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Pharmacokinetics of Serelaxin in Patients With Severe Renal Impairment or End-Stage Renal Disease Requiring Hemodialysis: A Single-Dose, Open-Label, Parallel-Group Study

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Abstract

Serelaxin, a recombinant human relaxin-2 hormone, is in clinical development for treating acute heart failure. This open-label, parallel-group study investigated serelaxin pharmacokinetics (PK) after a single 4-hour intravenous infusion (10 μg/kg) in patients with severe renal impairment (n = 6) or end-stage renal disease (ESRD) requiring hemodialysis (PK on the day of dialysis [n = 6] or during dialysis-free interval [n = 6]), compared with matched healthy subjects (n = 18). In all participants, serum serelaxin concentration peaked at the end of infusion and subsequently declined with mean terminal elimination half-life of 6.5–8.8 hours. Compared with healthy subjects, a moderate decrease in serelaxin systemic clearance (37%–52%) and increase in its exposure (30%–115%) were observed in all patients. During the 4-hour hemodialysis in ESRD patients, 30% serelaxin was removed, with hemodialysis clearance constituting approximately 52% of total systemic clearance. Serelaxin was well tolerated with no deaths, serious adverse events (AE), or AE-related discontinuations. Antiserelaxin antibodies were not detected in any participant. Given the shallow dose-response relationship observed with serelaxin in clinical studies and its wide therapeutic window, the observed PK differences in patients with severe renal impairment compared with healthy subjects are unlikely to pose a safety risk and do not warrant a predefined dosage adjustment in such patients.

Serelaxin, currently in clinical development for the treatment of acute heart failure (AHF), is a recombinant peptide identical in structure to human relaxin-2, a naturally occurring hormone that is believed to mediate hemodynamic and vascular changes during pregnancy.[1] Serelaxin stimulates the vascular signaling pathways with short- and long-term effects on hemodynamics.[1,2] Data are available on the efficacy and safety of serelaxin in patients with AHF from 2 randomized, double-blind, placebo-controlled, parallel-group, multinational clinical trials—the phase 2 Pre-RELAX-AHF and the pivotal phase 3 RELAX-AHF.[3,4] In these studies, a 48-hour intravenous (IV) infusion of serelaxin, 30 μg/kg/day significantly improved dyspnea and was well tolerated with favorable effects on long-term clinical outcomes.[3,4] In addition, the RELAX-AHF trial demonstrated that serelaxin, along with standard therapy, significantly reduced the incidences of in-hospital worsening heart failure (WHF) through day 5 and all-cause and cardiovascular (CV) mortality through day 180; however, no significant effects were observed for days alive and out of hospital and CV death or readmission for heart failure (HF) or renal failure up to day 60.[3] An ongoing phase 3 trial, RELAX-AHF-2, will confirm the effects of serelaxin on reducing CV death over 180 days after treatment and in-hospital WHF through day 5 (primary endpoints), and to assess several important

Abbreviations: AE, adverse events; AHF, acute heart failure; AUC, area under the serum concentration-time curve; BMI, body mass index; C$_{max}$, maximum serum concentration; CI, confidence interval; CV, cardiovascular; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; ESRD, end-stage renal disease; GMR, geometric mean ratios; HF, heart failure; IV, intravenous; PK, pharmacokinetics; PR, pulse rate; RELAX-AHF, RELAXin in Acute Heart Failure; SAE, serious adverse event; SBP, systolic blood pressure; SD, standard deviation.

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clinical outcomes (secondary endpoints) in a large patient population of approximately 6800 patients with AHF (ClinicalTrials.gov identifier NCT01870778).

Patients with AHF are often diagnosed with renal insufficiency or experience worsening of renal function during hospitalization. A meta-analysis of HF studies showed that 63% of patients had some degree of renal dysfunction, with 29% having moderate to severe renal dysfunction.[5] An analysis of the Acute Decompensated Heart Failure National Registry (ADHERE) data base of 118,465 patients hospitalized with AHF showed that 91% of patients had some degree of renal dysfunction, of whom 64% had at least a moderate renal dysfunction.[6] In the Pre-RELAX-AHF and RELAX-AHF trials, the analyses of serelaxin systemic clearance in patients with renal dysfunction indicated no clinically relevant impact of impaired renal function on the pharmacokinetics (PK) of serelaxin. However, these were mostly patients with mild to moderate renal dysfunction and a relatively small number of patients with severe renal impairment (n = 12). In addition, the available serelaxin PK data in these 12 patients were limited to the steady-state serum concentrations at 12 hours during infusion (data on file). Therefore, it was deemed important to systematically assess the effects of severe renal dysfunction and renal failure on the PK of serelaxin. This aspect has been addressed in the present study (ClinicalTrials.gov identifier NCT01875523), which was conducted in a selected population of patients with severe renal impairment and those with end-stage renal disease (ESRD) requiring hemodialysis. The primary objective of this study was to evaluate the PK of serelaxin after a single 4-hour IV infusion of 10 μg/kg serelaxin in patients with severe renal impairment or ESRD requiring hemodialysis in comparison with healthy subjects. The secondary objectives were

to assess the safety, tolerability, and immunogenicity of serelaxin in all the participants.

Methods

Study Design and Participants

The study protocol was reviewed and approved by the Research Ethics Committee at Ethikkommission Schleswig-Holstein, Germany. The study was designed, conducted, and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, the applicable local regulations, and the Declaration of Helsinki. All participants provided written informed consent before any study procedures were conducted.

