic C1INH and C4 of healthy volunteers ranged from 150 to 350 µg/mL and from 732 to 1951 nM (150–400 µg/mL), respectively. C4 activation products were determined with an ELISA, as previously described.²⁰ Because the ELISA does not discriminate between C4b, C4bi, or C4c, the products detected in the assay are referred to as C4b/c (in healthy volunteers <8 nM). C1INH, C4, and C4b/c assays were performed at the Department of Immunopathology at Sanquin Research, The Netherlands. Immunogenicity analyses consisted of testing for anti-C1INH antibodies of IgM, IgG, and IgA subclasses, for antibodies neutralizing C1INH activity as well as for antibodies against rabbit milk protein (details of these assays will be published separately). Anti-C1INH antibodies were tested against rhC1INH or plasma-derived C1INH (only during screening).

Pharmacokinetic and pharmacodynamic analysis

Maximum concentration (C_{max}) above baseline (average of 2 predose values) was derived directly from the concentration-time profiles. The time span in which the functional C1INH concentration exceeded 0.4 U/mL was determined by using linear interpolation. The pharmacokinetic parameters of functional C1INH were determined by using a 1-compartment model with a constant infusion rate to account for the endogenous concentrations estimated within the model.²¹ Calculations were performed by using nonlinear mixed-effect modeling, as implemented in NONMEM Version V software (GloboMax LLC, Hanover, Md) with first-order conditional estimation throughout. Although nonlinear mixed-effect modeling itself provides estimates of the population average parameters and the

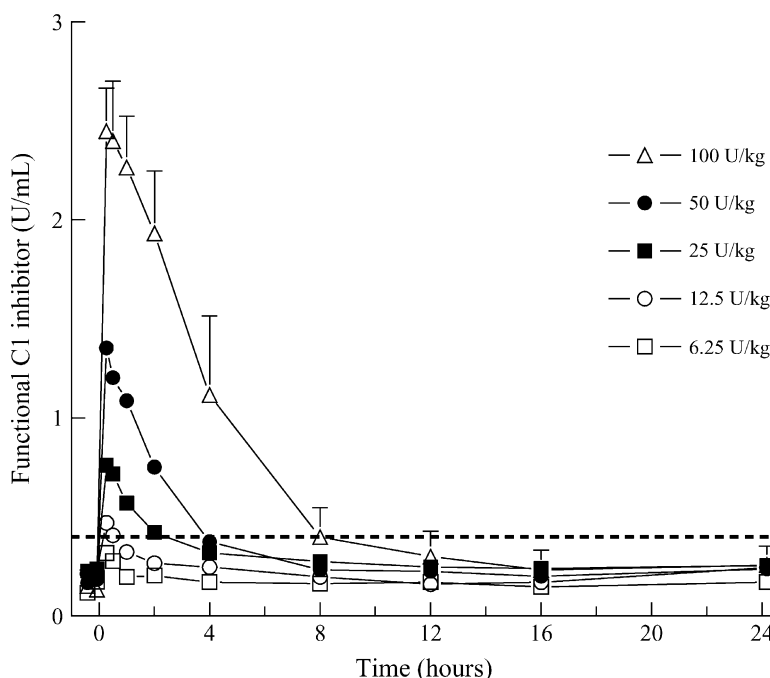


FIG 1. Time profiles of mean functional C1INH (in units per milliliter) in the distinct dosage groups. The SD in the highest dosage group (100 U/kg) is indicated by a bar. The dotted horizontal line indicates the 0.4 U/mL level.

TABLE II. Summary of model-independent pharmacokinetic parameters of functional C1INH in each dose group

Dose (U/kg)	N	Baseline (U/mL)	C _{max} (U/mL)	Dose normal C _{max} (U/mL/U/kg)	Time above 0.4 U/mL (min)
6.25	3	0.15 (0.04)	0.32 (0.11)	0.0275 (0.01354)	9.1 (0)
12.5	3	0.22 (0.11)	0.47 (0.14)	0.0201 (0.00300)	55.3 (22.6)
25	6	0.23 (0.10)	0.76 (0.12)	0.0213 (0.00319)	206.6 (150.3)
50	6	0.18 (0.12)	1.36 (0.31)	0.0236 (0.00468)	314.5 (210.3)
100	6	0.14 (0.08)	2.46 (0.22)	0.0232 (0.00198)	549.7 (215.2)

