

# Pharmacokinetics of C1-inhibitor protein in patients with acute myocardial infarction

**Objectives:** C1-inhibitor protein (C1-INH) purified from pooled human plasma is used for the treatment of patients with hereditary angioedema. Recently, the beneficial effects of high-dose C1-INH treatment on myocardial ischemia or reperfusion injury have been reported in various animal models and in humans. We investigated the pharmacokinetic behavior of C1-INH in patients with acute myocardial infarction to calculate the amount of C1-INH required for optimal efficacy.

**Methods:** Twenty-two patients received an intravenous loading dose, followed by 48 hours of continuous infusion of C1-INH. Changes in the endogenous production of C1-INH were evaluated in 16 control patients with acute myocardial infarction. A 2-compartment model was used to estimate the fractional catabolic rate constant (FCR), transcapillary escape rate constant (TER), and extravascular return rate constant (ERR) of C1-INH. Software designed to analyze and fit measured data to unknown parameters in a system of differential equations was used to fit the experimental data against the 3-parameter model.

**Results:** With fixed TER and ERR values ( $0.014 \text{ h}^{-1}$  and  $0.018 \text{ h}^{-1}$ , respectively), 20 of the 22 cases yielded well-determined FCR values, and simultaneous fitting resulted in a median FCR of  $0.011 \text{ h}^{-1}$  (95% confidence interval,  $0.010$  to  $0.012 \text{ h}^{-1}$ ) versus  $0.025 \text{ h}^{-1}$  as reported in healthy control patients. Simultaneous estimation of TER, ERR, and FCR demonstrated weakly defined TER and ERR values, whereas the median FCR value remained unchanged. The use of a 2-compartment model resulted in a significantly better fit compared with the 1-compartment model. Physiologic explanations are offered for discrepancies in the literature.

**Conclusions:** Dose calculation of C1-INH in patients treated with massive doses of C1-INH requires turnover parameters that differ from those found in healthy subjects, possibly because of suppression of continuous C1-INH consumption by target proteases. (Clin Pharmacol Ther 2002;72:498-504.)

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C1-inhibitor protein (C1-INH), a protein of approximately 104 kd, regulates the classical activation pathway of the complement system.<sup>1</sup> Patients with hereditary angioedema (HAE), in whom there is a congenital

lack of C1-INH, have attacks of uncontrolled complement activation that may lead to life-threatening laryngeal obstruction or swelling of the gastrointestinal mucosa.<sup>2</sup> Depending on the severity of the edematous attack, treatment of patients with HAE usually consists of one or more bolus injections of C1-INH purified from pooled human plasma.

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Other diseases in which complement inhibition might have beneficial effects are being explored, and the administration of C1-INH has been investigated in patients with sepsis,<sup>1</sup> rheumatoid arthritis,<sup>3</sup> and B-cell lymphoproliferative disorders.<sup>4</sup> Recent studies in various animal models have shown reduction of myocardial ischemia or reperfusion injury after high-dose C1-INH therapy.<sup>5-8</sup> To investigate the effects of C1-INH in patients with acute myocardial infarction, we recently performed a dose-escalating pilot study.<sup>9</sup> Patients were given an initial loading dose of C1-INH, followed by

Received for publication Dec 12, 2001; accepted June 18, 2002.

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0009-9236/2002/\$35.00 + 0 13/1/129320

doi:10.1067/mcp.2002.129320

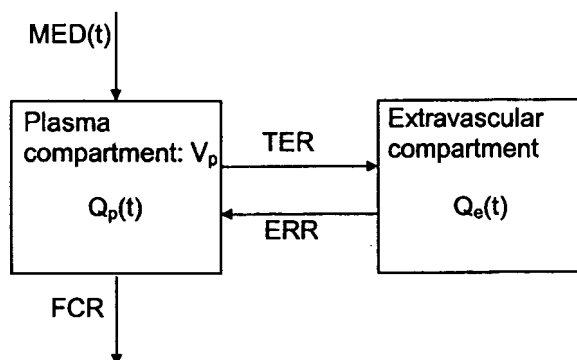
continuous infusion during the next 48 hours. Complement inhibition in this study proved to be strongly dose-dependent and necessitated accurate C1-INH dosage control. However, C1-INH was apparently eliminated more slowly than expected from available data of patients with HAE. Therefore we decided to study the pharmacokinetics of C1-INH in patients with acute myocardial infarction receiving high doses of this inhibitor.

