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Multicenter, Randomized, Double-Blinded, Placebo-Controlled Phase II Study of Serelaxin in Japanese Patients With Acute Heart Failure

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Background: Serelaxin, a recombinant form of human relaxin-2, is in development for treating acute heart failure (AHF) and a Phase II study in Japanese AHF patients was conducted.

Methods and Results: A randomized, double-blind, placebo-controlled study of serelaxin at 10 and 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ continuous intravenous infusion for up to 48 h, added to standard care for Japanese AHF patients. Primary endpoints were adverse events (AEs) through Day 5, serious AEs (SAEs) through Day 14, and serelaxin pharmacokinetics. Secondary endpoints included changes in systolic blood pressure (SBP) and cardiorenal biomarkers. A total of 46 patients received the study drug and were followed for 60 days. The observed AE profile was comparable between the groups, with no AEs of concern. Dose-dependent increase in the serum concentration of serelaxin was observed across the 2 dose rates of serelaxin. A greater reduction in SBP was observed with serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ vs. placebo (–7.7 [–16.4, 1.0] mmHg). A greater reduction in NT-proBNP was noted with serelaxin (–50.8% and –54.9% for 10 and 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$, respectively at Day 2).

Conclusions: Serelaxin was well tolerated in this study with Japanese AHF patients, with no AEs of concern and favorable beneficial trends on efficacy. These findings support further evaluation of serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ in this patient population. (*Circ J* 2015; **79:** 1237–1247)

Key Words: Efficacy; Pharmacokinetics; Safety; Serelaxin; Tolerability

Similar to Europe and the USA, the prevalence of heart failure (HF) in Japan is likely to increase in the future because of an aging population, and it is estimated that the number of patients with HF will increase by 0.6% every year over at least the next 30 years.[1–6] Signs and symptoms of decompensation at the onset of acute HF (AHF) as a result of worsening congestion and/or hypoperfusion are associated with a complex heterogeneous pathophysiology involving multiple organ systems.[7–9] Although currently recommended standard of care (SoC) treatments, including intravenous diuretics, vasodilators, inotropes, circulatory devices, etc, can improve the decompensation signs and symptoms during hospitalization, none of the existing pharmacological therapies have been shown to reduce mortality and nor have clinical trials in recent decades demonstrated clinically meaningful benefits.[10–14]

Serelaxin is a recombinant form of human relaxin-2, which is a naturally occurring peptide hormone that increases during pregnancy and is believed to mediate the maternal physiological cardiovascular (CV) and renal adaptations to pregnancy.[15,16] Serelaxin has a unique mechanism of action that could potentially address the complex pathophysiology of AHF through multiple pathways, possibly leading to organ protection.[15–17] Pre-RELAX-AHF, a Phase II dose-finding

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study, showed that serelaxin at the dose of 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ on top of SoC resulted in an improvement in dyspnea in AHF patients.[18] The study showed that serelaxin was well tolerated and had a favorable effect on clinical outcomes, including reduction of in-hospital worsening HF (WHF), and mortality through 180 days. Furthermore, a Phase III RELAX-AHF study demonstrated that serelaxin significantly improved dyspnea, measured by a visual analog scale (VAS), through Day 5 as well as significantly reducing in-hospital WHF through Day 5, and all-cause and CV death by 37% through Day 180.[19] These clinical benefits are likely mediated through organ protection as demonstrated by reduced levels of N-terminal prohormone of B-type natriuretic peptide (NT-proBNP), cardiac troponin T and cystatin-C.[20]

Because both the Pre-RELAX-AHF and RELAX-AHF clinical trials were conducted predominantly in Caucasian AHF patients, there is currently limited data regarding the efficacy and safety of serelaxin in Asian AHF patients, including Japanese AHF patients. This is being addressed by an ongoing Phase III study, RELAX-AHF-ASIA (ClinicalTrials.gov identifier NCT02007720). Although a previous study in healthy Japanese and Caucasian subjects showed no apparent difference between the 2 ethnic groups with regard to pharmacokinetics (PK), safety, and tolerability of serelaxin,[17] this Phase II study was conducted to evaluate serelaxin in Japanese AHF patients prior to enrolling them in the larger RELAX-AHF-ASIA trial.

Methods

Study Design and Participants

The present study was a multicenter, randomized, double-blind and placebo-controlled Phase II clinical trial conducted in 15 sites (Table S1) in Japan (ClinicalTrials.gov Identifier: NCT 02002702).

