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A Phase I Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Recombinant Human Hyaluronidase PH20 Administered Intravenously in Healthy Volunteers

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ABSTRACT

Background: Recombinant human hyaluronidase PH20 (rHuPH20) is used in subcutaneous formulations (eg, RITUXAN HYCELA [rituximab and hyaluronidase human], HERCEPTIN HYLECTA [trastuzumab and hyaluronidase-oysk], PHESGO [pertuzumab/trastuzumab/hyaluronidase-zzxf], and Darzalex FASPRO [daratumumab and hyaluronidase-fihj]) to increase the dispersion and absorption of coadministered therapeutics. Although unlikely, subcutaneous products that include rHuPH20 could be mistaken for the intravenous formulation of the corresponding drugs (eg, RITUXAN [rituximab], HERCEPTIN [trastuzumab], and DARZALEX [daratumumab]). To understand the potential effects of inadvertent intravenous injection of rHuPH20, we investigated the safety profile, pharmacokinetics (PK), and pharmacodynamics (PD) of rHuPH20 administered intravenously.

Objectives: This Phase I, open-label, single-center study in healthy volunteers was designed to assess the safety profile, tolerability, PK, and PD of rHuPH20 administered intravenously.

Methods: Healthy volunteers received 5 mL intravenous infusion of either 10,000 U (n = 12) or 30,000 U (n = 12) rHuPH20 over 5 minutes. Blood samples for PK and PD analysis were obtained at baseline and at various times after initiation of infusion. Adverse events and laboratory parameters were measured to assess the safety profile and tolerability of the intravenous infusion. The PK of rHuPH20 was assessed using both an enzymatic assay and a mass-based immunoassay, and plasma hyaluronan concentrations were measured as a PD marker using an HPLC–MS/MS disaccharide assay.

Results: All 24 volunteers (mean age = 36.5 years) completed the study, and no serious adverse events were reported in either treatment group. Overall, 2 adverse events (both Grade 1) were reported; catheter site pain in the 10,000 U group and hypotension in the 30,000 U group. Plasma concentrations of rHuPH20 increased during the 5-minute intravenous infusion (median t_{max} = 6 minutes from intravenous initiation) followed by a rapid plasma clearance ($t_{1/2}$ ~10 minutes from intravenous initiation). Plasma hyaluronan concentrations increased with dose and time (t_{max} range = 45–120 minutes from intravenous initiation) and returned to baseline within 1 week of administration. Changes in both PK and PD measurements appeared proportional to dose.

Conclusions: The study demonstrated that intravenous administration of up to 30,000 U rHuPH20 was well tolerated, rapidly cleared from the plasma, and did not appear to be associated with any serious adverse effects at doses used in subcutaneous therapeutic products. (*Curr Ther Res Clin Exp*. 2020; 81)

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Introduction

Hyaluronidases have been used in clinic settings for the past 70 years to modify tissue permeability through the depolymerization of hyaluronan (HA), a large glycosaminoglycan and key component of the extracellular matrix (ECM).[1,2] The first hyaluronidases used in clinical practice were derived from crude extracts of ovine or bovine testicular tissue.[3,4] To reduce the potential risk of allergenicity and immunogenicity associated with animal-derived hyaluronidases, a highly purified recombinant human form of hyaluronidase PH20 (rHuPH20) was developed.[5] In 2005, rHuPH20 was approved in the United States as hyaluronidase human injection (HYLENEX Recombinant; Halozyme Therapeutics, Inc, San Diego, California) in subcutaneous fluid administration for achieving hydration, to increase the dispersion and absorption of other injected drugs, and for subcutaneous urography for improving resorption of radiopaque agents.[6]

rHuPH20 facilitates the delivery of volumes that are normally administered intravenously as subcutaneous route by transiently removing the barrier to bulk fluid flow, which limits conventional subcutaneous administrations to small volumes.[7,8] Clinical experience has demonstrated that rHuPH20 can also improve the pharmacokinetic (PK) profile of coadministered subcutaneous therapeutic agents by increasing absorption, bioavailability, and C_{max}, as well as accelerating t_{max} compared with subcutaneous administration without rHuPH20.[9] To date, rHuPH20 is approved in more than 100 countries, including the United States and several European countries, in subcutaneous coformulation with 3 anticancer agents, including trastuzumab, rituximab, and daratumumab, and also in a single, fixed-dose subcutaneous administration of 2 anticancer agents (pertuzumab and trastuzumab), and for sequential administration with immunoglobulin for the treatment of primary immunodeficiency (Table 1).[10–22]

rHuPH20 facilitates subcutaneous tissue permeability through depolymerization of HA in the ECM, and is not intended for intravenous administration.[5,23] However, it is possible that a subcutaneous formulation of a drug could be mistaken for the intravenous formulation of the drug (eg, mistaking HERCEPTIN HYLECTA for HERCEPTIN [Genentech, Inc, South San Francisco, California]), or that incorrect placement of a needle could result in inadvertent injection of rHuPH20 into a vein during subcutaneous administration. Hypersensitivity reactions have been attributed to animal hyaluronidases, and this safety concern would be expected to be mitigated with the use of recombinant human hyaluronidases. At the time of this publication, there had been no reports of severe hypersensitivity reactions attributable to rHuPH20. The safety and tolerability of rHuPH20 in the systemic circulation has not previously been assessed in a clinical study. The PK of animal-derived hyaluronidase has been explored,[24] and plasma concentrations of rHuPH20 following subcutaneous administration have been below the level of detection (0.6 ng/mL)[25]; therefore, no human PK data for rHuPH20 were available. Consequently, this Phase I study in healthy volunteers was conducted to assess the safety profile, tolerability, PK, and pharmacodynamics (PD) of rHuPH20 when administered intravenously.