## METHODS

Twenty-two patients (12 men and 10 women; median age, 60.5 years) with acute myocardial infarction were admitted to the Intensive Coronary Care Unit, University Hospital Maastricht, The Netherlands. After informed consent was obtained for patients to participate in an open-label pilot study, treatment with C1-INH was started 6 hours after the onset of symptoms by means of 3 different dosage schemes as follows: an initial loading dose of 50 U/kg body weight, followed by a continuous infusion of  $1.25 \text{ U} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ; an initial loading dose of 100 U/kg body weight, followed by a continuous infusion of  $1.25 \text{ U} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ; or an initial loading dose of 100 U/kg body weight, followed by a continuous infusion of  $2.00 \text{ U} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  during the next 48 hours. Loading doses were infused at a rate of 250 U/min. The administered amounts were chosen in an effort to reach concentrations between 200% and 300% of normal. No presumptions were made about reaching steady-state conditions. C1-INH (Cetor; CLB, Amsterdam, The Netherlands) was purified (>95%) from human plasma according to good manufacturer's practice guidelines and was tested for viral safety and clinical efficacy. The lyophilized protein was reconstituted in water before administration.

Blood samples were collected before administration of the loading dose and at 1, 3, 6, 9, 12, 15, 18, 24, 36, 48, and 72 to 96 hours after the start of loading dose administration. Clotting was prevented with ethylenediaminetetraacetic acid (EDTA), and plasma samples obtained by immediate routine centrifugation were stored at  $-70^\circ\text{C}$  until further analysis.

Changes in endogenous C1-INH production after acute myocardial infarction were assessed by measuring the course of plasma C1-INH in 16 control patients with acute myocardial infarction who did not receive C1-INH. These patients were admitted to the Department of Cardiology, VU University Medical Center, Amsterdam, The Netherlands.<sup>10</sup> Blood samples were collected at admission and at 2, 6, 12, 24, 36, 48, 60, and 72 hours thereafter. After centrifugation, plasma



**Fig 1.** Two-compartment model.  $Q_p(t)$ , Amount of C1 inhibitor (C1-INH) in plasma (in units) at time  $t$ ;  $Q_e(t)$ , amount of extravascular C1-INH (in units) at time  $t$ ;  $V_p$ , plasma volume;  $Med(t)$ , input function of C1-INH;  $TER$ , transcapillary escape rate constant;  $ERR$ , extravascular return rate constant;  $FCR$ , fractional catabolic rate constant.

was collected and stored in aliquots at  $-70^\circ\text{C}$  until assays were performed.

C1-INH concentrations were determined with the use of a commercially available functional assay based on a chromogenic assay (Berichrom C1 inhibitor assay; Behringwerke AG, Marburg, Germany). Values were expressed in units per liter, with 1 unit equal to the activity of C1-INH in 1 mL of pooled plasma obtained from healthy donors. The specific activity of C1-INH was 3.8 U/mg protein.