The study protocol was reviewed by the independent ethics committee or institutional review board at each center, and the study was conducted according to the ethical principles of the Declaration of Helsinki. All subjects provided written informed consent before any study-specific procedures were conducted.

The trial design took into account prior clinical trial experience in the Pre-RELAX-AHF and RELAX-AHF studies.[18,19] Detailed inclusion and exclusion criteria are provided in Table S2. Importantly, we only enrolled patients who presented with a primary diagnosis of AHF, dyspnea at minimal exertion, radiological evidence of pulmonary edema, and BNP ≥350 pg/ml or NT-proBNP ≥1,400 pg/ml, as well as mild-to-moderate renal dysfunction (Japanese equation estimated glomerular filtration rate [eGFR] of 25–75 ml/min/1.73 m^2) and treatment with at least 40 mg intravenous furosemide before screening. Patients were randomized to the study treatment within 16 h of presentation. Patients with systolic blood pressure (SBP) <125 mmHg, concurrent usage of intravenous vasoactive drugs or inotropes (except intravenous nitrate ≤0.1 $mg \cdot kg^{-1} \cdot h^{-1}$ in patients with SBP at screening >150 mmHg) or mechanical support within 2 h or longer before screening were excluded.

The study primary endpoints were: (1) non-serious adverse events (AEs) through Day 5 and serious AEs (SAEs) through Day 14; and (2) PK assessments (0–56 h). Secondary efficacy endpoints were: (1) area under the curve (AUC) for changes in SBP through 48 h of infusion and through Day 5; and (2) change from baseline in cardiorenal biomarkers through Day 14. The exploratory efficacy endpoints are listed in Supplementary File 1 and included a clinical composite endpoint of "treatment success" at Day 2, "treatment failure" and "unchanged" through Day 5 as used as primary endpoints in RELAX-AHF-ASIA; dyspnea assessed by Likert scale and physician's assessment of dyspnea on exertion, orthopnea, edema, rales and jugular venous pulse (JVP) at Day 2; dyspnea by VAS-AUC change from baseline through Day 5.

Procedures

Eligible patients were randomized, within 16 h of presentation, in a 1:1:1 ratio to receive an a continuous intravenous infusion of serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ or 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ or placebo for up to 48 h in addition to SoC therapy in a double-blind manner. Serelaxin and a matching placebo were supplied to the study sites in identical masked kits. The randomization scheme and the list of kit numbers assigned to treatment were generated by an independent supplier (Bell Medical Solutions Inc, Tokyo, Japan). Once an eligible patient was identified, trained study staff (principal investigator, sub-investigator, or study coordinator) requested assignment of study drug kits for each 24-h period of dosing via the registration web site. Patients, all site personnel, including those preparing or administering study drug, and those undertaking study-related assessments, were masked to treatment assignments until after the clinical database lock.

As predefined by the protocol, if SBP decreased by more than 40 mmHg from baseline (hour 0, prior to study drug treatment) but was greater than 100 mmHg, the study drug infusion rate was halved for the remainder of the infusion period. As soon as SBP went below 100 mmHg, study drug infusion had to be permanently stopped. Use of additional SoC treatments for AHF was left to the treating physician's discretion. Safety assessments included physical examinations, vital signs, ECG, laboratory evaluations, and AE monitoring throughout the course of the study. If a BP decrease that met protocol-defined BP-related study drug adjustment or discontinuation was symptomatic, this event was mandated to be also reported as an AE. Serum samples for PK, immunogenicity testing and biomarker analyses were collected and analyzed through a central laboratory. Heart rate and BP were monitored frequently during study drug infusion and through 48 h after study drug termination.

Ad verbatim AE terms were coded using version 17.0 of MedDRA (Medical Dictionary for Regulatory Activities) and presented by MedDRA System Organ Class and Preferred Term for each treatment group.