This was a phase 1, open-label, parallel-group study conducted in patients with severe renal impairment and those with ESRD requiring hemodialysis. The control group comprised demographically matched healthy subjects with normal renal function. As shown in Figure 1, the study participants were grouped according to their renal function status as follows: patients with severe renal impairment (group 1), patients with ESRD requiring hemodialysis with PK assessments on the day of dialysis (group 2), and patients with ESRD requiring hemodialysis with PK assessments during the dialysis-free interval (group 3). Healthy subjects (group 4) were matched to individual patients in groups 1 to 3.

Male or female participants aged between 18 and 75 years, with body weight ≥50 kg, body mass index (BMI) of 18 to 35 kg/m^2, and an ability to communicate well with the investigator were included in the study. In addition, for patients in group 1, inclusion in the study required evidence of severe renal impairment as indicated by clinically significant abnormal creatinine and
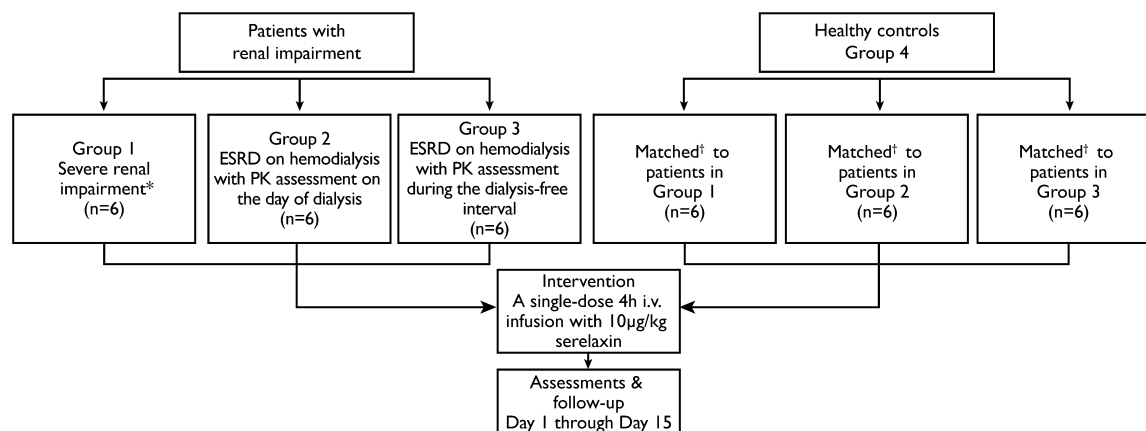
*15 mL/min/1.73 m^2 ≤ eGFR < 30 mL/min/1.73 m^2.
†Matched to patients with renal impairment or ESRD on the basis of the demographic characteristics (race, sex, age, and BMI).
BMI, body mass index; eGFR, estimated glomerular filtrate rate; ESRD, end-stage renal disease; i.v., intravenous; PK, pharmacokinetics.

Figure 1. Study design—a phase 1, open-label, parallel-group study.

creatinine clearance levels calculated according to the simplified Modification of Diet in Renal Disease formula ($15 \text{ mL}/[\text{min} \cdot 1.73 \text{ m}^2] \leq$ estimated glomerular filtration rate [eGFR] $<30 \text{ mL}/[\text{min} \cdot 1.73 \text{ m}^2]$). Patients in groups 2 and 3 were included if they had ESRD requiring hemodialysis. The vital signs for patients in groups 1 to 3, at screening and baseline visits, were required to have the following: systolic blood pressure (SBP) ranging from 110 to 170 mm Hg, diastolic blood pressure (DBP) ranging from 60 to 105 mm Hg, and pulse rate (PR) between 45 and 100 bpm. The corresponding inclusion criteria for healthy subjects were eGFR $\geq 90 \text{ mL}/(\text{min} \cdot 1.73 \text{ m}^2)$; matching in terms of race, age (± 10 years), sex, and BMI ($\pm 15\%$) to an individual patient with renal impairment in groups 1, 2, or 3; SBP ranging from 100 to 150 mm Hg; DBP ranging from 60 to 95 mm Hg; and PR between 50 and 100 bpm.

The key exclusion criteria were as follows: (1) clinically significant electrocardiogram abnormalities; (2) history of hypersensitivity to the study drug or to drugs of a similar class; (3) use of other investigational drugs at the time of study enrollment; (4) presence of any noncontrolled and clinically significant disease, surgical, or medical condition that could have affected the study outcome or that would have placed the patient at an undue risk, as judged by the investigator; and (5) baseline and other laboratory parameters at screening outside acceptable limits as judged by the investigator. In addition, patients with severe renal impairment/ESRD were excluded from the study if they had hemoglobin levels $<9.0 \text{ g/dL}$ at screening and received treatment with any cytostatic drug or nitrate. Healthy subjects were excluded if they had used any prescription drugs within 4 weeks prior to the initial dosing and/or over-the-counter medications within 2 weeks prior to the initial dosing, or had tested positive for hepatitis B or C.

Patients in groups 1 to 3 were enrolled in parallel into the study. Healthy subjects were matched pairwise and enrolled after the corresponding patient with renal impairment/ESRD had completed serelaxin treatment and PK blood sample collection.

Study participants received a single open-label, 4-hour IV infusion of $10 \mu g/kg$ serelaxin on day 1. This dosing regimen (instead of $30 \mu g/kg/day$ for 48 hours that was implemented in the serelaxin clinical development studies, including the ongoing phase 3 RELAX-AHF2 study) was chosen due to the feasibility challenges with regard to the timing of PK assessments and the intermittent hemodialysis (every other day) and for safety considerations. A short infusion was selected over an IV bolus to minimize the potential risks associated with a high initial serum concentration following an IV bolus. For patients with ESRD undergoing PK assessments on the day of hemodialysis, the dialysis procedure was started 2 hours (± 15 minutes) after the end of infusion and lasted for approximately 4 hours. The dialysis machines

used were Fresenius 4008s (Dialysator FX80, surface area 1.8 m^2), Fresenius 5008s (Dialysator FX80, surface area 1.8 m^2), Fresenius 5008s (Dialysator FX1000, surface area 2.2 m^2), and Gambro AK 200 ultra (Dialysator Polyflux P210H). For ESRD patients with PK assessments performed during the dialysis-free interval, serelaxin infusion was started after an interval of at least 12 hours following the previous hemodialysis with PK sample collection and safety/tolerability assessments being performed over the following 48 hours (until day 3) and prior to the subsequent hemodialysis. All participants were required to remain at the study site for approximately 3 days to facilitate baseline, PK, and safety/tolerability assessments, with an additional ambulatory end-of-study visit on day 15.