Methods

Study design

HALO 104-104 was a Phase I, open-label, single-center study in healthy volunteers to assess the safety profile, tolerability, PK, and PD of rHuPH20 administered intravenously. A total of 24 healthy volunteers were enrolled for the study at the Healthcare Discoveries Center in San Antonio, Texas. This study was conducted in accordance with the Declaration of Helsinki and approved by an institutional review board (IntegReview IRB, Austin, Texas). All volunteers signed an approved informed consent form before study participation and were compensated for their participation.

Two doses of rHuPH20 were investigated: 10,000 U and 30,000 U. The dose levels were selected to be consistent with the range of rHuPH20 doses administered in the currently approved subcutaneously coformulated products.[14,17] Before dosing, intravenous catheters were placed in both arms (Abbocath; Abbott, Chicago, Illinois). Each volunteer received a 5 mL intravenous infusion of either 10,000 U (n = 12) or 30,000 U (n = 12) rHuPH20 over 5 minutes. The selection of infusion time and doses consistent with those used in currently approved products allows for an assessment of outcomes if healthy people were accidentally given rHuPH20 coformulated products as intravenous infusions in clinical practice.[10,11] Intravenous infusions driven by a syringe pump (Medfusion 2010i; Medex Inc, Duluth, Georgia) were given via an indwelling catheter on the contralateral side of the peripheral intravenous line used for PK/PD blood draws.

The study was conducted in 2 stages (Figure 1). In Stage 1, a total of 6 volunteers received a 5 mL intravenous infusion over 5 minutes containing either 10,000 U (n = 3) or 30,000 U (n = 3) rHuPH20 diluted in normal saline. An interim analysis of the safety profile and PK data from the first 3 volunteers

Table 1
Agents approved in combination with recombinant human hyaluronidase PH20 (rHuPH20).

Agent	Product	Indication	Country			Marketing authorization holder
			United States	Europe	Canada	
Pertuzumab and trastuzumab	PHESGO[10]	HER2-positive breast cancer	2020	_	_	Genentech, Inc, South San Francisco, California
Daratumumab	DARZALEX FASPRO, DARZALEX SC[11,12]	Multiple myeloma	2020	2020	_	Janssen Biotech Horsham, Pennsylvania
Trastuzumab*	HERCEPTIN HYLECTA, HERCEPTIN SC[13–15]	HER2-positive breast cancer	2019	2013	2018	Genentech, Inc, South San Francisco, California Roche Indianapolis, Indiana
Rituximab*	RITUXAN HYCELA, RITUXAN SC, MABTHERA SC[17–19]	Chronic lymphocytic leukemia (All, Follicular lymphoma and diffuse large B-cell lymphoma (US only), Non-Hodgkin's lymphoma (Europe and Canada)	2017	2014	2018	Comarketed by Biogen and Genentech, South San Francisco, California Roche Indianapolis, Indiana
Immunoglobulin	HYQVIA, HyQvia[21,22]	Primary immunodeficiency	2014	2013	_	Takeda Deerfield, Illinois

HER2 = human epidermal growth factor receptor 2; SC = subcutaneous.
* The SC formulations of HERCEPTIN and RITUXAN/MabThera with rHuPH20 are approved in >100 countries worldwide.[16,20]

