Supplemental material:

METHODS

Study participants and design

Patients aged 18 years and older suffering from AE attacks with at least two attacks per month during the last six months despite prophylactic treatment with four times the standard daily dose of antihistamines, and no known cause for AE were eligible for inclusion. Additionally, C4 levels and C1-esterase inhibitor (C1-INH) function of functional levels were required (C4>0.1 g/L and C1-INH function > 0.63 U/ml). Patients were excluded in cases of accompanying wheals; pregnancy or breastfeeding; a history of rabbit allergy; ACE-inhibitor use in the past six months; recent or current use of methotrexate, azathioprine, mycophenolic acid, omalizumab or cyclosporine. Patients with clinically relevant conditions that had the potential to compromise the safety of the patient such as renal or hepatic insufficiency or malignancies or when another diagnosis was deemed more likely (e.g. allergic AE, drug-hypersensitivity, mastocytosis or HAE) were also excluded. All patients continued to use 4dd antihistamines in order to reduce the risk of bias due to alterations in treatment other than the initiation of rhC1-INH. Patients were allowed to use rescue medication during acute AE attacks including antihistamines, oral steroids and intramuscular adrenaline.

All patients provided written informed consent, and the study was approved by the local ethics committee (protocol number 17-139).

All enrolled patients completed a four-week observation period, followed by an eight-week treatment period with rhC1-INH (Recombinant human C1 esterase inhibitor (rhC1-INH), Conestat alfa/Ruconest®; Pharming Technologies; Leiden , The Netherlands), followed by another four-week observation period (Supplemental figure 1). Throughout the entire trial, patients continued using 4 doses daily antihistamines. The total dose of rhC1-INH was calculated based on body weight (50 IU/kg; max 4200 IU) and was twice-weekly administered intravenously over a time course of five minutes.

A detailed medical history and physical examination were recorded at first visit and adverse events and concomitant drug use were registered at each following visit.

The attack frequency and severity were recorded in the AE activity score (AAS) form.[1] Weekly AAS results (AAS7) were combined into AAS per four weeks (AAS28). An attack was separated from a subsequent attack when there had been an AE free period between reported swellings on subsequent days as scored on the AAS form. Due to explorative nature of this study, power calculation and statistical significance analysis were not performed. The primary endpoint of the study was a reduction in attack frequency of 50% in the treatment period compared to the cumulative observation period. Secondary outcomes were AAS28 and AE Quality of Life (AE-QoL) scores during the treatment period compared to observation period. Outcomes were assessed per case. Post-trial follow-up data was collected by analysing patient records. InH-AAE patients were treated according to the AE and urticaria local treatment protocol,[2] which is in line with international guidelines, but shared decision between the patient and physician allowed for protocol deviation.

Collection of blood samples and laboratory assessments

Blood was obtained at visit 2,4 and 17 (prior to C1-INH dose administration) and at 18 (follow-up visit). C1-INH function was measured with a chromogenic assay (Sanquin, the Netherlands). C4 levels and total IgE were determined at visit 1. In women <50 years of age, pregnancy was excluded via a urine dipstick βHCG test. CRP, d-dimer and leukocyte count were determined with routine assays. C1-INH, FXII, PK and HK levels were visualised using immunoblot. For this, EDTA plasma was diluted 40 times in four times reducing sample buffer (15.5% glycerol, 96.8 mM Tris-HCL, 3.1% SDS, and 0.003% bromophenol blue, 25 mM DTT), boiled for 10 minutes, and 5 µL per sample was loaded and ran on a 4-12% Bis-Tris gel at 165V for 60 minutes and transferred onto Immobilon-FL membranes at 125V for 55 minutes. For detection, polyclonal goat anti human IgG antibodies (anti-human FXII CI20055AP, anti-human PK CI20090A, anti-human HK CI20027AP, anti-human C1-INH CL200323AP, Cedarlane, Burlington, Canada) and Alexa Fluor 680 donkey anti-sheep IgG (lot#1878516, Dako, Glostrup, Denmark) were used.

Levels of cHK in EDTA plasma, an indirect marker for bradykinin release, were determined with ELISA as described above.[3] The upper normal limit was assessed using values of ~50 healthy individuals for cHK and ~20 healthy controls for C1-INH complexes.