Statistical Analysis

All analyses were performed using SAS version 9.3. Safety analyses included all patients who received the study drug and had at least 1 post-baseline safety assessment (safety set). Efficacy analyses included all randomized patients (excluding mis-randomized patients) according to the study treatment they had been assigned, following the intention-to-treat principle (full analysis set). PK analyses included all patients who received the study drug with at least 1 set of evaluable PK data and no major protocol deviations affecting the PK data. Because the time points of PK sample collection in the study were widely spaced and few in number, only C_{max} (maximum observed serum concentration), C_{ss} (serum concentration at steady state, estimated based on concentration prior to the end of 48-h infusion), and CL (systemic clearance calculated based on dose rate and C_{ss}) were calculated for each individual patient. Pharmacodynamic and exploratory efficacy endpoints were analyzed for the full analysis set. Standardized AUC for

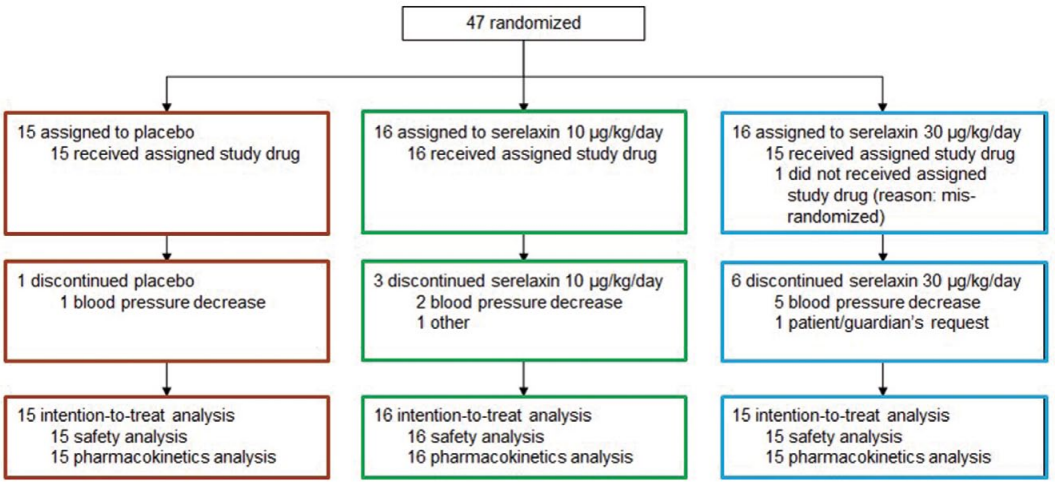

Figure 1. Trial profile. The 47 patients with acute heart failure were randomized to serelaxin 10 or 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ or placebo.

changes in SBP through 48 h and 5 days were analyzed using analysis of covariance (ANCOVA) with treatment group as a factor and baseline SBP as a covariate. The trapezoidal rule was used to calculate the AUC for SBP, which was standardized by dividing by the length of respective time ranges. Differences between the serelaxin and placebo groups were determined using least-square (LS) means. For biomarkers, the geometric mean of the ratio of post-baseline values to baseline values was calculated by treatment. In addition, descriptive statistics for the baseline values, the post-baseline values, and the change from baseline were provided by treatment group. Analyses for exploratory endpoints are summarized in **Supplementary File 1**.

A sample size of 15 patients per arm was considered appropriate so that the 90% confidence interval (CI) interval of the geometric mean would extend no more than 1 ng/ml and 3 ng/ml above the geometric mean of C_{max} for IV infusion of serelaxin 10 and 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$, respectively, for 48 h with more than 80% probability when we assume that C_{max} follows a log-normal distribution with a geometric mean of 3.8 ng/ml for serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ and 11.5 ng/ml for serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ and the estimated intra-subject standard deviation on the natural log scale is 0.448. This sample size was not calculated with regard to safety or for statistical comparisons of treatment effects on exploratory efficacy endpoints.

Results

Baseline Characteristics

The trial profile is summarized in **Figure 1**. A total of 47 patients were randomized; 46 received the study drug (serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ [n=16]; 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ [n=15]; and placebo [n=15]) and completed the study. One patient was randomized in the serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ group but did not receive any study drug because of being diagnosed with acute myocardial infarction before initiating therapy. Patients were randomly assigned to groups at a mean of 8.3±4.6 h of presentation and were treated with study drug within 1.1±0.6 h of randomization. Mean duration of study drug infusion was 45.6±9.2 h for patients in the placebo group, 42.6±13.2 h for those receiving serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ and 32.8±19.3 h for the 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ group.