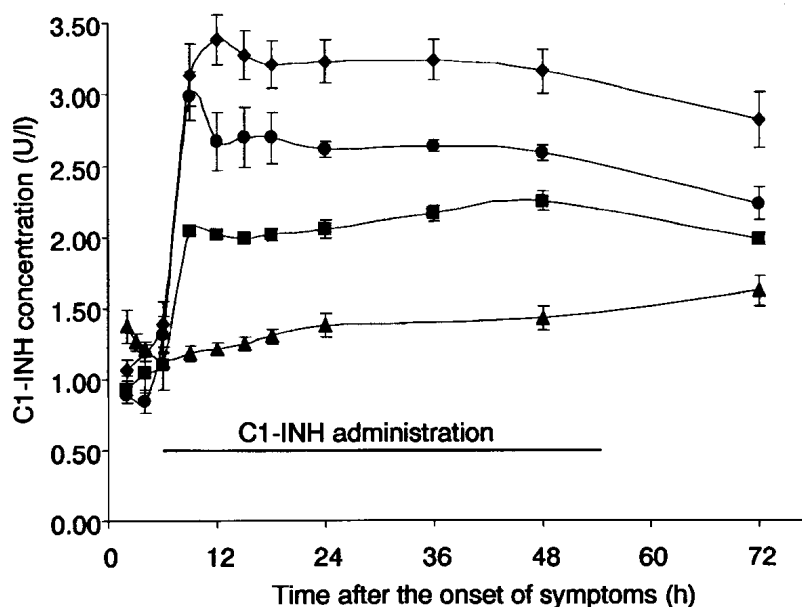
A 2-compartment model, as described in Fig 1, was used to estimate individual pharmacokinetic parameters of C1-INH. Time-related changes in plasma and extravascular C1-INH pools are given by the following equations:

$$dQ_p(t)/dt = MED(t) - (FCR + TER) \times Q_p(t) + ERR \times Q_e(t) \quad (1a)$$

$$dQ_e(t)/dt = TER \times Q_p(t) - ERR \times Q_e(t) \quad (1b)$$

in which  $Q_p(t)$  and  $Q_e(t)$  are the plasma and extravascular pools, respectively, expressed in units at time  $t$ ,  $MED(t)$  is the input function of C1-INH,  $FCR$  is the fractional catabolic rate constant (per hour) for the elimination of C1-INH from the plasma,  $TER$  is the transcapillary escape rate constant (per hour) of C1-INH for extravasation of C1-INH to the extravascular compartment, and  $ERR$  is the extravascular return rate constant (per hour) for the return of C1-INH from the extravascular compartment to plasma.

Equation 2, used as an initial condition, presents steady-state conditions for the amount of C1-INH in the extravascular compartment at time 0.  $Q_p(0)$  is known



**Fig 2.** C1-INH plasma curves of 3 infusion groups and control patients (mean with standard error). Group 1 (*squares*): Loading dose, 50 U/kg; infusion rate,  $1.25 \text{ U} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  ( $n = 6$ ). Group 2 (*circles*): Loading dose, 100 U/kg; infusion rate,  $1.25 \text{ U} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  ( $n = 5$ ). Group 3 (*diamonds*): Loading dose, 100 U/kg; infusion rate,  $2.00 \text{ U} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  ( $n = 11$ ). Control patients (*triangles*): No C1-INH administered ( $n = 16$ ).

by measurement of the C1-INH plasma concentration at time 0 (ie, before C1-INH administration).

$$Q_e(0) = [\text{TER}/\text{ERR}] \times Q_p(0) \quad (2)$$

All plasma concentrations were converted to total amounts in units by multiplying the concentrations in units per liter by the individual plasma volume in liters. Plasma volume was calculated from the initial increase in C1-INH concentration after administration of the loading dose.