Role of the funding source

The study was designed and performed by the study team of the UMC Utrecht at the Dermatology and Allergology clinic, and partly financed by Pharming Technologies, which was informed about the study protocol and was notified regarding inclusion of patients and progress in order to organize drug delivery. The company donated the required rhC1-INH for the treatment period and also donated rhC1-INH for patient 1 for six months treatment after the last study visit. Omalizumab treatment was given under standard insurance coverage.

Laboratory results

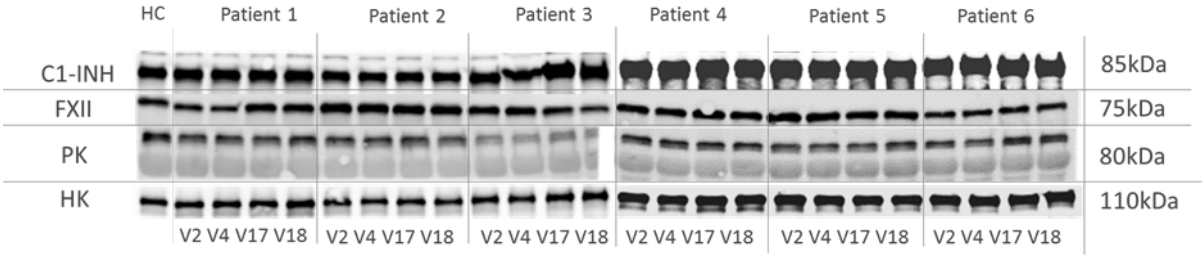

Figure S1
Immunoblot of the proteins of the contact system.
Legend: Immunoblot of the proteins of the contact system C1-INH (C1-esterase inhibitor), HK (high molecular weight kininogen), PK (plasma kallikrein) and FXII (factor XII). Healthy Control (HC) represents a healthy pooled plasma sample. Material was taken pre- and post-treatment at visit V2 and V18 in the observation period and during treatment at visits 4 and 17(see also figure 1), with samples being collected prior to rhC1-INH administration.

Figure S2
cHK, d-dimer, CRP and leukocyte laboratory assessment
A) % Cleaved kininogen (cHK). 100% indicates cleavage of the total HK pool**.** Material was taken pre- and post-treatment at visit V2 and V18 in the observation period and during treatment at visits 4 and 17(see also figure 1), with samples being collected prior to rhC1-INH administration.. Dotted lines represent upper range or normal ranges. Data V02 Patient 5 missing. Reference range <20% **B) D-dimer levels in mg/L over time.** Reference range 0.00-0.50 mg/L. **C) CRP (mg/L) over time during study.** Reference range <10mg/L. **D) Leukocytes (x10^9/L) over time during study**. Reference range 4.0-10.0 x10^9/L

A2M	F2	KLK4	PTGS1
ACE	HRH1	KLK5	PTGS2
ANGPT1	HRH3	KLK6	SERPINA1
BDKRB1	HRH4	KLK7	SERPINA4
BDKRB2	KLK1	KLK8	SERPINB2
CPB2	KLK10	KLK9	SERPINE1
CPM	KLK11	KLKB1	SERPINF2
CPN1	KLK12	KNG1	SERPING1
CPN2	KLK13	MASP1	TFPI
DPP4	KLK14	MASP2	VEGFA
F11	KLK15	PLAU	XPNPEP1
F12	KLK2	PLAUR	XPNPEP2
F13B	KLK3	PLG	MYOF
			HS3ST6