Baseline characteristics of all patients are summarized in **Table 1**. Notably, the majority of patients were male (73.9%), and the average age was 75.3 years (range: 52–94). Mean SBP at screening was 144±17 mmHg but lowest in the serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ group (137.0±10 mmHg) and highest in the placebo group (148±18 mmHg), although these were not statistically different. Mean eGFR was 49.2±13.2 ml/min/1.73 m^2 across the treatment groups. Overall, patients had a high prevalence of comorbidities such as hypertension (82.6%), mitral regurgitation (50.0%), ischemic heart disease (47.8%), atrial fibrillation (43.5%) or angina pectoris (34.8%), and 52.2% had a prior history of HF and 37.0% were hospitalized for HF within the past 12 months. There was no loss to follow-up over the 60-day study.

Safety and Tolerability

All treatment-emergent AEs (ie, non-serious AEs) through Day 5 and SAEs through Day 14 as a primary variable are summarized in **Table 2**. A total of 10 patients (62.5%) treated with serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$, and 11 (73.3%) with serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$, and 9 (60.0%) with placebo experienced at least 1 AE. SAEs were reported in 1 patient (6.3%, atrial fibrillation, cardiac failure congestive, and multi-organ failure) treated with serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ and in 3 (20.0%, [1] gastric cancer, esophageal carcinoma, metastases to lymph nodes, metastases to liver, and metastases to peritoneum, [2] non-occlusive intestinal ischemia and cardiac failure aggravated, [3] non-sustained ventricular tachycardia and coronary artery stenosis) with serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$; there were no SAEs in the placebo group. All SAEs occurred in severely ill patients, who suffered from either long-term sequelae or serious complications of their underlying HF condition, and in 1 case from gastric cancer, thereby providing a potential alternate explanation of the events. Of note, none of the SAEs was considered to be causally related to the study drug by the investigators. There were 11 patients who met the BP-related study drug dose adjustment or discontinuation rule:

Table 1. Baseline Characteristics

	Placebo (n=15)	Serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ (n=16)	Serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ (n=15)	P value
Male	12 (80.0)	12 (75.0)	10 (66.7)	0.773
Age (years)	76.4 (12.1)	70.2 (11.8)	79.7 (9.0)	0.061
Body weight (kg)	62.8 (11.2)	71.1 (17.4)	60.4 (11.8)	0.089
Body-mass index (kg/m^2)	24.2 (3.4)	26.4 (5.6)	24.6 (4.8)	0.382
SBP (mmHg)	148 (18)	145 (20)	137 (10)	0.176
<140 mmHg	5 (33.3)	9 (56.3)	10 (66.7)	–
Heart rate (beats/min)	86 (18)	90 (17)	91 (20)	0.639
Medical history				–
Ischemic heart disease	10 (66.7)	7 (43.8)	5 (33.3)	0.198
Hypertension	14 (93.3)	12 (75.0)	12 (80.0)	0.491
Diabetes mellitus	9 (60.0)	11 (68.8)	8 (53.3)	0.697
Mitral regurgitation	9 (60.0)	5 (31.3)	9 (60.0)	0.186
Atrial fibrillation	4 (26.7)	9 (56.3)	7 (46.7)	0.244
Atrial flutter	2 (13.3)	1 (6.3)	2 (13.3)	0.724
HF history	11 (73.3)	5 (31.3)	8 (53.3)	0.077
Time since diagnosis of HF				0.327
0–3 months	4 (26.7)	1 (6.3)	0	–
>1–2 years	2 (13.3)	0	0	–
>2–5 years	2 (13.3)	1 (6.3)	3 (20.0)	–
>5 years	3 (20.0)	3 (18.8)	5 (33.3)	–
Most recent ejection fraction (%)	39.8 (16.6) (n=8)	33.5 (13.4) (n=2)	47.3 (14.1) (n=6)	0.494
NYHA class 30 days before admission				0.358
I	1 (6.7)	1 (6.3)	0	–
II	1 (6.7)	2 (12.5)	4 (26.7)	–
III	6 (40.0)	1 (6.3)	2 (13.3)	–
IV	3 (20.0)	1 (6.3)	1 (6.7)	–
Admission to hospitalization for HF in past year	8 (53.3)	3 (18.8)	6 (40.0)	1.000
Treatments at baseline				–
ACEI	4 (26.7)	0	4 (26.7)	0.065
ARB	5 (33.3)	4 (25.0)	6 (40.0)	0.678
β-blocker	5 (33.3)	6 (37.5)	9 (60.0)	0.367
PO loop diuretic	7 (46.7)	3 (18.8)	7 (46.7)	0.165
Other PO diuretics	3 (20.0)	5 (31.3)	3 (20.0)	0.752
Calcium-channel blocker	6 (40.0)	2 (12.5)	3 (20.0)	0.215
Nitrates (oral/topical)	3 (20.0)	7 (43.8)	1 (6.7)	0.050
Time from presentation to randomization (h)	7.1	8.5	9.3	0.431
Time from randomization to study drug administration (h)	0.9	1.1	1.4	0.034
Intravenous nitrates at randomization	2 (13.3)	2 (12.5)	0	0.526
NT-proBNP (pg/ml)*	5,863 (3,851)	5,144 (4,335)	6,709 (4,280)	0.583
eGFR (ml/min/1.73 m^2)**,†	52.6 (13.8)	49.4 (13.0)	45.6 (12.8)	0.353
Serum creatinine (μmol/L)†	94.9 (26.2)	103.6 (38.6)	102.0 (24.1)	0.703
BUN (mg/dl)†	22.6 (4.6)	22.1 (9.0)	21.7 (7.1)	0.938
Sodium (mEq/L)†	141.7 (2.6)	140.3 (2.9)	141.7 (2.3)	0.221
Hemoglobin (g/dl)†	11.4 (2.1)	12.8 (2.4)	11.6 (1.8)	0.143