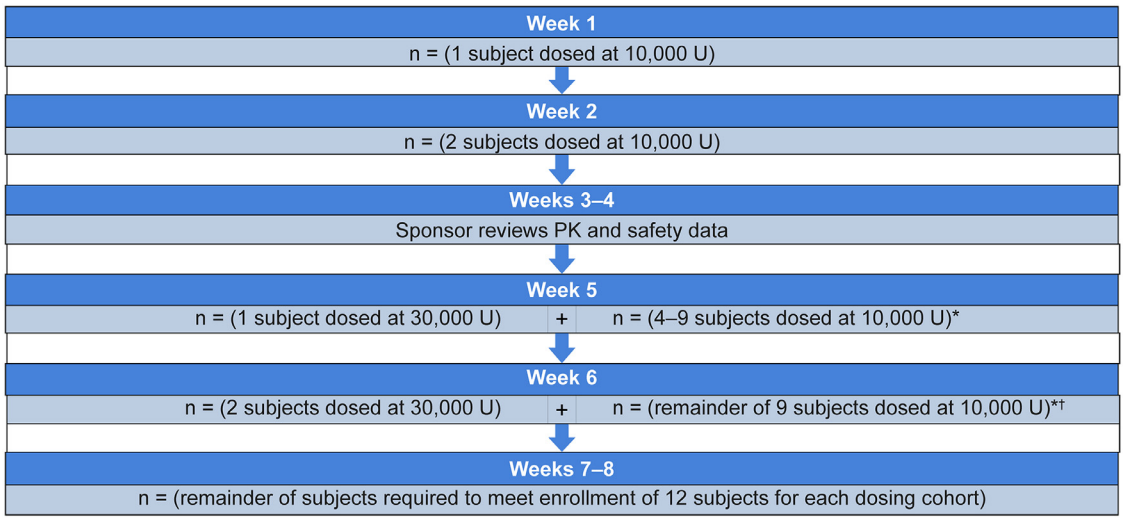

Figure 1. Enrollment schema.
PK = pharmacokinetics.
* Number of subjects dosed was dependent on clinic space and subject availability.
† If all 12 subjects from the 10,000 U treatment group had been dosed by Week 6, the remaining subjects from the 30,000 U treatment group were dosed in Week 6.

who received 10,000 U was conducted before proceeding with administration of the 30,000 U dose to a second group of volunteers. Within the second group, the first volunteer received the 30,000 U dose, and at least 1 week later 2 additional volunteers received 30,000 U rHuPH20. In Stage 2, conducted once the safety and tolerability profiles of the 10,000 U and 30,000 rHuPH20 doses were established, 9 additional volunteers were enrolled for each dose group and received rHuPH20.

Key inclusion and exclusion criteria

Eligible volunteers were healthy men or women aged 18 to 65 years, who had adequate venous access in both arms and normal vital signs, echocardiograph (ECG), and metabolic parameters. Volunteers were excluded if they had a known allergy to hyaluronidase or any other ingredient of the study formulation, or a prior exposure to hyaluronidase; contraindication to intravenous heparin lock or hypersensitivity to heparin; creatinine clearance <60 mL/min; known clinically significant illness; or major systemic disease. Female volunteers could not be pregnant or breastfeeding. Volunteers who reported the use of tobacco, nicotine, or alcohol, or who reported drug abuse, were not eligible for the study.

Outcome measures

The primary end points were safety profile and tolerability of rHuPH20 administered intravenously. The secondary end point was the PK profile of rHuPH20 administered intravenously, and the exploratory end point was the PD profile of plasma HA.

Safety profile assessment

The safety population was defined as all volunteers who received a dose of rHuPH20. The safety profile was assessed primarily through investigator-assessed adverse events (AEs) at the clinic. The incidence of treatment-emergent AEs was summarized by treatment group according to the Medical Dictionary for Regulatory Activities (version 12) preferred term and system organ class. Other safety assessments included ECGs, hematology, clinical chemistry, urinalysis, physical examination findings, vital signs, medical history, concomitant medications, and pregnancy test results.

PK and PD assessments

Blood samples for plasma analysis to assess PK/PD were collected 30 and 5 minutes before starting the intravenous infusion, and at 1, 3, 4.5, 6, 8, 10, 12, 15, 17, 20, 25, 30, and 45 minutes, and 1, 2, 4, and 24 hours after initiation of infusion. In addition, samples were collected on Day 2, Day 7, and at the follow-up visit when applicable. Whole blood samples for PK/PD assessments were collected in 4 mL tubes with tri-potassium (K_3) EDTA as the anticoagulant. Filled collection tubes were centrifuged, and plasma was aliquoted into vials and frozen at −70°C until shipped to the referral labs for analysis.

The plasma concentrations of rHuPH20 were determined by 2 orthogonal methods: one based on measurement of functional enzyme activity and a second that measured the concentration of enzyme using a sandwich immunoassay. Plasma concentration versus time data were analyzed by noncompartmental PK analyses with Phoenix WinNonlin version 6.3 (WinNonlin; Pharsight Corp, Mountain View, California) using an intravenous infusion model. Nominal dose(s), actual infusion times, and actual sampling times were used in the analysis. Descriptive PK parameters for rHuPH20 were summarized, including AUC_{0-last}, $AUC_{0-\infty}$, C_{max}, t_{max}, and $t_{1/2}$. The PD effect of rHuPH20 was evaluated by characterizing the change in plasma HA concentration from predose measurements.

Enzymatic hyaluronidase assay

Plasma hyaluronidase activity was determined using a modified microtiter plate-based assay for rHuPH20 using a biotinylated-hyaluronic acid (bHA) substrate.[26] Briefly, this hyaluronidase assay was used to quantify the enzymatically active rHuPH20 in K_3-EDTA plasma samples by measuring the degree of digestion of a large molecular weight (~1.2 megadalton) bHA substrate, noncovalently bound to plastic multiwell microtiter plates. Plates were coated with 100 µg/mL bHA (Halozyme Therapeutics, Inc, San Diego, California) in 0.5 M carbonate-coating buffer (Mallinckrodt Pharmaceuticals, Dublin, Ireland) at pH 9.6, and incubated overnight at 4°C. After a series of washes in phosphate-buffered saline with 0.05% polysorbate 20, nonspecific binding sites were blocked for 1 hour with 1.0% bovine serum albumin, Fraction V (all Sigma-Aldrich, St Lous, Missouri) in phosphate-buffered saline. Diluted plasma samples, rHuPH20 calibrators, and quality control samples were incubated at 37°C in the coated and blocked plates

Table 2
Demographic and baseline characteristics.