Pharmacokinetic Assessments and Analyses

Serum samples for PK analysis were collected during the treatment and follow-up periods (time points: pretreatment, 15 minutes, and 1, 2, 3, 4, 4.25, 5, 6, 7, 8, 9, 10, 11, 12, 16, 24, 28, 36, and 48 hours after the start of infusion) and on day 15 (end of study). Specifically for group 2 patients, who had hemodialysis performed from 6 to 10 hours post–serelaxin administration (4-hour dialysis period), serum samples for PK analyses were collected from both the line entering (LINE IN) and that exiting (LINE OUT) the dialyzer. In addition, hemodialysis fluid samples were collected from these patients 6 hours prior to the start of dialysis and as fractions during the 4-hour dialysis period for assesment of serelaxin concentrations. The dialysate was collected in 20-L bottles, with 20-mL aliquots (or 0.1% of the respective fraction) being taken from each bottle, pooled, and stored at 3°C to 5°C for the duration of dialysis. At the end of the procedure, 3-mL samples of the thoroughly mixed pooled dialysate aliquots were frozen at -70°C and later shipped on dry ice for analysis.

The serum serelaxin concentrations were determined by a validated, commercially available enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, Minnesota). Briefly, the ELISA method used a monoclonal antibody specific for relaxin-2 as the capture reagent and an enzyme-linked polyclonal antibody specific for relaxin-2 as the detection reagent. The lower limit of quantification was 15.6 pg/mL. The same kit was used to determine the serelaxin concentration in the dialysate for group 2, following validation, to ensure no matrix interference to the assay. The lower limit of quantification in the dialysate was 31.3 pg/mL.

Noncompartmental PK analysis was performed using WinNonlin Phoenix (Version 6.2). The evaluated PK parameters are described in Table 1. The key PK parameters to compare serelaxin exposure between the patient groups and matched healthy subjects were area under the serum concentration-time curve (AUC) from time 0 to infinity (AUC_∞) and the observed maximum

Table 1. Pharmacokinetic Parameters Assessed

Parameters	Description
AUC_∞	The area under the serum concentration-time curve from time 0 to infinity (mass $\times$ time/volume)
$AUC\tau\_in$	The area under the serum concentration-time curve during the dialysis interval based on samples collected at the entry of the dialyzer
$AUC\tau\_out$	The area under the serum concentration-time curve during the dialysis interval based on samples collected at the exit of dialyzer
CD	The concentration of the drug in the pooled dialysate during the dialysis interval
C_{max}	The observed maximum serum concentration following drug administration (mass/volume)
CL	The systemic (or total-body) clearance from serum following IV administration (volume/time)
CLHD*	Hemodialysis clearance (volume/time)
DE	Dialysis efficiency
f	Fraction of drug eliminated during dialysis
MRT	Mean residence time (time)
QBIN	Blood flow to the dialyzer (volume/time)
τ	Dialysis interval (time)
T_{max}	Time to reach the maximum concentration after drug administration (time)
$T_{1/2}$	The terminal elimination half-life (time)
VD	Volume of the pooled dialysate during the dialysis interval (volume)
V_z	The volume of distribution during the terminal elimination phase following IV administration (volume)
V_{ss}	The volume of distribution at steady state following IV administration (volume)

*As per the protocol, CLHD was calculated as (CD $\times$ VD)/$AUC\tau\_in$ (Method 1). However, to avoid a potential underestimation because of adsorption, and subsequent under-recovery of serelaxin in the dialysate, an alternative calculation was performed where CLHD $=$ QBIN $\times$ R $\times$ f, where R is the blood-to-serum drug concentration ratio, which was assumed to be 55% (equivalent to the hematocrit as serelaxin does not bind to blood cells). IV, intravenous; PK, pharmacokinetics.

serum concentration following drug administration (C_{max}). In addition, hemodialysis clearance (CLHD) was calculated as (CD $\times$ VD)/$AUC\tau\_in$ Method 1, where CD is the concentration of the drug in pooled dialysate during the dialysis interval, VD is the volume of pooled dialysate during the dialysis interval and $AUC\tau\_in$ is the area under the serum concentration-time curve during the dialysis interval on the basis of the samples collected at the entry of the dialyzer. To avoid a potential underestimation due to adsorption and subsequent underrecovery of serelaxin in the dialysate, an alternative calculation (Method 2) was performed where CLHD $=$ QBIN $\times$ R $\times$ f, where QBIN is the blood flowing into the dialyzer; R is the blood-to-serum drug concentration ratio, which was assumed to be 0.55 as serelaxin does not bind to blood cells; and f is the fraction of drug eliminated during hemodialysis, calculated as $(AUC\tau\_in - AUC\tau\_out)/AUC\tau\_in$, where $AUC\tau\_out$ is the area under the serum concentration-time curve during the dialysis interval on the basis of the samples collected at the exit of the dialyzer.