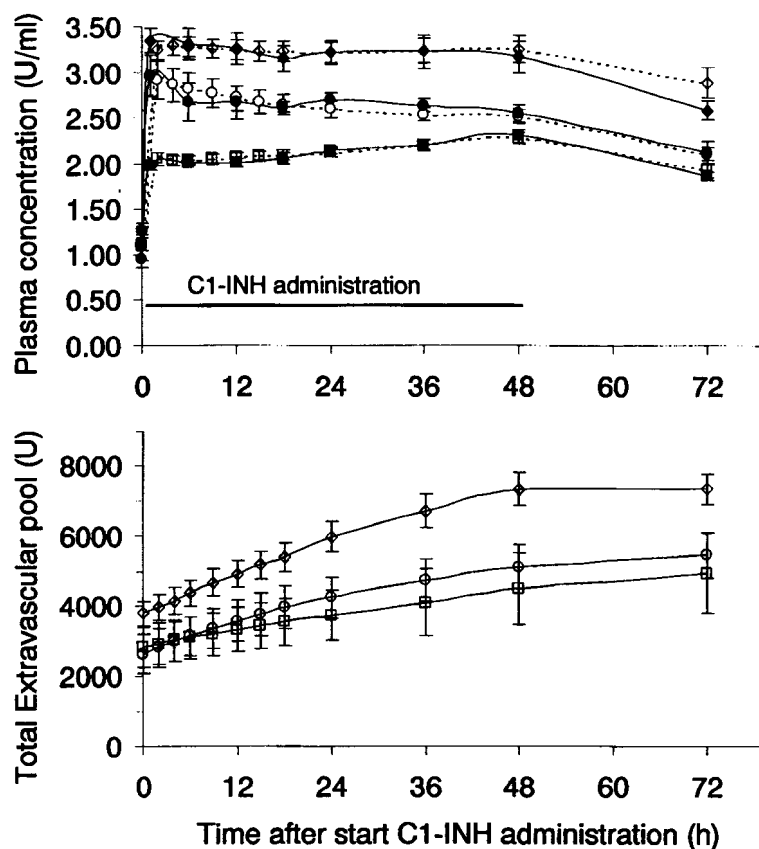
A software package called spIds,<sup>11</sup> developed for estimation of unknown parameters in time-dependent dynamic systems,<sup>12</sup> was used to solve the system defined by the differential algebraic equations 1a and 1b and the initial condition given by equation 2. It uses the algebra package Maple V (Waterloo Maple Inc, Ontario, Canada) to derive variational equations from the differential equations. These variational equations are then used in the process by combining them with the initial differential equations. The Levenberg-Marquardt method was used to minimize the total sum of squared discrepancies between calculated and measured values of  $Q_p$ . A confidence interval (CI) at a user-defined level  $\alpha$  (ie, .05) is calculated for each estimate. The program also gives a detailed analysis of the reliability and interdependencies of the parameters. This is used to

evaluate whether each parameter independently contributes to a significant decrease in the total sum of squares. Thus we can verify whether the use of a 2-compartment model is to be preferred over a 1-compartment model.

## RESULTS

Fig 2 shows mean plasma C1-INH concentrations for the 3 patient groups with different dosage schemes and for the control patients. A clear dose-concentration profile is visible in the 3 groups that received C1-INH therapy. A decrease in C1-INH is shown in the group of control patients in the first 6 hours after the onset of symptoms, and a slow but continuing increase in C1-INH concentration after 6 hours can be noted in the same group.

Fig 3 demonstrates the mean observed and fitted C1-INH plasma concentration curves, as well as the calculated extravascular C1-INH pool, for each dosage group. After a rapid increase caused by the loading dose, a plateau phase is visible for the duration of the infusion. When the infusion was stopped, a gradual decline in the plasma concentration was seen, while the extravascular pool continued to increase, albeit with diminishing speed. The latter finding indicates that no steady-state conditions were reached.



**Fig 3.** Time course of C1-INH plasma concentration and extravascular content after treatment (mean with standard error [ $n = 22$ ]). *Upper panel*, Plasma concentrations (in units per milliliter) in group 1 (squares), group 2 (circles), and group 3 (diamonds). *Solid lines*, Measured C1-INH plasma concentrations; *dotted lines*, fitted C1-INH plasma concentrations. *Lower panel*, Calculated extravascular C1-INH pool (in units) in group 1 (squares), group 2 (circles), and group 3 (diamonds).