Characteristic	10,000 U rHuPH20 (n = 12)	30,000 U rHuPH20 (n = 12)	Total (N = 24)
Age, y			
Mean (SD)	36.3 (10.3)	36.6 (8.9)	36.5 (9.4)
Median (min, max)	34 (24, 60)	37 (26, 54)	36 (24, 60)
Sex*			
Male	3 (25.0)	11 (91.7)	14 (58.3)
Female	9 (75.0)	1 (8.3)	10 (41.7)
Ethnicity*			
Hispanic or Latino	6 (50.0)	6 (50.0)	12 (50.0)
Not Hispanic or Latino	6 (50.0)	6 (50.0)	12 (50.0)
Race*			
Caucasian	9 (75.0)	9 (75.0)	18 (75.0)
African American	2 (16.7)	3 (25.0)	5 (20.8)
Asian	1 (8.3)	0 (0)	1 (4.2)

rHuPH20 = recombinant human hyaluronidase PH20.
* Values are presented as n (%).

for 1 hour. After a series of washes, the remaining intact, bound bHA was visualized using a streptavidin horseradish peroxidase conjugate (Thermo Fisher Scientific, Waltham, Massachusetts), followed by incubation with 3,3',5,5'-tetramethylbenzidine (Sigma, St. Louis, Missouri), and the reaction was stopped with 1 N sulfuric acid. Quantitation of the soluble yellow reaction product was determined by reading the absorbance at 450 nm using a microtiter plate spectrophotometer (SpectraMax, Molecular Devices, San Jose, California). Interpolated hyaluronidase activity values were corrected for sample dilution factor and reported in units per milliliter.

Sandwich immunoassay for rHuPH20 in plasma

Concentrations of immunoreactive rHuPH20 in K_3-EDTA plasma were determined using a format-validated sandwich immunoassay based on electrochemiluminescent (ECL) technology and the MesoScale Discovery platform (Rockville, Maryland).[25] High binding capacity 96-well plates (MesoScale Discovery) were coated with a mixture of 3 unique mouse anti-rHuPH20 monoclonal antibodies (Halozyme Therapeutics) at 1.5 µg/mL total antibody concentration. The rHuPH20 in diluted plasma samples, rHuPH20 calibrators, and quality control samples were captured on the plates by a 2-hour incubation with orbital shaking. After a series of washes with 0.05% polysorbate 20, a biotinylated rabbit anti-rHuPH20 polyclonal antibody (Halozyme Therapeutics) was used for detection. Plates were then washed with 0.05% polysorbate 20, and a Sulfo-TAG-conjugated streptavidin reagent (MesoScale Discovery) was applied for 1 hour. After a final wash step, 1X Read Buffer T with surfactant (MesoScale Discovery) was applied and the ECL signal (proportional to bound immunoreactive rHuPH20) was acquired using a SECTOR 2400 instrument (MesoScale Discovery). Interpolated concentrations of rHuPH20 were corrected for sample dilution factor and reported as total immunoreactive rHuPH20 in nanograms per milliliter.

Detection of rHuPH20-reactive antibodies

rHuPH20-reactive antibodies are prevalent in approximately 5% of the healthy US adult population.[27] The presence of rHuPH20-reactive antibodies was determined using a format-validated bridging ECL immunoassay.[28] Briefly, plasma samples were incubated overnight with biotin-conjugated rHuPH20 and Sulfo-TAG-conjugated rHuPH20. The resulting immune complex was captured onto streptavidin-coated plates and detected in a SECTOR 2400 instrument (MesoScale Discovery) using ECL read buffer.

LC-MS HA disaccharide assay

Plasma HA concentration was measured using HPLC–MS/MS total disaccharide assay. This assay has been previously validated and provides accurate HA quantification independent of chain length.[29] In short, the K_3-EDTA plasma was digested with chondroitinase ABC (Sigma) to liberate HA disaccharides. The HA disaccharides were recovered by precipitation with ethanol and subsequently derivatized with 4-nitrobenzylhydroxylamine. Excess derivatized reagent was removed by a solvent wash step and then the extracts were separated by HPLC using a Synergi MAX-RP column (Phenomenex, Torrance, California). The mobile phase was nebulized, and ionized compounds were detected via MS/MS. A deuterium-labeled 4-nitrobenzylhydroxylamine derivative of HA disaccharide was incorporated as an internal standard.

Statistical methods

The sample size of 12 per rHuPH20 dose would result in a 70% chance of detecting an AE with a true underlying rate of 10%, and a 90% chance of detecting an AE with a true underlying rate of 20%. The total sample size of 24 results in an >80% chance of detecting AEs with a true underlying rate of 7%.