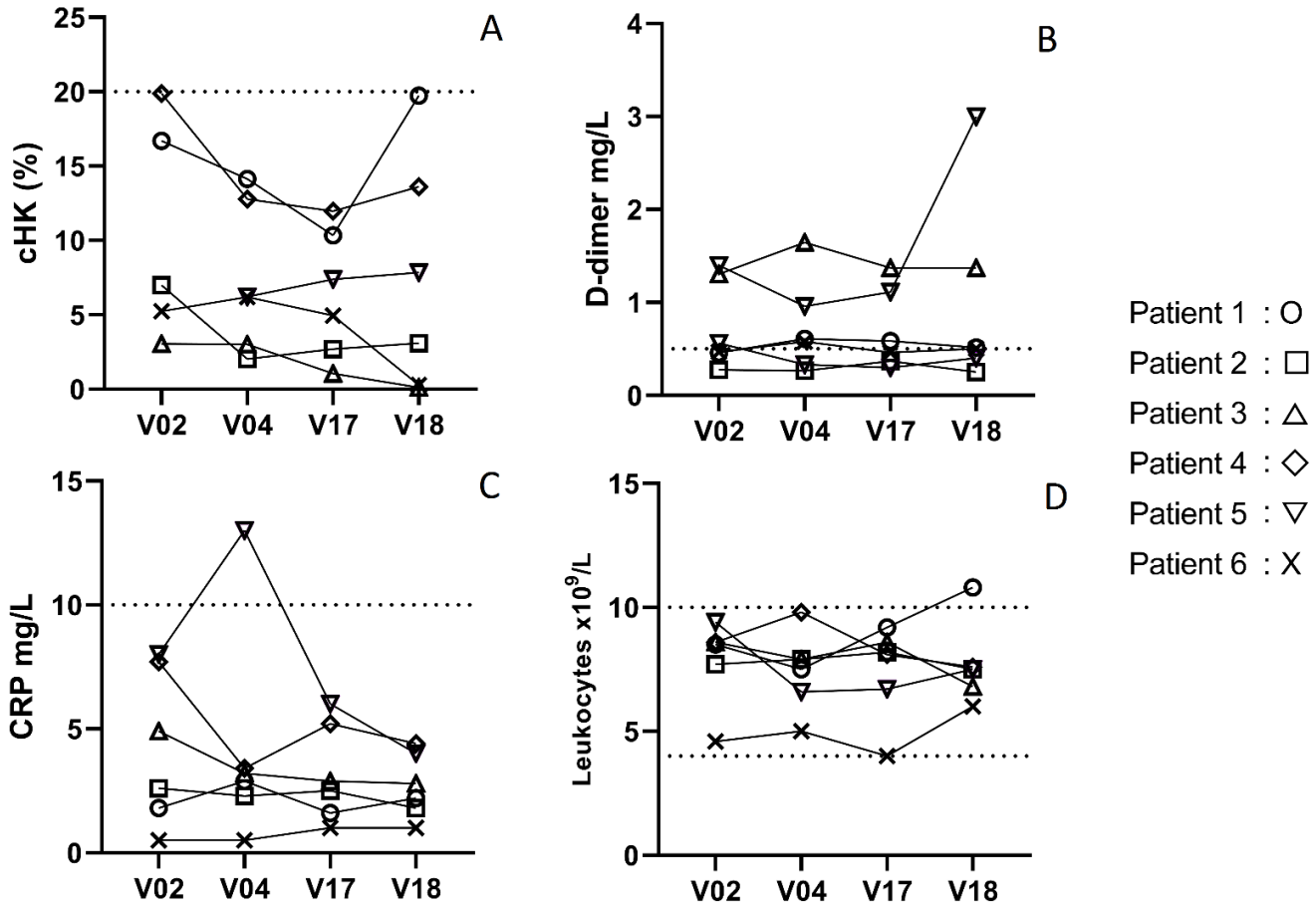

Table S1
Gene panel analysed in patient 1 (rhC1-INH responder).

References used in supplemental material

1. Weller K, Groffik A, Magerl M, Tohme N, Martus P, Krause K, et al. Development, validation, and initial results of the Angioedema Activity Score. Allergy. 2013;68(9):1185-92.
2. Dekkers C, Alizadeh Aghdam M, de Graaf M, Knulst AC, Meijer Y, van den Reek J, et al. Safety and effectiveness of omalizumab for the treatment of chronic urticaria in pediatric patients. Pediatr Allergy Immunol. 2021;32(4):720-6.
3. Hofman ZLM, de Maat S, Suffritti C, Zanichelli A, van Doorn C, Sebastian SAE, et al. Cleaved kininogen as a biomarker for bradykinin release in hereditary angioedema. J Allergy Clin Immunol. 2017;140(6):1700-3 e8.