Time above 0.4 U/mL: time span in which the functional C1INH concentration exceeded 0.4 U/mL. SDs are shown in parentheses.

associated between-subject variability in these parameters, NONMEM also provides so-called empiric Bayesian estimates, which are individual estimates conditional on the previously obtained population information. These empiric Bayesian estimates allow the generation of individual predicted profiles and allow an initial assessment of, for instance, dose dependence of pharmacokinetics (PK) parameters.²¹

Because the initial analysis indicated possible nonlinear kinetics, likely caused by a saturable elimination mechanism, an additional model assuming Michaelis-Menten elimination was also applied. This model provided estimates of V_{max}, Michaelis-Menten constant, volume of distribution, and endogenous infusion rate of functional C1INH.

Because baseline C4 concentrations were highly variable between subjects, these concentrations were first normalized by expressing them as the percentage change from baseline for each individual, where baseline (average of values at 25 and 5 minutes before dosing) was set at 100%. C4 was characterized by baseline, C_{max}, and time of maximum concentration (T_{max}) (obtained after the first 4 hours) and additionally by minimum concentration (C_{min}) and time of

minimum concentration (T_{min}). C4b/c was summarized by using baseline, C_{min}, and T_{min}.

The relationship of PK and pharmacodynamics parameters with dose was investigated by using the Jonckheere-Terpstra nonparametric test for ordered differences among classes with 2-sided exact *P* values. This nonparametric test for trend with dose was used because variability for some parameters tended to fluctuate markedly with increasing dose, making regression or ANOVA techniques unsuitable.

Analyses were performed with SAS for Windows V8.2 (SAS Institute, Inc, Cary, NC).

RESULTS

The study population consisted of 8 male and 4 female subjects. All subjects completed the study. The adverse events reported in the course of this study were graded mild or moderate, except for one severe HAE attack. None of the adverse events were considered related to the study drug.

TABLE III. Summary of model-dependent pharmacokinetic parameters (empiric Bayesian estimates) of functional C1INH in each dose group (initial model)

Dose (U/kg)	N	Initial model		
		Clearance (mL/min)	Volume (L)	Half-life (min)
6.25	3	71.4 (10.2)	2.75 (1.03)	28.0 (13.4)
12.5	3	59.5 (11.8)	3.29 (0.62)	40.1 (13.7)
25	6	33.9 (9.7)	3.50 (0.85)	73.1 (13.7)
50	6	22.8 (7.3)	3.03 (0.79)	93.7 (8.5)
100	6	12.7 (2.5)	3.10 (0.72)	172.1 (36.1)

SDs are shown in parentheses.

TABLE IV. Summary of model-dependent pharmacokinetic parameters (empiric Bayesian estimates) of functional C1INH in each dose group (Michaelis-Menten elimination)

Dose (U/kg)	N	Michaelis-Menten elimination		
		V_{max} (U/min)	Volume (L)	Endogenous infusion rate (U/min)
6.25	3	48.4 (0.92)	3.20 (0.224)	11.3 (2.31)
12.5	3	50.5 (3.01)	3.53 (0.308)	13.1 (4.39)
25	6	48.8 (6.10)	3.57 (0.753)	13.8 (4.39)
50	6	46.9 (14.97)	3.12 (0.784)	13.1 (7.66)
100	6	39.8 (3.98)	3.22 (0.758)	10.7 (3.36)

The Michaelis-Menten constant (K_m) was estimated at 0.57 U/mL, with an interindividual variability of 0% indicating that the analysis is incapable of distinguishing different values for this parameter between subjects. SDs are shown in parentheses.

TABLE V. Summary of model-independent pharmacodynamic parameters of C4

Dose (U/kg)	N	Baseline (nmol/L)	C_{max} (%)	T_{max} (min)	C_{min} (%)	T_{min} (min)
6.25	3	800.0 (491.07)	127.9 (2.82)	640.0 (138.56)	94.7 (6.24)	70.0 (45.83)
12.5	3	479.7 (192.51)	121.4 (10.87)	640.0 (138.56)	93.7 (4.93)	25.3 (8.08)
25	6	736.8 (349.27)	142.0 (24.58)	640.0 (195.96)	88.3 (9.77)	35.3 (20.11)
50	6	538.7 (184.37)	175.5 (81.93)	640.0 (123.94)	80.0 (2.05)	35.3 (20.11)
100	6	510.9 (202.48)	201.0 (89.14)	720.0 (0.00)	76.3 (4.30)	50.3 (39.53)

C_{max} , maximum concentration; T_{max} , time of maximum concentration; C_{min} , minimum concentration; T_{min} , time of minimum concentration. SDs are shown in parentheses.