Results

Demographics and baseline characteristics

The study enrolled 24 healthy adult volunteers with a mean age of 36.5 years (range = 24–60 years) between September and December 2013. Volunteers were divided equally between the 2 dose groups: half (n = 12) received a single 5 mL intravenous infusion containing 10,000 U rHuPH20, and the other half (n = 12) received a single 5 mL intravenous infusion containing 30,000 U rHuPH20. There were more women (75.0%) in the 10,000 U rHuPH20 group, and more men (91.7%) in the 30,000 U rHuPH20 group. A summary of the baseline characteristics is shown in Table 2.

Safety assessments

All 24 volunteers completed the study and were included in the safety analysis. No serious AEs were reported in either of the treatment groups and there were no deaths or discontinuations due to AEs (Table 3). Overall, 2 AEs were reported for 1 subject each: catheter site pain (in the 10,000 U group) and hypotension (in the 30,000 U group). Both AEs were transient and were assessed by the investigator as Grade 1 and considered unlikely to be related to the study treatment.

The volunteer who presented with a nonserious AE of Grade 1 hypotension was a 42-year-old woman of Hispanic/Latino ethnicity with a body mass index of 22.9. This volunteer had no no-

Table 3
Summary of adverse events (AEs).

Event*	10,000 U rHuPH20 (n = 12)	30,000 U rHuPH20 (n = 12)	Total (N = 24)
All AEs	1 (8.3)	1 (8.3)	2 (8.3)
Hypotension†	0	1 (8.3)	1 (4.2)
Catheter site pain†	1 (8.3)	0	1 (4.2)
SAEs	0	0	0
AEs leading to discontinuation	0	0	0
Deaths	0	0	0

AE = adverse event; rHuPH20 = recombinant human hyaluronidase PH20; SAE = serious adverse event.
* Values are presented as n (%).
† Grade 1, unlikely related to the study treatment as determined by the investigator.

Table 4
Pharmacokinetic (PK) parameters for plasma recombinant human hyaluronidase PH20 (rHuPH20) following a single 5-minute intravenous infusion of rHuPH20 10,000 U or 30,000 U in healthy volunteers.

PK parameter	Enzymatic rHuPH20		Immunoreactive rHuPH20	
	10,000 U (n = 11)*	30,000 U (n = 12)	10,000 U (n = 11)*	30,000 U (n = 12)
AUC_{0-last}†				
hour x U/mL	0.32 (20.8)	1.03 (18.7)		
hour x ng/mL			1.62 (23.6)	5.04 (20.3)
$AUC_{0-\infty}$†				
hour x U/mL	0.36 (16.5)	1.08 (17.6)		
hour x ng/mL			1.64 (23.7)	5.06 (20.3)
C_{max}†				
U/mL	2.50 (19.8)	6.80 (17.6)		
ng/mL			11.7 (21.5)	37.3 (24.7)
t_{max},‡§ minutes	6.00 (5.57, 7.38)	6.00 (5.25, 7.98)	6.00 (4.50, 7.38)	6.00 (6.00, 7.98)
$t_{1/2}$,‡† minutes	3.70 (22.8)	5.64 (16.9)	6.60 (40.9)	10.4 (37.7)

AUC_{0-last} = area under the plasma concentration curve from time 0 to the time of last measurable concentration; $AUC_{0-\infty}$ = area under the plasma concentration curve from time 0 to infinity; C_{max} = maximum plasma concentration; $t_{1/2}$ = plasma elimination half-life; t_{max} = time to achieve maximum plasma concentration.
* One PK profile was excluded from the analysis.
† Values are presented as arithmetic mean (percent coefficient of variation).
‡ Summary units were converted from hours to minutes for this summary table.
§ Values are presented as median (min, max).

table medical history and was not taking any medications at baseline. The volunteer's blood pressure at screening was 106/65 mm Hg and 95/58 mm Hg predose on Day 1. Per study, she was administered 30,000 U rHuPH20 over 5 minutes. When measured 4 hours after study drug administration, her blood pressure was 76/48 mm Hg. When measured 59 minutes after the hypotensive event, her blood pressure was 92/61 mm Hg. No action was taken for the event. The final blood pressure recorded on Study Day 1 was 99/67 mm Hg. On Study Days 2 and 7, the volunteer's blood pressure was 93/60 mm Hg and 90/69 mm Hg, respectively. While on study, all ECGs were normal, and no laboratory tests were deemed by the investigator to be clinically significant.

Laboratory results and vital signs did not reveal clinically significant abnormalities with either dose of rHuPH20, suggesting that intravensous rHuPH20 was well tolerated. Grade 1 or Grade 2 changes in laboratory assessments (hematology, clinical chemistry, or urinalysis) occurred occasionally, but none were considered clinically significant. Similarly, no clinically significant changes in vital signs were observed. There were no clinically significant ECG changes identified during the study.