Data are mean (SD), n (%), or geometric mean (95% CI). *Measured locally by a study-specified device at screening for eligibility. **eGFR calculated by Japanese equation. †Serum sample collected at baseline. P values are for the baseline comparability of treatment groups. ACEI, angiotensin-converting enzyme inhibitor; AHF, acute heart failure; ARB, angiotensin-receptor blocker; BUN, blood urea nitrogen; CCB, calcium-channel blocker; CI, confidence interval; eGFR, estimated glomerular filtration rate; HF, heart failure; NT-proBNP, N-terminal prohormone of B-type natriuretic peptide; NYHA, New York Heart Association; PO, per os; SBP, systolic blood pressure.

3 (18.8%) in the serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ group, 6 (40.0%) in the serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$, and 2 (13.3%) in the placebo group. The occurrence of BP decrease after exposure to serelaxin was to be anticipated because of the vasodilatory properties of serelaxin. Of these patients, 2 in the serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ group experienced symptomatic events. In one of those patients, no additional treatment for the event was needed. In the other patient, SBP dropped to 99 mmHg and

Table 2. AEs

	Placebo (n=15)	Serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ (n=16)	Serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ (n=15)
Non-SAEs through Day 5 and SAEs through Day 14			
No. of patients	9 (60.0)	10 (62.5)	11 (73.3)
Constipation	1 (6.7)	0	4 (26.7)
Cardiac failure	3 (20.0)	0	2 (13.3)
Hypokalemia	2 (13.3)	0	2 (13.3)
BP decreased and systolic BP decreased	0	1 (6.3)	2 (13.3)
Ventricular tachycardia	0	0	2 (13.3)
Dehydration	0	1 (6.3)	1 (6.7)
Atrial fibrillation	1 (6.7)	1 (6.3)	0
Protocol-defined BP-related study drug adjustment or discontinuation			
No. of patients	2 (13.3)	3 (18.8)	6 (40.0)
Dose adjusted	1 (6.7)	1 (6.3)	2 (13.3)*
Permanently discontinued	1 (6.7)	2 (12.5)	5 (33.3)

Common (occurred in at least 2 patients in total) AEs are presented. Data are number (%) unless otherwise stated. *Including 1 patient whose dose was reduced by 50%, but finally discontinued. AEs, adverse events; AHF, acute heart failure; BP, blood pressure; SAEs, serious adverse events.

Table 3. Pharmacokinetic Parameters

	Serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ (n=12)	Serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ (n=8)
CL ($ml \cdot h^{-1} \cdot kg^{-1}$)	55.0 (26.4)	67.5 (28.9)
C_{max} (ng/ml)	7.76 (27.3)	18.6 (29.3)
C_{ss} (ng/ml)	7.58 (26.4)	18.5 (28.9)

Data are geometric mean (coefficient of variation, %). AHF, acute heart failure; CL, systemic clearance from serum following intravenous administration; C_{max}, maximum serum concentration after drug administration; C_{ss}, steady state of serum concentration following intravenous administration.

intravenous inotrope (dobutamine) at 1.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ was initiated and BP recovered to 121 mmHg soon after. In both cases, the event was assessed as non-serious and reported as AEs as mandated by the study protocol. All other BP decreases that met protocol-defined BP-related study drug adjustment or discontinuation were asymptomatic and resolved spontaneously without the need for drug therapy. Across the treatment groups, no clinically meaningful differences with respect to clinical laboratory assessments were noted during the study. There were no anti-serelaxin antibodies detected in any of the patients at either baseline or 14 days post-dose.