No clinically significant increases in anti-C1INH or anti-rabbit milk protein antibodies were observed, and none of the patients showed evidence of antibodies neutralizing C1INH activity. Vital signs, ECG results, and routine laboratory parameters were virtually unchanged.

Pharmacokinetics of rhC1INH

The profiles of functional C1INH showed a dose-dependent increase in C_{max} ($P < .0001$) and the time span in which the functional C1INH concentration exceeded 0.4 U/mL ($P < .0001$, Fig 1 and Table II). After dosing at 100 U/kg, the functional C1INH level remained greater than 0.4 U/mL for 9.2 ± 3.6 hours (Table II). Dose-normalized C_{max} appeared constant ($P = .48$, Table II), indicating dose proportionality, but the standard PK analysis showed that clearance and elimination half-lives were dependent on dose (Table I and III). Therefore the profiles were also analyzed by using a 1-compartment model assuming Michaelis-Menten elimination. This showed that the data could be adequately described and revealed dose-independent estimates of V_{max} , Michaelis-Menten constant (0.57 U/mL), volume of distribution, and endogenous infusion rate (Table IV). All results and considerations presented in this article on the pharmacokinetics of rhC1INH are based on the assay of functional C1INH in plasma (ie, on the detection of C1 esterase inhibiting activity in plasma).

Results and analyses obtained from the assay of C1INH antigen were similar to those obtained through the detection of functional C1INH and therefore were not considered of sufficient interest to be presented here.

Pharmacodynamics of rhC1INH

After infusion of rhC1INH, an initial dose-dependent decrease in mean normalized C4 concentration was observed ($P < .0001$; Table V), followed by a dose-dependent increase ($P = .0034$, Fig 2). The response was highly variable within dosage groups and is summarized in Table V. The C4 peak levels occurred at approximately 12 hours after infusion and thereafter gradually decreased to baseline levels. At the 100 U/kg dose, a maximal increase of $201\% \pm 89\%$ was observed.

An immediate dose-dependent decrease of C4b/c concentration was observed ($P < .0001$; Table VI). In addition, the duration of C4b/c decrease appeared dose dependent (Fig 3). Figs 4 and 5 illustrate the inverse relationship between functional C1INH and C4b/c. After infusion of rhC1INH at 6.25, 12.5, 25, 50, and 100 U/kg, the lowest mean C4b/c levels of 56.7, 28.7, 17.8, 7.1, and 2.2 nM coincided with mean functional C1INH levels of 0.32, 0.41, 0.76, 1.35, and 2.27 U/mL, respectively (Figs 1 and 3). Although clear immediate C4b/c reductions were observed after the infusion of rhC1INH at all dosages, C4b/c levels in the normal range were, with few exceptions, only observed when the functional C1INH levels

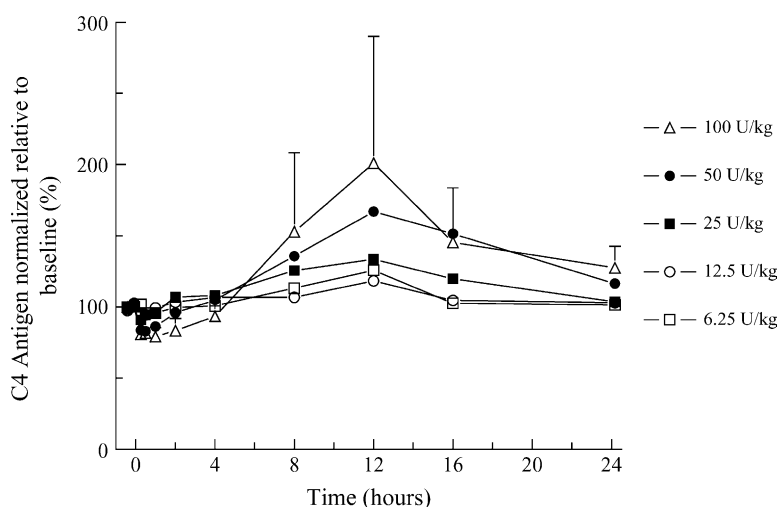


FIG 2. Time profiles of mean normalized C4 antigen (in percentages) in the distinct dosage groups. The SD in the highest dosage group (100 U/kg) is indicated by a bar.