Presence of rHuPH20-reactive antibodies at baseline

The presence of rHuPH20-reactive antibodies at baseline was detected in 2 volunteers, both men, 1 in each of the treatment groups. In 1 volunteer (30,000 U treatment group), the presence of rHuPH20-reactive antibodies had no apparent effect on the observed time–concentration profile of rHuPH20, and the data from this volunteer were included in the PK analysis. However, the time-concentration profile of the second volunteer was dissimilar to that for the mean of the 10,000 U treatment group, and the data from this volunteer were excluded from the PK analysis. Consistent with prior reports, no unusual safety observations were associated with the presence of rHuPH20-reactive antibodies.[28]

PK of rHuPH20 after a single intravenous dose

Following a single 5-minute intravenous infusion of rHuPH20, there was a rapid increase in plasma rHuPH20 concentration, peaking on average at 6 minutes postinitiation of infusion (Table 4) and quickly declining to below the quantification limit (BQL) (0.3 U/mL and 0.03 ng/mL) within 2 hours (Figure 2). In the enzymatic rHuPH20 assay, mean rHuPH20 concentration declined to BQL after 30 minutes (Figure 2A). However, in the more sensitive ECL immunoreactive rHuPH20 assay, mean rHuPH20 concentration declined to BQL after 45 minutes and 90 minutes at 10,000 U and 30,000 U dose levels, respectively (Figure 2B). Although the median t_{max} was similar in both PK assays for rHuPH20 concentration, the apparent mean $t_{1/2}$ values were statistically significantly shorter for enzymatic data (3.7 and 5.6 minutes) versus the immunoreactive mass-based data (6.6 and 10.4 minutes) following intravenous infusion of 10,000 U and 30,000 U rHuPH20, respectively ($P < 0.05$) (Table 4). Data from each bioanalytical method showed that plasma rHuPH20 concentrations increased proportionally with the dose, suggesting linear PKs (Figure 2 and Table 4).

The shape of the terminal phase after t_{max} was different for the enzymatic and immunoreactive rHuPH20 data, with the enzymatic data displaying a more rapid (approximately monophasic) decline compared with the immunoreactive rHuPH20 (biphasic) data set (Figure 2 and Table 4).

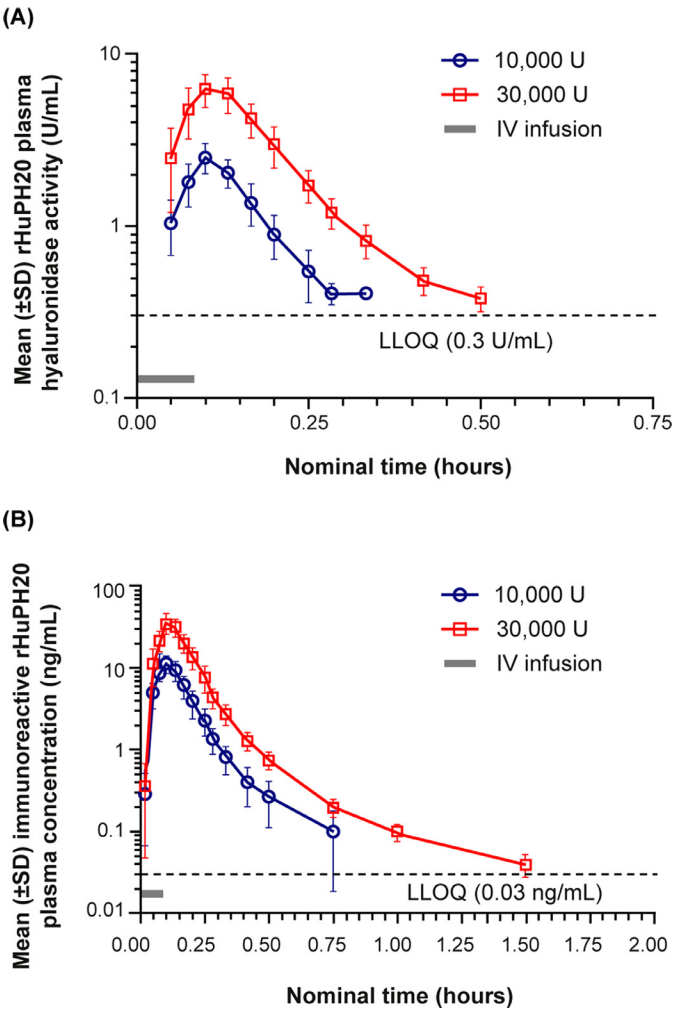

Figure 2. Plasma recombinant human hyaluronidase PH20 (rHuPH20) concentration following a single 5-minute IV infusion of rHuPH20 in healthy volunteers. Plasma rHuPH20 concentration in healthy volunteers following a single 5 mL IV infusion of 10,000 U (n = 11) or 30,000 U (n = 12) rHuPH20, quantified as (A) plasma rHuPH20 hyaluronidase activity (enzymatic rHuPH20), and (B) immunoreactive rHuPH20 plasma concentration. IV, intravenous; LLOQ = lower limit of quantification.