Safety Assessments and Analyses

Safety assessments included regular monitoring of hematology, blood chemistry, coagulation, and urinalysis, performed at a local laboratory; and regular assessments of body weight, vital signs, physical condition, and electrocardiography. Adverse events (AEs) were sought by nondirective questioning of participants during the study and were also recorded if volunteered by the participants between visits or through the above-listed safety assessments. The frequency of serious AEs (SAEs),

their intensity, and their relationship to the study drug were evaluated for all participants.

Immunogenicity Assessments and Analyses

Serum samples for immunogenicity analysis were collected before the start of treatment and on day 15 (end of study). Antiserelaxin antibodies were evaluated in serum in a 4-tiered approach with validated assays. The details of these assays have been described previously.[7,8] Samples were initially screened for potential immunogenicity in a screening assay. Any positive screening results required confirmation using an immunodepletion assay. If a sample was immune depleted, it was considered positive and the sample would then move to a third-tier titration assay. In addition, any confirmed positive samples required testing for neutralization of serelaxin biological activity using a validated cell-based bioassay. If the sample was not confirmed as being positive in the immunodepletion assay, the titration and neutralization analyses were not performed.

Statistical Analyses

A sample size of 6 patients in each of the renal impairment groups matched with 18 healthy subjects was calculated to have 89% power to detect an observed ratio of 2-fold change at 10% level of significance, assuming an interparticipant coefficient of variation of 40% (based on a prior study, where a 40% coefficient of variation was obtained for the AUC).[8]

Participant demographics (age, sex, ethnicity, race, weight, and height) and baseline characteristics were

Table 2. Demographics of the Study Population

Parameters	Group 1 (n = 6)	Group 2 (n = 6)	Group 3 (n = 6)	All Patients (Groups 1, 2, and 3; n = 18)	Group 4 (n = 18)	Total Study Population (Groups 1, 2, 3, and 4; n = 36)
Age (mean ± SD), years	58.7 ± 10.1	46.5 ± 12.0	52.3 ± 15.8	52.5 ± 13.1	52.0 ± 12.0	52.3 ± 12.4
Males, %	83.3	66.7	83.3	77.8	77.8	77.8
White, %	100	100	100	100	100	100
BMI (mean ± SD), kg/m²	27.9 ± 4.0	25.5 ± 2.0	26.2 ± 6.1	26.6 ± 4.2	26.0 ± 2.8	26.3 ± 3.5

Group 1, patients with severe renal impairment; group 2, patients with ESRD requiring hemodialysis and assigned to receive serelaxin infusion on the day of dialysis; group 3, patients with ESRD requiring hemodialysis and assigned to receive serelaxin infusion during the dialysis-free interval; and group 4, matched healthy subjects.
BMI, body mass index; ESRD, end-stage renal disease; SD, standard deviation.

summarized (Table 2). Data from all the participants were used for PK and safety analyses. The primary statistical analyses were performed on the following PK parameters of serelaxin: C_{max} and AUC_∞. Parameters were compared between each renal impaired group and the respective matched healthy subjects. Log-transformed PK parameters were analyzed using a linear mixed-effects model, with the healthy subject group as a fixed effect and the matching pair as a random effect. Least-squares means for each group as well as the estimated difference between patients with renal impairment and respective matched healthy subjects with corresponding 90% confidence intervals (CI) on the log-scale were calculated. These estimates were back-transformed to obtain the ratio of geometric means (GMR) and the associated 90% CI for the comparison of the renal impaired group vs the matched healthy subjects. In addition, PK parameters (C_{max} and AUC_∞) obtained on the day of hemodialysis and during the dialysis-free interval were compared between the 2 groups of patients with ESRD to evaluate the impact of hemodialysis on the PK of serelaxin, with the healthy subject group as a fixed effect. Safety parameters were analyzed descriptively.

Results

Study Population and Demographics
A total of 36 participants, 18 patients with renal impairment/ESRD (6 each in groups 1–3) and 18 matched healthy subjects (group 4), were enrolled into the study. All 36 participants completed the study, and their data were included in the PK, immunogenicity, and safety/tolerability analyses.

The demographics were generally well balanced across the patient and healthy subjects groups (Table 2).

Pharmacokinetics of Serelaxin in Patients With Severe Renal Impairment and ESRD
Following the start of IV infusion, serum serelaxin concentrations increased rapidly and reached peak concentrations at the end of the 4-hour infusion. On completion of infusion, serum serelaxin concentrations declined rapidly, with a mean terminal elimination half-life ranging from 6.5 to 8.8 hours (Figure 2). Patients with severe renal impairment or ESRD demonstrated a moderate decrease (37%–52%) in systemic serelaxin clearance and a slight increase (10%-22%) in the apparent volume of distribution at steady state compared with the matched healthy controls (Table 3).

Compared with the healthy subjects, serelaxin reached a higher C_{max} in patients with renal impairment. The
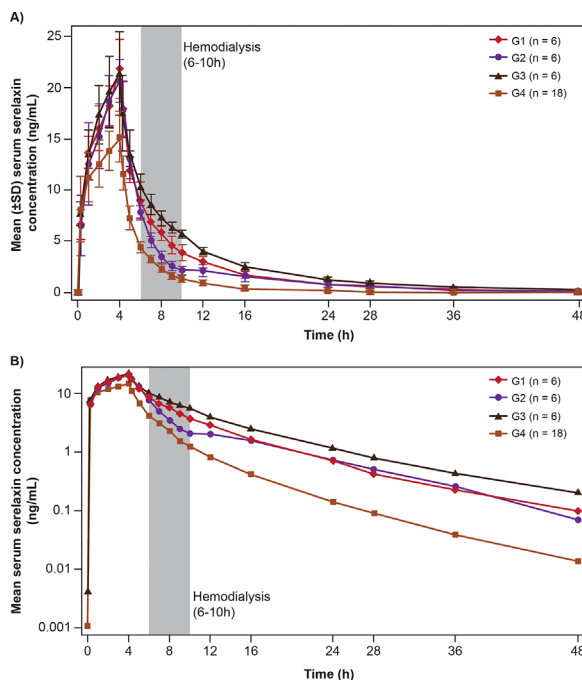