PK

The estimated PK parameters are summarized in Table 3. Overall, the serelaxin profiles with the different doses were comparable to those observed in previous studies in Caucasian AHF patients (data on file), with a fast initial increase of serum concentration within the first few hours, a steady state achieved around 24 h, and a quick terminal decline phase after the end of the infusion. Dose-dependent increases in both C_{max} and C_{ss} were observed across the 2 dose rates of serelaxin in Japanese AHF patients, consistent with observations from previous studies.[21,22] Group mean steady state concentrations were 7.8 and 19.1 ng/ml for 10 and 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$, respectively.

Pharmacodynamic and Exploratory Efficacy Results

The assessment of the pharmacodynamic effects of serelaxin compared with placebo included changes in BP and cardiorenal biomarkers (NT-proBNP, high-sensitivity cardiac troponin T, cystatin-C, neutrophil gelatinase-associated lipocalin (NGAL) and aldosterone). The changes in systolic and diastolic BP during study drug infusion are shown in Figure 2. BP progressively decreased during the first 6 h of infusion, with stabilization during the remaining infusion period. In general, patients receiving serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ demonstrated a numerically greater SBP decrease during the 48-h infusion period than those in the serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ and placebo groups. The LS mean treatment differences [95% CI] for the change from baseline AUC of SBP for 48 h were 2.7 [–5.8, 11.1] mmHg (P=0.527) and –7.7 [–16.4, 1.0] mmHg (P=0.080) for the serelaxin 10 and 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ groups, respectively. Similar results were obtained for the change from baseline AUC for SBP through Day 5 (serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$: 6.0 [–2.0, 14.0] mmHg, P=0.141, 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$: –7.4 [–15.6, 0.9] mmHg, P=0.078). The changes in BP in patients treated with placebo, 10 and 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ serelaxin suggest that serelaxin exerted pharmacodynamic effects in Japanese AHF patients as expected. Changes in NT-proBNP, an indicator of the degree of cardiac wall stress and congestion, through Day 14 are shown in Figure 3. NT-proBNP (geometric mean) was lowest at baseline in the 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ serelaxin group (placebo: 5,030 pg/ml; serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$: 3,918 pg/ml; serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$: 5,927 pg/ml). Compared with placebo, reductions in NT-proBNP were earlier and higher in the 2 serelaxin groups through Day 5. At both Day 2 (end of infu-