TABLE VI. Summary of model-independent pharmacodynamic parameters of C4b/c

Dose (U/kg)	N	Baseline (nmol/L)	C _{min} (nmol/L)	T _{min} (min)
6.25	3	128.0 (48.59)	48.3 (24.79)	30.7 (25.40)
12.5	3	78.8 (24.83)	20.6 (15.25)	35.3 (22.48)
25	6	103.5 (62.00)	15.9 (9.73)	25.7 (17.73)
50	6	107.4 (58.51)	5.3 (1.90)	40.3 (39.63)
100	6	99.9 (30.80)	1.9 (0.39)	95.0 (39.87)

C_{min}, Minimum concentration; T_{min}, time of minimum concentration. SDs are shown in parentheses.

were in the normal range or higher (Figs 3 and 5). Thus a normal or increased functional C1INH concentration prevents cleavage of C4 (ie, the formation of C4b/c).

DISCUSSION

This is the first clinical study with any form of recombinant C1INH. Twelve asymptomatic patients with HAE received 2 escalating intravenous doses of rhC1INH to characterize its pharmacokinetics and biologic effects. rhC1INH appeared safe and was well tolerated because no drug-related adverse events or clinically significant changes in ECG results, vital signs, and laboratory parameters were observed. All subjects were exposed to rhC1INH on 2 occasions without evidence of allergic-hypersensitivity reactions or clinically significant antibody responses to rhC1INH.

The pharmacokinetics of rhC1INH revealed dose proportionality for C_{max} and for the time span in which the functional C1INH level was greater than 0.4 U/mL (Fig 1 and Table II). After the infusion of rhC1INH at 100 U/kg, functional C1INH levels in plasma remained greater

than 0.4 U/mL for about 9 hours (Figure 1 and Table II). Activation of the cascade systems releasing inflammatory mediators responsible for the generation of edema is thought to be sufficiently controlled, with functional C1INH levels exceeding 0.4 U/mL. No HAE attacks are observed with functional C1INH levels exceeding approximately 0.4 U/mL.^{6,9}

Furthermore, rhC1INH showed a dose-dependent clearance, most likely caused by a saturable mechanism of elimination, and its half-life was approximately 3 hours after administration of rhC1INH at 100 U/kg (Table III). The half-life of rhC1INH appeared reduced compared with that of human plasma-derived C1INH, which reportedly is at least 24 hours.^{15,16,22-24} This is probably related to differential glycosylation of the 2 C1INH species.¹⁹ The presence of oligomannose and hybrid type N-linked glycans and a low degree of sialylation in rhC1INH results in the exposure of terminal galactose groups, mannose groups, or both, which are recognized and bound by specific receptors, most notably on hepatocytes.²⁵ Thus uptake of rhC1INH by hepatic galactose and mannose receptors likely represents the major clearance pathway of native rhC1INH. A minor portion of rhC1INH in asymptomatic HAE⁵ might clear through receptors specific for serpin-enzyme complexes.²⁶

Functional C1INH is assumed to indirectly affect C4 levels through inhibition of C1 esterase, which can cleave C4 into C4b/c. Recombinant C1INH displayed biologic activity in the patients through a dose-dependent increase in C4 antigen and an immediate and dose-dependent decrease in C4b/c concentration (Tables V and VI and Figs 2 and 3). C4 peak concentrations at 12 hours after infusion of rhC1INH at 100 U/kg in this study in asymptomatic patients with HAE were similar to the increase seen in C4 concentrations after administration of plasma-derived C1INH at 25 U/kg to patients with acute HAE attacks.¹⁶ Furthermore, a number of studies reported