Figure 2. Arithmetic mean (SD) serum serelaxin concentration–time profiles by group. (A) Linear and (B) semilogarithmic views of serum serelaxin concentration-time profiles. Data are shown as arithmetic mean with SD in linear and semilogarithmic views for patients with severe renal impairment (G1), ESRD requiring hemodialysis (with PK assessment on the day of dialysis [G2] and during the dialysis-free period [G3]) compared with healthy controls (G4). ESRD, end-stage renal disease; G, group; PK, pharmacokinetics; SD, standard deviation.

Table 3. Statistical Summary of Serum Serelaxin PK Parameters per Group

Parameters	Group 1 (n = 6)	Group 2 (n = 6)	Group 3 (n = 6)	Group 4 (n = 18)
AUC_∞ (ng · h/mL)	144 (13.0)	131 (12.5)	173 (10.5)	82.2 (13.3)
C_{max} (ng/mL)	21.7 (13.4)	20.5 (9.4)	21.2 (18.1)	15.4 (13.5)
$T_{1/2}$ (hours)	7.8 (10.1)	8.1 (15.8)	8.8 (11.3)	6.4 (22.4)
C_L (mL/[h · kg])	69.7 (13.0)	76.3 (12.5)	57.7 (10.5)	122 (13.3)
V_{ss} (mL/kg)	391 (13.3)	432 (21.9)	424 (22.2)	355 (20.7)
V_z (mL/kg)	787 (14.6)	886 (14.7)	730 (18.6)	1120 (21.1)
MRT (hours)	5.6 (13.4)	5.7 (24.8)	7.4 (15.9)	2.9 (19.3)

All values are presented as geometric means (coefficient of variation, %).

Group 1, patients with severe renal impairment; group 2, patients with ESRD requiring hemodialysis with PK assessment on the day of dialysis; group 3, patients with ESRD requiring hemodialysis with PK assessment during the dialysis-free interval; and group 4, matched healthy subjects.

AUC_∞, area under the serum concentration-time curve from 0 to infinity; C_{max}, observed maximum serum concentration following drug administration; C_L, systemic clearance from serum; ESRD, end-stage renal disease; MRT, mean residence time; PK, pharmacokinetics; $T_{1/2}$, terminal elimination half-life; V_{ss}, volume of distribution at steady state; V_z, volume of distribution during the terminal elimination phase following intravenous administration.

GMR (in comparison with healthy subjects) for C_{max} were 1.39 (90%CI 1.26, 1.53), 1.42 (90%CI 1.29, 1.56), and 1.30 (90%CI: 1.18, 1.43) in groups 1, 2, and 3, respectively (Table 4). Furthermore, compared with the matched healthy subjects, patients with renal impairment had a higher AUC_∞ with the following GMRs: 1.61 (90%CI 1.46, 1.77), 1.70 (90%CI 1.54, 1.87), and 2.15 (90%CI 1.95, 2.36) for groups 1, 2, and 3, respectively (Table 4).

Serelaxin PK differences were much smaller than those among patients with renal impairment. Patients with ESRD undergoing PK assessments during the hemodialysis-free period had the slowest systemic serelaxin clearance, followed by patients with severe renal impairment and then by patients with ESRD undergoing PK assessments on the day of hemodialysis (mean ± standard deviation [SD]: 58.0 ± 5.92 mL/[h · kg] in group 3, 76.8 ± 9.71 mL/[h · kg] in group 2, and 70.1 ± 8.38 mL/[h · kg] in group 1; Figure 2 and Table 3).

To determine whether the PK of serelaxin was affected by the timing of hemodialysis relative to serelaxin administration, the arithmetic mean serum concentration-time profiles from both ESRD groups (groups 2 and 3) were compared. As shown in Figure 3, the mean serum concentration-vs-time profiles for both groups overlapped at all early time points prior to 6 hours and diverged only after the start of hemodialysis in group 2 patients, wherein serum serelaxin concentrations declined much faster during the 4-hour hemodialysis period compared with the equivalent 4-hour period in group 3 patients. Upon completion of dialysis, the terminal elimination phases were again in parallel with each other between the 2 groups. Statistical analysis confirmed that although C_{max} was similar for groups 2 and 3 (GMR 0.97, 90%CI 0.83, 1.12), a 4-hour dialysis on the day of infusion reduced the overall exposure by 24% versus infusion during the dialysis-free period (GMR for AUC_∞ 0.76; 90%CI 0.67, 0.85; Table 5).

Table 4. Statistical Analysis of Serum Serelaxin PK Parameters, Comparison of Patients With Renal Impairment (Groups 1, 2, and 3) vs Matched Healthy Subjects (Group 4)

Parameters	Patient Group	Adjusted Geometric Means*		Observed Ratio of Geometric Means, Patient/Healthy (90%CI)*
		Patient	Matched Healthy Subject	
C_{max} (ng/mL)	Group 1	21.7	15.6	1.39 (1.26–1.53)
	Group 2	20.5	14.4	1.42 (1.29–1.56)
	Group 3	21.2	16.3	1.30 (1.18–1.43)
AUC_∞ (ng · h/mL)	Group 1	144	89.2	1.61 (1.46–1.77)
	Group 2	131	77.1	1.70 (1.54–1.87)
	Group 3	173	80.6	2.15 (1.95–2.36)

*All values are back-transformed from the log-scale. Log-transformed PK parameter data were analyzed using a linear mixed-effects model, with subject group as a fixed effect and matched pair as a random effect.