On the basis of plausible fixed TER and ERR values ( $0.014 \text{ h}^{-1}$  and  $0.018 \text{ h}^{-1}$ , respectively; see Discussion section), the FCR for individual patients was estimated. The results listed in Table I demonstrate that 20 of the 22 cases had well-determined FCR values, with CIs of less than 55%. The 2 cases with CIs greater than 100% both underwent sampling for only a short period of time (<30 hours) because of difficulties with blood sampling (not drug-related). Simultaneous fitting of the remaining 20 patients with fixed TER and ERR values yielded the same FCR as the calculated median ( $0.011 \text{ h}^{-1}$ ), but this value was better defined (Table I).

Estimation of all 3 parameters resulted in almost the same FCR value,  $0.012 \text{ h}^{-1}$  (CI,  $0.002$  to  $0.022 \text{ h}^{-1}$ ). The TER and ERR values were  $0.021 \text{ h}^{-1}$  (CI,  $-0.018$  to  $0.060 \text{ h}^{-1}$ ) and  $0.019 \text{ h}^{-1}$  (CI,  $-0.055$  to  $0.093 \text{ h}^{-1}$ ), respectively, but the large CIs indicate that no precise TER and ERR values could be determined.

## DISCUSSION

### Recovery of C1-INH

From the plasma volumes and individual body weights listed in Table I, a median plasma volume of  $48 \text{ mL/kg}$  body weight was calculated. Comparison with the normal plasma value of  $41 \text{ mL/kg}$  (corrected for sex, weight, and age)<sup>13</sup> showed an apparent incomplete recovery (85%) of C1-INH, probably caused by consumption of C1-INH in the acute phase. This finding is supported by the initial decrease in C1-INH in control patients (Fig 2).

### Validation of model used

On the basis of the independency of the parameters (data not shown), adding a second compartment to the model caused a significant reduction in the total sum of squares. This means that, although the data obtained lack extravascular measurements, the use of a

**Table I.** Results of 2-compartment model on patients with acute myocardial infarction

Patient no.	Sex	Age (y)	Body weight (kg)	$V_p$ (mL)	FCR and 95% CI ( $h^{-1}$ )*
1	F	74	75	2953	0.009 (0.005 to 0.013)
2	M	51	107	5144	0.009 (0.008 to 0.010)
3	M	60	85	4474	0.007 (0.005 to 0.009)
4	M	58	105	4953	0.005 (0.003 to 0.007)
5†	F	55	55	2500	0.900 (-0.470 to 2.270)
6	F	38	71	3170	0.011 (0.010 to 0.012)
7	M	61	88	3411	0.012 (0.009 to 0.015)
8	F	46	60	2970	0.003 (0.002 to 0.004)
9	F	70	56	2857	0.011 (0.008 to 0.014)
10	M	57	75	5068	0.010 (0.005 to 0.015)
11	M	40	78	3000	0.021 (0.017 to 0.025)
12	M	51	80	4420	0.010 (0.006 to 0.014)
13	M	63	70	3784	0.017 (0.012 to 0.022)
14	F	83	64	2795	0.016 (0.014 to 0.018)
15	F	78	73	3106	0.016 (0.011 to 0.021)
16	M	59	70	3590	0.025 (0.021 to 0.029)
17†	F	74	58	2929	0.002 (-0.004 to 0.008)
18	M	61	100	6329	0.017 (0.010 to 0.024)
19	M	61	82	4059	0.007 (0.004 to 0.010)
20	M	68	93	4769	0.009 (0.007 to 0.011)
21	F	57	96	3254	0.012 (0.005 to 0.019)
22	F	68	87	3750	0.011 (0.009 to 0.013)
Median (n = 20)		60.5	77	3500	0.011 (0.008 to 0.014)

$V_p$ , Plasma volume; FCR, fractional catabolic rate constant; CI, confidence interval; F, female; M, male.

\*Simultaneous fit of 20 patients (fixed transcappillary escape rate constant [ $0.014 h^{-1}$ ] and extravascular return rate constant [ $0.018 h^{-1}$ ]): FCR =  $0.011 h^{-1}$  (95% CI, 0.010 to  $0.012 h^{-1}$ ). Individual fit of 20 patients (estimation of all 3 parameters): FCR =  $0.012 h^{-1}$  (95% CI, 0.002 to  $0.022 h^{-1}$ ).