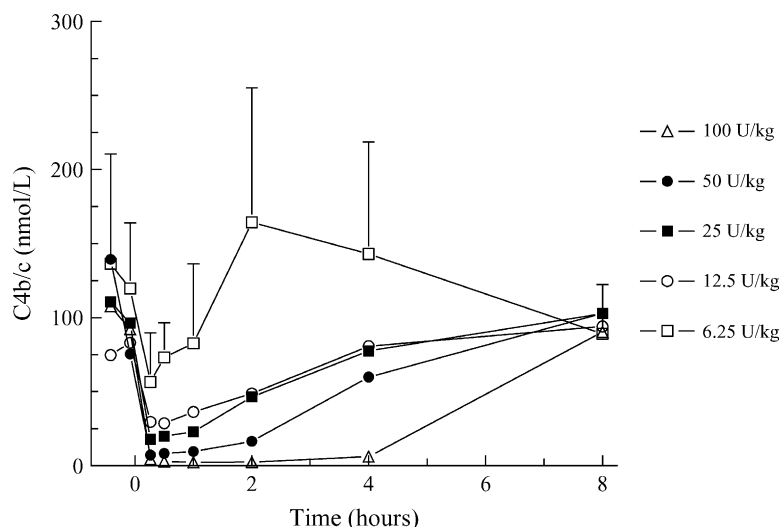


FIG 3. Time profiles of mean C4b/c (in nanomoles per milliliter) in the distinct dosage groups during the first 8 hours after infusion. The SD in the lowest dosage group (6.25 U/kg) is indicated by a bar.

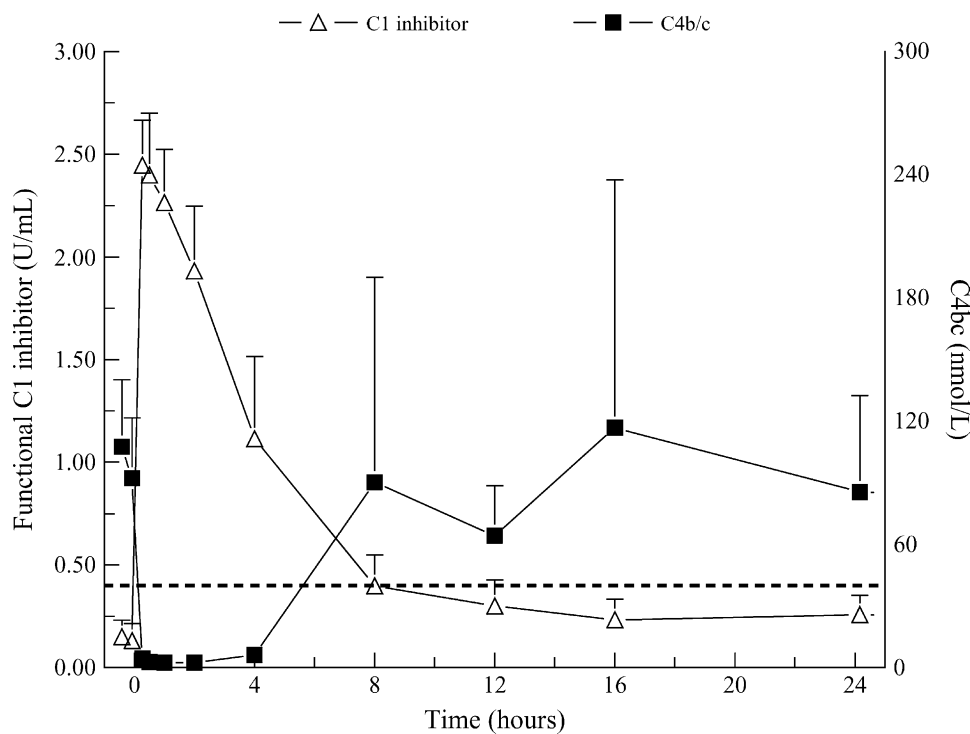


FIG 4. Mean (SD) functional C1INH and C4b/c time profiles in the 100 U/kg dosage group.

a similar lag time (ranging from 6-24 hours) between C1INH administration and C4 increases in patients with HAE.^{9,13,22,23} The late increase in C4 concentration therefore seems to be a common phenomenon after the administration of C1INH, likely reflecting the rate of *de novo* C4 synthesis.

The immediate dose-dependent inhibition of C4 cleavage after the administration of rhC1INH (Fig 3) coincided

with initial dose-dependent decreases in C4 levels (Fig 2). In our experience polyclonal antibodies often contain antibodies against epitopes not present on the native antigen.^{20,27,28} Polyclonal anti-C4 antibodies used in the antigenic C4 assay might have a lower affinity for native than for cleaved C4. Consequently, underestimation of total C4 levels at normal or increased functional C1INH levels when cleavage of C4 is inhibited (Fig 1 and 5) might