Group 1, patients with severe renal impairment; group 2, patients with ESRD requiring hemodialysis with PK assessment on the day of dialysis; group 3, patients with ESRD requiring hemodialysis with PK assessment during the dialysis-free interval; and group 4, matched healthy subjects.

AUC_∞, area under the serum concentration-time curve from 0 to infinity; CI, confidence interval; C_{max}, observed maximum serum concentration following drug administration; ESRD, end-stage renal disease; PK, pharmacokinetics.
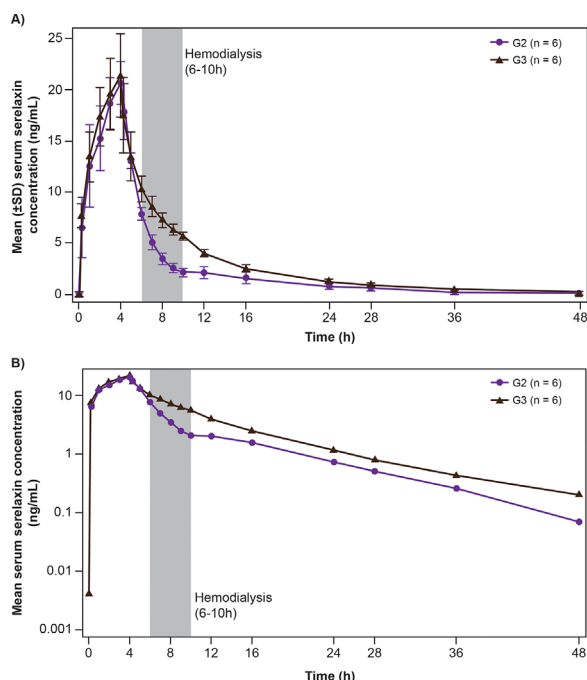
Figure 3. Arithmetic mean (SD) serum serelaxin concentration-time profiles for the ESRD groups requiring hemodialysis. (A) Linear and (B) semilogarithmic views of serum serelaxin concentration-time profiles. Data are shown as arithmetic mean with SD in linear and semilogarithmic views for patients with ESRD requiring hemodialysis with PK assessment on the day of dialysis (G2) and during the dialysis-free period (G3). ESRD, end-stage renal disease; G, group; PK, pharmacokinetics; SD, standard deviation.

Further, analysis of blood entering and leaving the dialyzer revealed that serum serelaxin concentrations were lower in the line exiting than in the line entering the dialyzer (Figure 4). The fraction of serelaxin eliminated during the 4-hour hemodialysis period was estimated to be approximately 30% (geometric mean 0.297). Based on the 2 methods of calculation described in the Methods section, the geometric mean for CLHD was either 552 mL/h (Method 1) or 3020 mL/h (Method 2; Table 6).

Safety and Tolerability
Overall, a single 4-hour IV infusion of 10 μg/kg serelaxin was well tolerated in all participants. No deaths, SAEs, or

severe AEs were reported during the study period, and none of the participants discontinued the study due to an AE. Overall, AEs of mild intensity were reported in 4 participants: 2 in predose and 2 in postinfusion. The former AEs were increased lipase and increased blood creatine phosphokinase, reported in 2 healthy subjects, and the latter included a mild increase in blood creatine phosphokinase in 1 patient in group 1 and headache in 1 healthy subject. Headache was the only AE suspected by the investigator to be related to the study drug. All of these AEs were reported to have resolved by the end of the study period. Changes in hematology or clinical chemistry, none of which were clinically significant, were reported in few of the healthy subjects. No trends or systematic changes in SBP, DBP, and PR were observed across the groups with a single 4-hour IV infusion of 10 μg/kg serelaxin. There were small changes in SBP, DBP, and PR, which were not clinically significant. Importantly, no apparent differences were observed in any of these changes between patients with renal impairment and the matched controls (Figure 5).

Immunogenicity
Antiserelaxin antibodies were not detected in any of the participants on either day 1 (predose) or on day 15 (post–serelaxin treatment).

Discussion
This study was designed to evaluate the PK, safety, and tolerability of a single 4-hour IV infusion of 10 μg/kg serelaxin in patients with severe renal impairment and those with ESRD requiring hemodialysis compared with matched healthy subjects, and to inform whether potential serelaxin dosage adjustments are needed in patients with severe renal impairment.

The dosing regimen in this study was chosen based on practical and safety considerations as mentioned earlier. Although the dose rate (60 μg/kg/day for a 10 μg/kg dose infused over 4 hours) was higher than the nominal serelaxin clinical dose for patients with AHF (30 μg/kg/day), both dose rates are well within the linear range

Table 5. Statistical Analysis of the Effect of Timing of Hemodialysis on Serum Serelaxin PK Parameters in Patients With ESRD Undergoing PK Assessment on the Day of Dialysis (n = 6; Group 2) vs Those Undergoing PK Assessment During Dialysis-Free Interval (n = 6; Group 3)

| Parameters | Patient Group | Adjusted Geometric Means* | | Observed Ratio of Geometric Means, Dialysis/Dialysis-Free (90%CI)* |
		PK on Day the of Dialysis	PK During the Dialysis-Free Interval	
C$_{max}$ (ng/mL)	ESRD	20.5	21.2	0.97 (0.83–1.12)
AUC$_\infty$ (ng·h/mL)	ESRD	131	173	0.76 (0.67–0.85)

*All values are back-transformed from the log-scale. Log-transformed PK parameter data were analyzed using a linear mixed-effects model with subject group as fixed effect and matched pair as random effect.