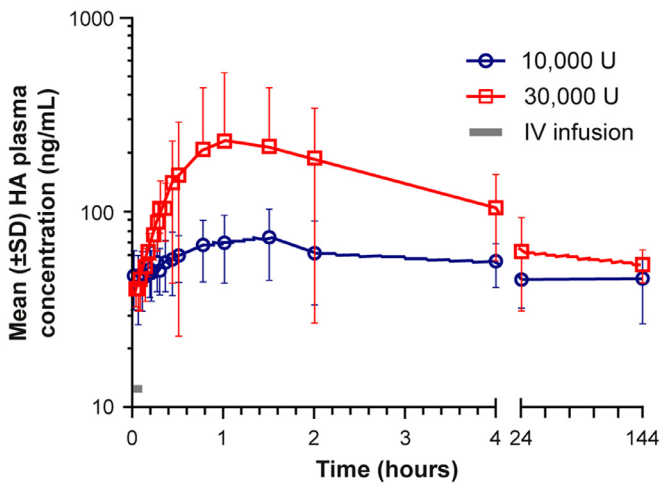

Figure 3. Plasma hyaluronan (HA) concentrations following a single dose of intravenous recombinant human hyaluronidase PH20 (rHuPH20) in healthy volunteers. Plasma HA concentration in healthy volunteers following a single 5 mL IV infusion of 10,000 U (n = 12) or 30,000 U (n = 12) rHuPH20 over time.

Table 5
Parameters describing the systemic concentration of plasma hyaluronan (HA) following a single 5-minute intravenous infusion of recombinant human hyaluronidase PH20 (rHuPH20) 10,000 U or 30,000 U in healthy volunteers.

Parameter	10,000 U rHuPH20 (n = 12)	30,000 U rHuPH20 (n = 12)
AUC_{0-last},* hour x ng/mL	3100 (120.0)	8570 (42.7)
C_{max},* ng/mL	79 (36.4)	244 (116.0)
t_{max},†,‡ minutes	90 (45, 120)	60 (45, 120)

AUC_{0-last} = area under the plasma concentration curve from time 0 to the time of last measurable concentration; C_{max} = maximum plasma concentration; t_{max} = time to achieve maximum plasma concentration.
* Values are presented as arithmetic mean (percent coefficient of variation).
† Value is presented as median (min, max).
‡ Summary units were converted from hours to minutes for this summary table.

Plasma HA as a PD marker for rHuPH20

Baseline plasma HA concentrations were detected in some of the volunteers before the infusion of rHuPH20 (n = 5, 10,000 U and n = 8, 30,000 U). In these volunteers, baseline plasma HA concentrations ranged from 31.4 to 83.2 ng/mL across the 2 treatment groups. The mean plasma HA concentrations increased following a single intravenous infusion of rHuPH20 at 10,000 U or 30,000 U (Figure 3), and the median plasma HA t_{max} was reached 60 to 90 minutes after dosing (Table 5), which was delayed relative to the peak of plasma rHuPH20 (t_{max} = 6 min) (Table 4). Plasma HA concentrations returned to baseline within 1 week of dosing in both groups. The range of t_{max} values (45–120 minutes) was similar between the 2 dose groups, but the variability in plasma HA concentrations between volunteers was greater with the higher dose (116.0%) than the lower dose (36.4%) (Table 5).

Discussion

The HALO 104-104 study assessed the safety profile, tolerability, PK, and PD of rHuPH20 administered intravenously in healthy adult volunteers. The study demonstrated that intravenous rHuPH20 was well tolerated in healthy adults. No serious AEs, deaths, or discontinuations due to AEs were reported, and no clinically significant abnormal laboratory findings were observed. The prevalence of rHuPH20-reactive antibodies at baseline (8%) in the studied volunteers was within the range of a larger study of normal volunteers (5%)[27] and of previously studied disease-state populations (3%–12%).[28]

Plasma concentrations of rHuPH20 were analyzed using an enzymatic assay and an immunoreactive assay to provide orthogonal methods for characterization of rHuPH20 PK. Both assays confirmed a rapid clearance of rHuPH20 from the plasma after intravenous infusion (mean $t_{1/2}$ range = 3.7–10.4 minutes) and a return to undetectable concentrations within 1 to 2 hours after dosing. Although the time-concentration profiles were similar for the 2 assays, the half-life of rHuPH20 was significantly shorter for the enzymatic data versus the immunoreactive mass-based data. The difference in observed half-life and shape of the terminal phase are presumed a function of the varying assay sensitivities, which differ by more than 50-fold. The dose-proportional increase in plasma concentrations using both assays suggests linear PK between 10,000 U and 30,000 U doses used in current commercial products. Regarding the 1 volunteer noted to have an unusual time-concentration profile for rHuPH20, it is unclear whether the apparent difference in the PK of rHuPH20 for this volunteer was due to direct interference/enhancement by the rHuPH20-reactive antibodies (titer of 640) in the mass immunoassay, or due to differences in actual PK of rHuPH20 in this volunteer. It should be noted that the time-concentration profiles obtained with the

enzymatic assay were similar to the profile obtained with the mass immunoassay for this volunteer.