†Sampling period <30 hours; therefore patient's data were excluded from calculations.

2-compartment model still results in a better fit of the plasma curves compared with a 1-compartment model.

### TER and ERR values

The choice of plausible fixed values for TER ( $0.014 h^{-1}$ ) and ERR ( $0.018 h^{-1}$ ) in this study was based on an overview of data on the behavior of circulating proteins in humans.<sup>14</sup> These data, in most cases obtained from turnover studies of radiolabeled proteins, show an average extravasation rate of about 1.4% of the plasma pool per hour for proteins with a molecular weight exceeding 100 kd. Moreover, it was found that the extravascular pool of such proteins is somewhat smaller than the plasma pool, as also follows from the equilibrium relation  $E/P = TER/ERR = 0.78$ , where  $E/P$  is the extravascular pool/plasma pool ratio.

The fact that no precise TER and ERR values for C1-INH could be determined is mainly caused by the lack of extravascular measurements. The small number of observations (2 or 3) made after the end of infusion enlarges the imprecision of TER and ERR because it is primarily this last part of the plasma curve that expresses these values.

### Estimation of FCR: Comparison with data from literature

Table II presents an overview of C1-INH turnover studies in healthy subjects and in patients with various diseases. Apart from this study, all studies used single intravenous bolus injections of C1-INH. With the exception of the study of Kunschak et al,<sup>15</sup> in which the total observation time was limited to 24 hours, C1-INH concentrations were measured for at least 72 hours.

In a number of these studies<sup>15-17</sup> the apparent disappearance rate constant ( $k_d$ ) rather than the true fractional catabolic rate constant for the disappearance of C1-INH from plasma was determined. For instance, Brackertz et al<sup>16</sup> found a biphasic disappearance of injected C1-INH in control subjects and reported a plasma half-life of 64 hours for the final slow disappearance phase from 3 to 8 days after injection. However, the corresponding apparent disappearance rate constant  $k_d = (\ln 2)/64 = 0.011 h^{-1}$  also incorporates the return of extravascular C1-INH to plasma, which suggests that the FCR would be even lower than  $0.011 h^{-1}$ .

Another situation occurred in the study of Kunschak

**Table II.** Data from literature on human C1 inhibitor turnover

Reference	Subjects	C1-INH preparation	$k_d$ ( $h^{-1}$ )	FCR ( $h^{-1}$ )	TER ( $h^{-1}$ )	ERR ( $h^{-1}$ )	TER/ERR = E/P	Method
Brackertz et al <sup>16</sup> (1975)	Controls (n = 3) Hereditary angioedema (n = 3)	Iodine label	0.011 0.010	— —	— —	— —	— —	Exponential fit ( $k_d$ from second phase)
Quastel et al <sup>18</sup> (1983)	Controls (n = 9) Hereditary angioedema (n = 5)	Iodine label	— —	0.025 0.035	— —	— —	0.60 1.26	Matthews (2-compartment model) or Nosslin (non-compartmental model)
Woo et al <sup>3</sup> (1985)	Controls (n = 10) Rheumatoid arthritis (n = 13)	Iodine label	— —	0.025 0.028	— —	— —	— —	Matthews (2-compartment model)
Melamed et al <sup>4</sup> (1986)	LPD (n = 5)	Iodine label	—	0.053	—	—	1.54	Matthews (2-compartment model)
Waytes et al <sup>17</sup> (1996)	Hereditary angioedema (n = 6)	Concentrate	0.015	—	—	—	—	Half-life estimation over 72 h
Kunschak et al <sup>15</sup> (1998)	Hereditary angioedema (n = 10)	Concentrate	0.018 0.029	— —	— —	— —	— —	$t_{1/2}$ activity $t_{1/2}$ antigen ( $k_d$ first phase)
This study	Acute myocardial infarction (n = 22)	Concentrate	—	0.011 0.012 0.015	0.014 0.021 —	0.018 0.019 —	0.78 1.11 —	2-Compartment fit: TER, ERR (fixed) TER, ERR (free) Corrected for endogenous production