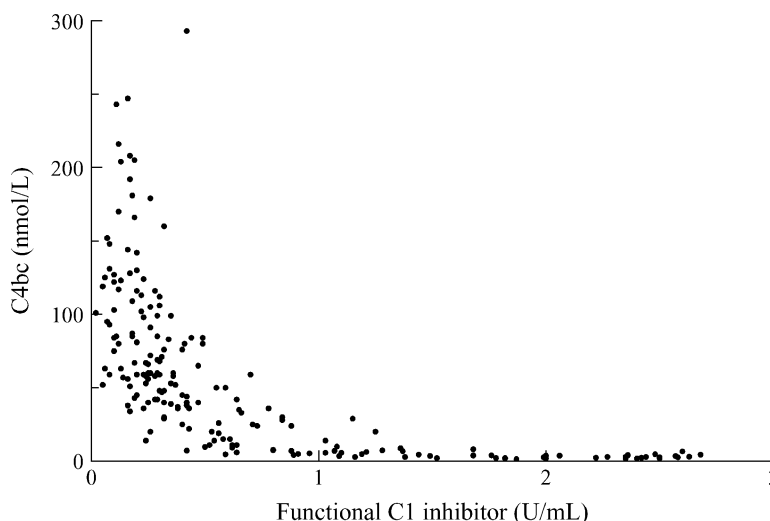


FIG 5. Scatterplot of all C1INH and C4b/c concentrations. The levels of individual functional C1INH in all serial plasma samples collected from patients with HAE who received rhC1INH at escalated doses between 6.25 and 100 U/mL were plotted against the corresponding plasma C4b/c concentrations.

explain the initial dose-dependent decreases in C4 levels after administration of rhC1INH.

The time profiles and inverse relationship of functional C1INH and C4b/c (Figs 1, 3, 4, and 5) show that cleavage of C4 occurs once the functional C1INH concentration is reduced. At baseline, a highly variable but significant proportion of total C4 is present in the cleaved form (Table V and VI). Although the sharp decrease of C4b/c concentration after the administration of C1INH provides clear evidence for the biologic activity of rhC1INH, it cannot be excluded that a major proportion of C4b/c in patient's plasma might be derived from *in vitro* cleavage of C4 (ie, after blood collection). If it is assumed that the sudden decrease in C4b/c concentration after the administration of rhC1INH (Fig 3) reflects only *in vivo* inhibition of C4 cleavage and a rapid clearance of cleaved C4, this would imply a very short half-life of C4b/c (<1 min), as well as a very high *de novo* C4 synthesis rate to arrive at the high predose C4b/c levels. The administration of rhC1INH at doses yielding normal or increased functional C1INH levels would then have to result in almost instant massive increases in C4. In contrast, initial dose-dependent decreases of C4 and late dose-dependent limited increases in C4 were observed. On the basis of the inverse relationship of functional C1INH and C4b/c and with the latter representing the sum of C4 cleaved *in vivo* and *in vitro*, it is concluded that the assay of C4 in plasma remains the most straightforward parameter for *in vivo* biologic activity of C1INH.

In conclusion, the findings from this study warrant clinical studies investigating the efficacy and safety of rhC1INH in symptomatic patients with HAE. It is conceived that an adequate dosage of rhC1INH will provide functional C1INH levels for a sufficiently long period to halt the progression of edema and allow its resolution. These studies will provide information of the minimum

requirements for level and duration of reconstitution with rhC1INH to achieve optimum efficacy in symptomatic patients with HAE.

REFERENCES

1. Cicardi M, Agostoni A. Hereditary angioedema. *N Engl J Med* 1996; 334:1666-7.
2. Landerman NS, Webster ME, Becker EL, Ratcliffe HE. Hereditary angioneurotic edema. II. Deficiency of inhibitor for serum globulin permeability factor and/or plasma kallikrein. *J Allergy* 1962;33:330-41.
3. Zuraw BL, Curd JG. Demonstration of modified inactive first component of complement (C1) inhibitor in the plasmas of C1 inhibitor-deficient patients. *J Clin Invest* 1986;78:567-75.
4. Nielsen EW, Johansen HT, Hogasen K, Willemin W, Hack CE, Mollnes TE. Activation of the complement, coagulation, fibrinolytic and kallikrein-kinin systems during attacks of hereditary angioedema. *Scand J Immunol* 1996;44:185-92.
5. Cugno M, Nuijens J, Hack E, Eerenberg A, Frangi D, Agostoni A, et al. Plasma levels of C1-inhibitor complexes and cleaved C1-inhibitor in patients with hereditary angioneurotic edema. *J Clin Invest* 1990;85: 1215-20.
6. 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.
7. Gelfand JA, Sherins RJ, Alling DW, Frank MM. Treatment of hereditary angioedema with danazol. Reversal of clinical and biochemical abnormalities. *N Engl J Med* 1976;295:1444-8.
8. Zurlo JJ, Frank MM. The long-term safety of danazol in women with hereditary angioedema. *Fertil Steril* 1990;54:64-72.
9. Agostoni A, Cicardi M. Hereditary and acquired C1-inhibitor deficiency: biological and clinical characteristics in 235 patients. *Medicine (Baltimore)* 1992;71:206-15.
10. Bork K, Pitton M, Harten P, Koch P. Hepatocellular adenomas in patients taking danazol for hereditary angio-oedema. *Lancet* 1999;353:1066-7.
11. Crampon D, Barnoud R, Durand M, Ponard D, Jacquot C, Sotto JJ, et al. Danazol therapy: an unusual aetiology of hepatocellular carcinoma. *J Hepatol* 1998;29:1035-6.
12. 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.