AUC$_\infty$, area under the serum concentration–time curve from 0 to infinity; CI, confidence interval; C$_{max}$, observed maximum serum concentration following drug administration; ESRD, end-stage renal disease; PK, pharmacokinetics.
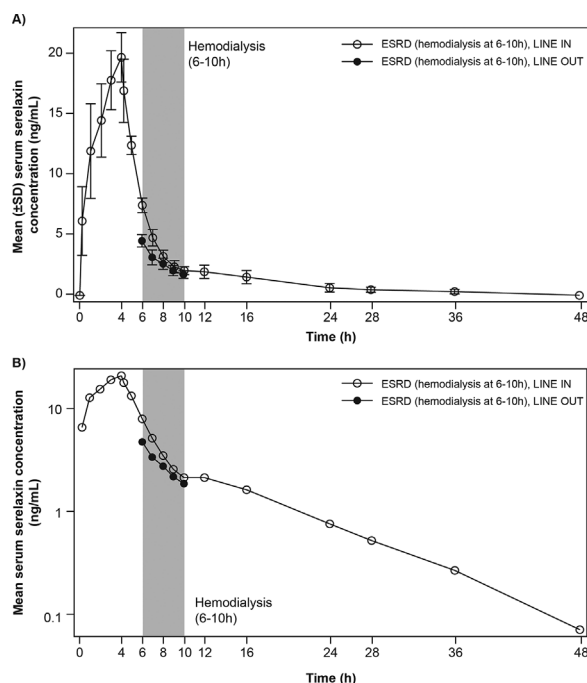
Figure 4. Arithmetic mean (SD) serum serelaxin concentration-time profiles for the ESRD group requiring hemodialysis and assigned to receive serelaxin infusion on the day of dialysis—LINE IN and LINE OUT. (A) Linear view and (B) semilogarithmic views of serum serelaxin concentration-time profiles. Data are shown as arithmetic mean with SD in linear and semilogarithmic views for patients with ESRD requiring hemodialysis and assigned to receive serelaxin infusion on the day of dialysis (G2)—LINE IN and LINE OUT. ESRD, end-stage renal disease; G, group; SD, standard deviation.

$(10-100\,\mu g/kg/day)$ for serelaxin PK based on existing data.[7] Thus, the essential PK characteristics generated in this study can be used to inform whether or not there is a need to adjust the clinical dose for patients with AHF.

Of note, serelaxin, being a recombinant peptide identical to the endogenous human relaxin-2, is expected to be cleared via the same pathways as the endogenous relaxin.[1,2,9] The main elimination pathway for serelaxin is most likely to be via catabolism by peptidases/proteases throughout the body, including the kidneys, liver, and other organs and tissues.[9,10] Given its small molecular mass (5.9 kDa), serelaxin is expected to be filtered via glomerular filtration in the kidneys and then metabolized via intraluminal metabolism or intracellular lysosomal metabolism in the tubular region of the kidneys. Therefore, it is not surprising to see a slower clearance and higher exposure of serelaxin in patients with severe renal impairment or ESRD when compared with matched healthy subjects. However, the differences are moderate, with the GMR of AUC_∞ ranging from 1.61 to 2.15. The moderate difference is partly due to the contribution of the catabolism of serelaxin in other tissues and organs outside the kidneys as well as the distribution of serelaxin into the extravascular space ($V_{ss} = 355$–$432\,mL/kg$). This is also consistent with the observed moderate impact of hemodialysis on the PK of serelaxin. During the 4-hour hemodialysis, 30% of serum serelaxin was eliminated by hemodialysis, which constituted approximately 52% of the overall systemic clearance, indicating that

Table 6. Summary Statistics for Dialysis in Group 2 (n = 6) of Serelaxin PK Parameters

Parameters	Statistics		
	Mean (SD)	Geometric Mean (CV%)	Median (Min, Max)
CLHD (mL/h)*	619 (308)	552 (57.7)	517 (242, 996)
CLHD_2 (mL/h)**	3210 (1260)	3020 (39.2)	2680 (2010, 5060)
CL (mL/h)	5870 (1130)	5780 (19.1)	5710 (4360, 7800)
CLHD/CL (%)	11.2 (6.9)	9.56 (69.2)	9.24 (4.13, 22.7)
CLHD_2/CL (%)	57.5 (29.3)	52.2 (50.0)	42.8 (32.3, 102)
F	0.312 (0.108)	0.297 (34.9)	0.271 (0.203, 0.452)
DE†	0.034 (0.0176)	0.0299 (63.9)	0.0287 (0.0115, 0.0554)
DE_2††	0.171 (0.0596)	0.163 (34.9)	0.149 (0.111, 0.248)
AUC_{τ_in} (ng·h/mL)	20.1 (2.93)	19.9 (15.6)	20.4 (15.2, 23.6)
AUC_{τ_out} (ng·h/mL)	13.8 (2.71)	13.6 (19.5)	13.2 (10.6, 17.9)
Amount dialyzed (ng)	12 200 (5880)	11 000 (56.4)	11 100 (4560, 22 000)
Blood flow rate (mL/min)	308.3 (20.41)	307.8 (6.3)	300 (300, 350)
Rate of dialysis (mL/min)	445 (68.63)	440.3 (16.2)	475 (360, 500)

*CLHD = amount dialyzed/AUCτ in.

**CLHD_2 = blood flow into the dialyzer × 55% × f.

†DE = CLHD/blood flow into the dialyzer.

††DE_2 = CLHD_2/blood flow into the dialyzer.

Group 2: patients with ESRD requiring hemodialysis and assigned to receive serelaxin infusion on the day of dialysis.