C1-INH, C1 inhibitor;  $k_d$ , apparent disappearance rate constant; TER, transcapillary escape rate constant; ERR, extravascular return rate constant; E/P, extravascular pool/plasma pool; LPD, lymphoproliferative disorder;  $t_{1/2}$ , half-life.

et al,<sup>15</sup> who reported half-lives of 37.8 to 24.0 hours for the initial disappearance phase of C1-INH in patients with HAE, corresponding to  $k_d$  values of 0.018 to 0.029  $h^{-1}$ . These values are indeed higher than the true FCR, because C1-INH is not only catabolized but also extravasating during the first 24 hours after a bolus injection.

Overall, Table II shows that an FCR value of 0.025  $h^{-1}$  was obtained in healthy subjects and that higher values are obtained in patients with various diseases that cause consumption of C1-INH as a result of complement activation, such as HAE, rheumatoid arthritis, and various forms of leukemia.

**Possible explanations for discrepancies in FCR values**

A first explanation for the relatively low FCR value could be a considerable increase in endogenous production of C1-INH in the days after acute myocardial infarction. Such acute phase reactant-like behavior of C1-INH could compensate for the extra consumption of C1-INH as a result of complement activation during the first 24 hours after acute myocardial infarction. However, the course of C1-INH plasma levels in the control patients did not support this explanation, given that levels initially decreased rather than increased (Fig 2),

suggesting increased consumption and not increased synthesis.

Furthermore, increased endogenous production of acute phase reactants usually starts after more than 24 hours, and Fig 2 indeed shows that C1-INH plasma concentrations in control patients are increasing slightly in the days after acute myocardial infarction. On the basis of data from these patients, it was estimated that this slow increase corresponded to an average increase in C1-INH synthesis of 17 U/h. When this production was added to equation 1a, it was found that the FCR changed only marginally to 0.015  $h^{-1}$  (CI, 0.013 to 0.017  $h^{-1}$ ) (Table II).

Another explanation for the discrepancy between healthy subjects and patients with acute myocardial infarction could be that continuous consumption of C1-INH caused by complex formation with activated proteases and subsequent rapid removal of complexes from plasma is a main determinant of C1-INH elimination in healthy subjects. If it is assumed that the disappearance of functional C1-INH proceeds via 2 almost equally contributing pathways, (1) inactivation resulting from complex formation and (2) slow hepatic removal of C1-INH with an FCR value of about 0.011  $h^{-1}$ , it is clear that in studies that used lower doses the

combination of 2 removal pathways could be mistaken for a higher FCR value.

An obvious difference between the other studies mentioned in Table II and this study is that we used bulk amounts of C1-INH instead of trace amounts of radiolabeled C1-INH. Patients with HAE are usually treated with doses of 1000 U. In contrast, the patients with acute myocardial infarction in this study received total doses of as much as 20,000 U. At these high doses, disappearance as a result of complex formation will play only a relatively minor role, as compared with hepatic removal of functional C1-INH. This could reveal the observed FCR value of  $0.011 \text{ h}^{-1}$ .

A similar low FCR value was found by Quastel et al<sup>18</sup> for a dysfunctional C1-INH protein, Ta. They reported that C1-INH Ta scarcely binds to C1. This too supports the idea of the important role of continuous C1-INH disappearance from plasma as a result of complex formation. The defective complex formation of Ta blocked one of the two pathways, thus decreasing the speed of disappearance and revealing the true FCR.