13. Agostoni A, Bergamaschini L, Martignoni G, Cicardi M, Marasini B. Treatment of Acute Attacks of Hereditary Angioedema with C1-Inhibitor Concentrate. *Ann Allergy* 1980;44:299-301.
14. Agostoni A, Cicardi M, Cugno M, Storti E. Clinical problems in the C1-inhibitor deficient patient. *Behring Inst Mitt* 1993;93:306-12.
15. 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.
16. Kunschak M, Engl W, Maritsch F, Rosen FS, Eder G, Zerlauth G, 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.
17. Visentin DE, Yang WH, Karsh J. C1-esterase inhibitor transfusions in patients with hereditary angioedema. *Ann Allergy Asthma Immunol* 1998;80:457-61.
18. Agostoni A, Aygoren-Pursun E, Binkley KE, Blanch A, Bork K, Bouillet L, et al. Hereditary and acquired angioedema: problems and progress: proceedings of the third C1 esterase inhibitor deficiency workshop and beyond. *J Allergy Clin Immunol* 2004;114(suppl):S51-131.
19. Koles K, Van Berkel PH, Pieper FR, Nuijens JH, Manneke ML, Vliegenthart JF, et al. N- and O-glycans of recombinant human C1 inhibitor expressed in the milk of transgenic rabbits. *Glycobiology* 2004;14:51-64.
20. Wolbink GJ, Bollen J, Baars JW, ten Berge RJ, Swaak AJ, Paardekooper J, et al. Application of a monoclonal antibody against a neoepitope on activated C4 in an ELISA for the quantification of complement activation via the classical pathway. *J Immunol Methods* 1993;163:67-76.
21. Schoemaker RC, Cohen AF. Estimating impossible curves using NONMEM. *Br J Clin Pharmacol* 1996;42:283-90.
22. Gadek JE, Hosea SW, Gelfand JA, Santaella M, Wickerhauser M, Triantaphyllopoulos DC, et al. Replacement therapy in hereditary angioedema—successful treatment of acute episodes of angioedema with partly purified C1-inhibitor. *N Engl J Med* 1980;302:542-6.
23. Bergamaschini L, Cicardi M, Tucci A, Gardinali M, Frangi D, Valle C, et al. C1 INH concentrate in the therapy of hereditary angioedema. *Allergy* 1983;38:81-4.
24. 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.
25. Minta JO. The role of sialic acid in the functional activity and the hepatic clearance of C1-INH. *J Immunol* 1981;126:245-9.
26. Perlmutter DH, Glover GI, Rivetna M, Schasteen CS, Fallon RJ. Identification of a serpin-enzyme complex receptor on human hepatoma cells and human monocytes. *Proc Natl Acad Sci U S A* 1990;87:3753-7.
27. Nuijens JH, Huijbregts CC, Eerenberg-Belmer AJ, Abbink JJ, Strack Van Schijndel RJ, Felt-Bersma RJ, et al. Quantification of plasma factor XIIa-C1-inhibitor and kallikrein-C1-inhibitor complexes in sepsis. *Blood* 1988;72:1841-8.
28. Hack CE, Paardekooper J, Smeenk RJ, Abbink J, Eerenberg AJ, Nuijens JH. Disruption of the internal thioester bond in the third component of complement (C3) results in the exposure of neodeterminants also present on activation products of C3. An analysis with monoclonal antibodies. *J Immunol* 1988;141:1602-9.