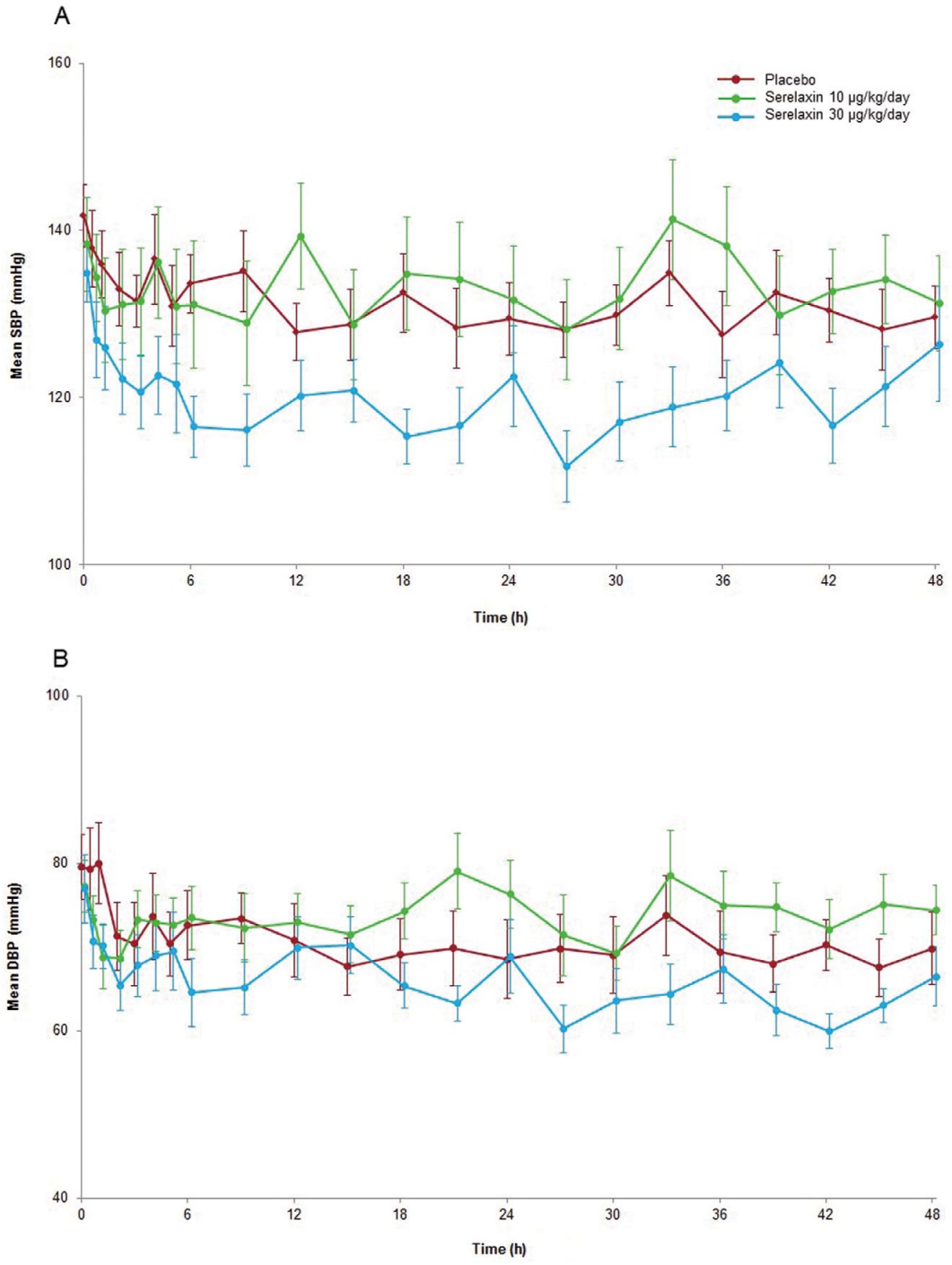

Figure 2. Changes in blood pressure (BP) during the study drug infusion. (**A**) Mean systolic BP (SBP; mmHg) and (**B**) mean diastolic BP (DBP; mmHg) (placebo in red, serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ in green, and 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ in blue). Error bars represent the standard errors.

sion) and Day 5, reductions were –50.8% (vs. placebo, P=0.439) and –61.2% (P=0.260) for the serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ group, –54.9% (P=0.247) and –61.4% (P=0.260) for the serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ groups, and –41.9% and –47.4% for the placebo group, respectively. For other biomarkers (high-sensitivity cardiac troponin T, cystatin-C, NGAL and aldosterone), relatively small changes were observed and comparable among treatment groups. Exploratory endpoints evaluated also included a clinical composite endpoint, which is being used as the primary endpoint in the ongoing RELAX-AHF-ASIA trial, defined as: (1) "treatment success" of moderate/marked improvement in dyspnea by Likert (moderate/marked improve-