In conclusion, pharmacokinetic data for C1-INH were estimated in patients with acute myocardial infarction receiving high doses of this inhibitor. The obtained FCR value ( $0.011 \text{ h}^{-1}$ ) is lower than that previously found ( $0.025 \text{ h}^{-1}$ ). Saturation of the normal steady-state kinetics of C1-INH may explain this finding. These data may help to design further clinical trials with this anti-inflammatory drug.

We thank Apple Kleine for collecting patient data, Wim Bleeker for his useful suggestions, and the Technology Foundation for the use of the program splds. Special thanks to Walter Stortelder for his valuable knowledge about programmatic details of splds and Jan Kok for his lessons in using this program.

## References

1. Caliezi C, Wuillemin WA, Zeerleder S, Redondo M, Eisele B, Hack CE. C1-esterase inhibitor: an anti-inflammatory agent and its potential use in the treatment of diseases other than hereditary angioedema. *Pharmacol Rev* 2000;52:91-112.
2. 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.
3. Woo P, Lachmann PJ, Harrison RA, Amos N, Cooper C, Rosen FS. Simultaneous turnover of normal and dysfunctional C1 inhibitor as a probe of in vivo activation of C1 and contact activatable proteases. *Clin Exp Immunol* 1985;61:1-8.
4. Melamed J, Alper CA, Cicardi M, Rosen FS. The metabolism of C1 inhibitor and C1q in patients with acquired C1-inhibitor deficiency. *J Allergy Clin Immunol* 1986;77:322-6.
5. Murohara T, Guo JP, Delyani JA, Lefer AM. Cardioprotective effects of selective inhibition of the two complement activation pathways in myocardial ischemia and reperfusion injury. *Methods Find Exp Clin Pharmacol* 1995;17:499-507.
6. Buerke M, Murohara T, Lefer AM. Cardioprotective effects of a C1-esterase inhibitor in myocardial ischemia and reperfusion. *Circulation* 1995;91:393-402.
7. Horstick G, Heimann A, Gotze O, Hafner G, Berg O, Boehmer P, et al. Intracoronary application of C1 esterase inhibitor improves cardiac function and reduces myocardial necrosis in an experimental model of ischemia and reperfusion. *Circulation* 1997;95:701-8.
8. Kleine A, Muller-Boumans M-L, Kop-Klaassen B, Koenderman A, Hack E, Hermens W. Cardioprotective effects of C1-inhibitor after coronary occlusion in dogs: comparison of effects in reperfused and non-reperfused hearts [abstract]. *Eur Heart J* 2000;21:78.
9. Diris J, Kleine A, Zwaan C, Hack C, Tissing M, Strengers P. Beneficial effects of C1-inhibitor treatment in patients with acute myocardial infarction [abstract]. *Clin Chem Lab Med* 2001;39:S183.
10. Lagrand WK. C-reactive protein and related inflammatory mediators in acute and chronic ischemic myocardial syndromes. Amsterdam: Free Univ. of Amsterdam; 2001.
11. Everaars C, Hemker P, Stortelder P. Manual of SPLDs, a software package for parameter identification in dynamic systems. Amsterdam: National Research Institute for Mathematics and Computer Science; 1995.
12. Stortelder W. Parameter estimation in dynamic systems. *Math Comput Simul* 1996;42:135-42.
13. Dagher FJ, Lyons JH, Finlayson DC, Shamsai J, Moore FD. Blood volume measurement: a critical study prediction of normal values: controlled measurement of sequential changes: choice of a bedside method. *Adv Surg* 1965;1:69-109.
14. Hermens WT, Willems GM, Visser MP. Quantification of circulating proteins. Boston: Martinus Nijhoff; 1982.
15. 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.
16. Brackertz D, Isler E, Kueppers F. Half-life of C1INH in hereditary angioneurotic oedema (HAE). *Clin Allergy* 1975;5:89-94.
17. 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.
18. Quastel M, Harrison R, Cicardi M, Alper CA, Rosen FS. Behavior in vivo of normal and dysfunctional C1 inhibitor in normal subjects and patients with hereditary angioneurotic edema. *J Clin Invest* 1983;71:1041-6.