The rapid plasma clearance of intravenous rHuPH20 provides additional explanation for the undetectable systemic concentrations measured after subcutaneous administration. In clinical studies of subcutaneous trastuzumab, subcutaneous trastuzumab/pertuzumab or subcutaneous tocilizumab co-formulated with rHuPH20, systemic concentrations of rHuPH20 at clinically relevant doses were not detected using the most sensitive analytical methods available.[9,25,30] PK modeling and simulation studies of rHuPH20 after subcutaneous administration provide further evidence that rHuPH20 systemic exposure is limited when administered subcutaneous at therapeutic doses.[31]

Results from this study also provide evidence for PK similarities between rHuPH20 and the animal-derived hyaluronidases that were studied in clinical trials decades ago. The rapid plasma clearance of rHuPH20 aligns with previous reports of dose-dependent PK of subcutaneous rHuPH20 in animal model studies, and the PK of intravenous bovine testicular hyaluronidase (BTH) previously reported in both preclinical and clinical studies.[23,24] Studies with intravenous BTH demonstrated a rapid clearance of BTH from the serum with a half-life $<$10 minutes in dogs, rats, and humans. In human patients, serum BTH fell below detection level within 20 minutes following intravenous administration of 500 U.[24]

Clinical studies in the 1970s–1990s suggested that intravenous BTH may aid in accelerating the recovery of cells in the necrotic area in patients with myocardial infarction or peripheral arterial occlusive disease.[32,33] Prior studies also postulated that coadministration of intravenous BTH may improve the anticancer activity of cytotoxic agents in a wide range of cancers.[3,34] Despite promising preliminary results in cancer patients, no survival benefit was observed with intravenous hyaluronidase in combination with cytostatic therapy in patients with high-grade astrocytoma.[3] The limited effect of intravenous BTH in these studies may be attributed to the rapid plasma clearance and associated with decreased in vivo exposure to hyaluronidase.

This study identified an initial increase in plasma HA concentration, peaking 45 to 120 minutes after intravenous rHuPH20 administration and declining to baseline within 1 week. Because HA is present in the ECM and pericellular matrix of multiple tissues,[35] increased release of HA into the circulation would be expected after systemic exposure to exogenous hyaluronidase. Dose-dependent increase of HA is consistent with enzymatic depolymerization of the hyaluronidase substrate, and these data support the use of plasma HA as a PD marker for rHuPH20. Plasma HA levels resolved toward baseline within 1 week after administration of rHuPH20, consistent with the transient effects previously reported for this enzyme.[36]

Limitations of the current study include the fact that, as the study population was restricted to healthy adult volunteers without any prior exposure to hyaluronidase, the conclusions may be limited to such a population. However, based on the local and transient action of rHuPH20 it is not expected that those with prior exposure to rHuPH20 would have any permanent changes that could affect response to subsequent exposure.

Conclusions

This study demonstrated that intravenously administered rHuPH20 was well tolerated and did not appear to be associated with any serious AEs when given at doses used to enable subcutaneous delivery of coadministered therapeutic agents in healthy adult volunteers without prior exposure to hyaluronidases. Therefore, accidental intravenous exposure to rHuPH20 is unlikely to cause clinically significant AEs at the doses used in approved subcutaneous coformulated products. PK characterization of intravenously administered rHuPH20 demonstrated that rHuPH20 was rapidly cleared from the plasma with a half-life of $\sim$10 minutes, and supports the use of plasma HA as a PD biomarker. The results of this study confirm previous clinical observations and modeling studies, which demonstrated that rHuPH20 systemic concentrations were below the level of detection when administered subcutaneously at therapeutic doses.

Conflicts of Interest Statement

The study was sponsored by Halozyme Therapeutics, Inc, and the data are held by the company. Additional information about the study and/or datasets can be obtained by contacting Halozyme Therapeutics, 11388 Sorrento Valley Rd, San Diego, CA 92121. E-mail: publications@halozyme.com.

The study sponsor was involved in the study design, data collection, data analysis, and preparation of the manuscript.

Marie A. Printz and David W. Kang are employees of Halozyme Therapeutics, Inc. Samuel S. Dychter, Barry J. Sugarman, Monica Zepeda, Jennifer Souratha, Rena Harrigan, and Daniel C. Maneval were employees of Halozyme Therapeutics Inc. at the time of the study. The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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B.J. Sugarman was responsible for conceptualization, formal analysis, writing the original draft, review, and editing. D.C. Maneval was responsible for conceptualization, methodology, formal analysis, writing the original draft, review, and editing. D.W. Kang was responsible for writing the original draft and formal analysis. E.P. DeNoia was responsible for resources, investigation, supervision, manuscript review, and editing. J. Souratha was responsible for methodology, investigation, formal analysis, manuscript review, and editing. M.A. Prinz was responsible for writing the original draft, investigation, formal analysis, and supervision. M. Zapeda was responsible for conceptualization, methodology, supervision, writing the original draft, manuscript review, and editing. R. Harrigan was responsible for manuscript review and editing, investigation, and supervision. S.S. Dychter was responsible for methodology, supervision, formal analysis, manuscript review, and editing. All authors approved the final version of the manuscript and are accountable for all aspects of the work.

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