AUCτ in/out, AUC during dialysis interval (τ) for serum samples collected at the entry/exit of the dialyzer; CL, systemic clearance; CLHD, hemodialysis clearance (calculated using two methods as CLHD and CLHD_2); CV, coefficient of variation; DE, dialyzer extraction ratio (calculated using two methods as shown below as DE and DE_2); ESRD, end-stage renal disease; f, fraction eliminated by dialysis; PK, pharmacokinetics; SD, standard deviation.
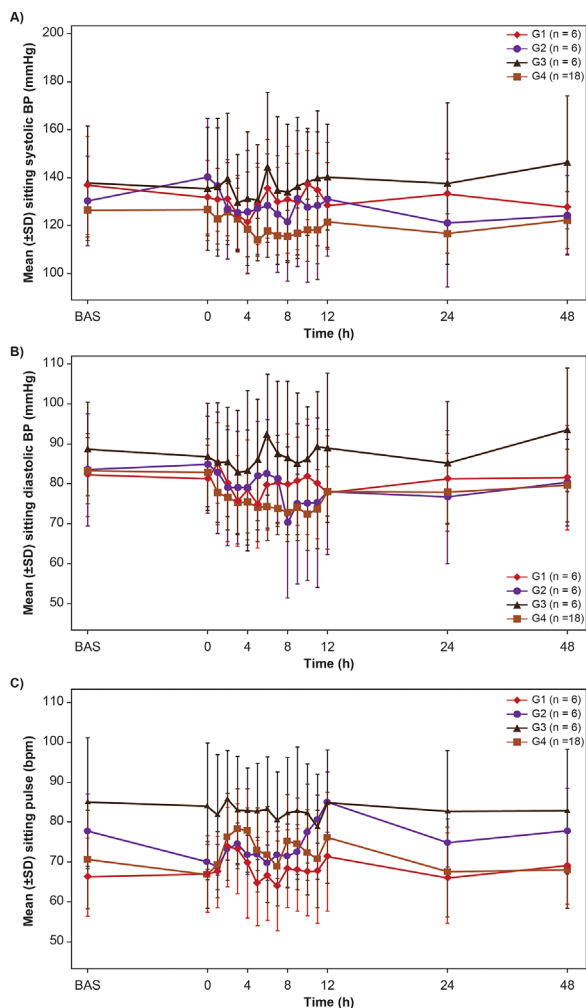
Figure 5. Arithmetic mean (SD) values over time per group for (A) sitting systolic blood pressure, (B) sitting diastolic blood pressure, and (C) pulse rate. Data are shown as arithmetic mean with SD for patients with severe renal impairment (G1), ESRD requiring hemodialysis (with PK assessment on the day of dialysis [G2] and during the dialysis-free period [G3]) compared with healthy controls (G4). BAS, baseline; BP, blood pressure; ESRD, end-stage renal disease; G, group; SD, standard deviation.

hemodialysis accelerates drug clearance from the body. Two different methods were used to calculate the estimated CLHD. Method 1 used the total amount of serelaxin recovered from the dialysate in the calculation and generated a lower value than the CLHD estimated using Method 2, which by definition is independent of the serelaxin concentration measurement in the dialysate. The discrepancy between these results was most likely due to underestimation of the total amount of serelaxin in the dialysate, potentially through adsorptive loss of serelaxin to the dialysis device and tubing. Therefore, the results provided by Method 2 are considered more reliable. Renal impairment and dysfunction are often noted in patients with HF.[3,4,6] Therefore, in order to evaluate the clinical

impact and relevance of the observed PK differences in this study, a population PK analysis was performed on the basis of the pooled data from subjects, including patients with AHF, with various degrees of renal function (normal, mild, moderate, and severe impairment and ESRD) who were treated with serelaxin across multiple studies. Data from this analysis showed that serelaxin clearance was reduced by 38% and 47% in patients with ESRD compared with patients with mild renal impairment and healthy subjects with normal renal function, respectively (data on file). Therefore, serelaxin PK differences between patients with severe renal impairment or ESRD and patients with mild to moderate renal impairment are smaller than the differences observed in this study when healthy subjects with normal renal function are used as comparators.

In addition, clinical data suggest that serelaxin has a wide therapeutic window and a shallow exposure/dose-response relationship.[4,11] Serelaxin doses of up to 960 μg/kg/day were administered to patients with AHF, and it was demonstrated that doses ranging from 10 to 100 μg/kg/day were generally well tolerated in patients with AHF.[4,11] Moreover, serelaxin was well tolerated in this study by all subjects, including patients with ESRD, regardless of their increased exposure to serelaxin. The PK differences observed in this study are not likely to pose a safety risk to patients with severe renal impairment or ESRD and do not warrant a predefined dosage adjustment based on renal function.

Possible limitations of this study are that it did not evaluate the effect of peritoneal dialysis on the PK of serelaxin, a factor that may be of particular relevance for patients with ESRD requiring dialysis. Second, the effect of dialysis on the PK of serelaxin was evaluated based on the comparison between the 2 parallel groups of patients (groups 2 and 3), and no intragroup comparisons were performed, where PK data are collected before and during dialysis from the same patients. Given the observed small interpatient serelaxin PK variability, and considering the serious health state of the patient population, the parallel-group design is a sound approach for the purpose of the study.

In summary, this study demonstrated a moderate decrease in clearance and an increase in exposure of serelaxin in patients with severe renal impairment or ESRD compared with healthy subjects. It also showed that serelaxin can be partially eliminated from circulation by hemodialysis. Serelaxin was well tolerated by patients with severe renal impairment, by those with ESRD requiring hemodialysis, and by healthy subjects. No antiserelaxin antibodies were detected in any participant. The observed serelaxin PK differences in patients with severe renal impairment compared with matched healthy subjects are unlikely to pose a safety risk and do not warrant a predefined dosage adjustment in such patients.

Acknowledgments

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