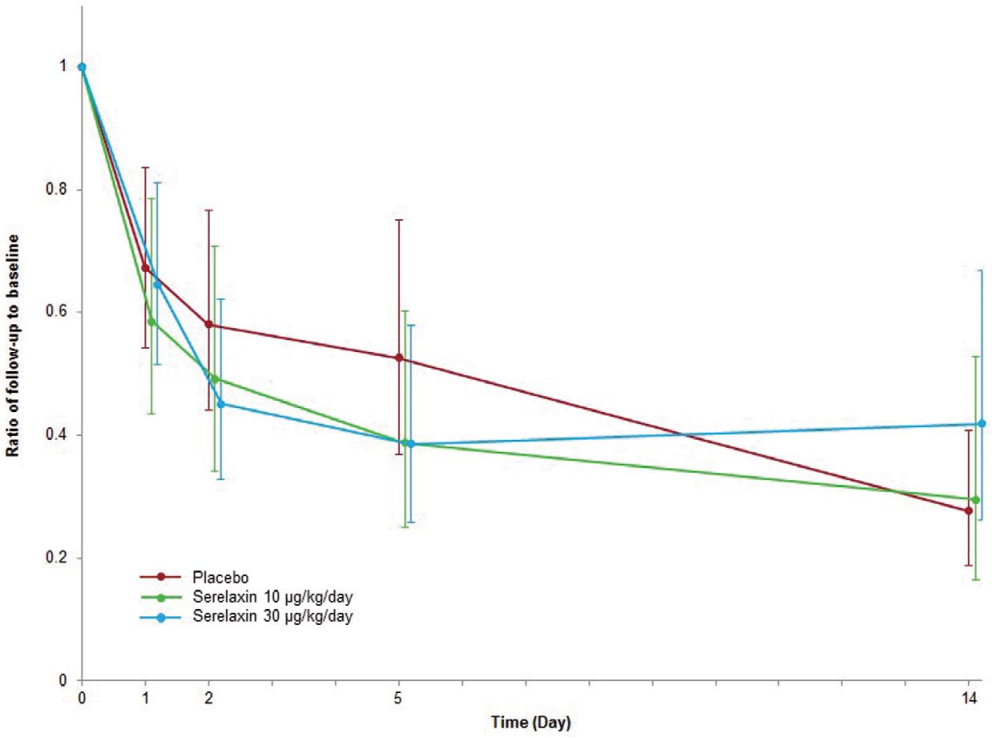

Figure 3. Changes in N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) from baseline through Day 14. Changes from baseline to each study day in NT-proBNP (placebo in red, serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ in green, and serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ in blue). Error bars represent the 95% confidence interval.

ment) and 2 or more points improvement in 2 or more out of 4 physician-assessed symptoms and signs of congestion (orthopnea, edema, rales and JVP) and none of them deteriorating at Day 2, (2) "treatment failure" of WHF, all causes of death or rehospitalization because of HF or renal failure through Day 5 and (3) neither as "unchanged". If a patient was determined as treatment failure through Day 5, then he/she was excluded from the assessment of treatment success or unchanged even if the patient experienced improved signs and symptoms at Day 2. There were 7 patients with "treatment success" at Day 2; 3 (18.8%) and 4 (26.7%) in the 10 and 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ serelaxin groups, respectively, and 1 (6.7%) in the placebo group. The proportion of patients with "treatment failure" through Day 5 was higher in the placebo group than in the serelaxin groups; 3 vs. 1 in each of the serelaxin groups. All "treatment failures" were investigator-reported WHF events (**Figure 4A**, vs. placebo, P=0.298 for serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$, P=0.149 for serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$). For each component of the clinical composite endpoint, patients in the serelaxin treatment groups experienced larger improvement at Day 2 (Hour 48) in the physician assessments of HF signs and symptoms (dyspnea on exertion, orthopnea, edema, rales and JVP), and also in patient-reported dyspnea by Likert scale (**Figures 4B,C**; **Table 4**), in addition, greater improvement in patient-reported dyspnea assessed by VAS was observed in the serelaxin groups. The LS mean change from baseline AUC for VAS through Day 5 was 1,814.1 h (vs. placebo, P=0.688), 1,880.5 h (P=0.646) and 1,422.1 h for the serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$, 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$, and placebo groups, respectively.

Discussion

This Phase II study examined the effects of intravenous 48-h infusion of 10 or 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ serelaxin compared with placebo, when added to SoC in Japanese patients with AHF presenting with SBP >125 mmHg and mild-to-moderate renal impairment. It is the first study to specifically evaluate serelaxin therapy in Japanese AHF patients.

The data showed that serelaxin was safe and well tolerated in these Japanese AHF patients. Overall, the AE profile was comparable between the 2 serelaxin dose groups and placebo group, and no deaths occurred. Importantly, there were no AEs of concern and the overall AE analysis did not suggest any deleterious effect of the 2 doses of serelaxin compared with the placebo group. Also, the dose-dependent increase in the serum concentration of serelaxin was observed across the 2 dose rates of serelaxin (10 and 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$), which was confirmed for the first time in Japanese AHF patients. The PK of serelaxin in the studied Japanese AHF patients was largely comparable to the one already observed in previous serelaxin studies of Caucasian AHF patients (data on file), reaffirming the conclusion reached in the previous ethnic sensitivity study in both Japanese and Caucasian healthy subjects[21] that there is no ethnic sensitivity for serelaxin. The additional analysis using available PK data (data on file) revealed the concentrations of serelaxin in the patients with discontinued infusion were not different from those who completed the infusion of serelaxin at the time points when the study drug was still infused. Therefore, there were no concerns about AEs related

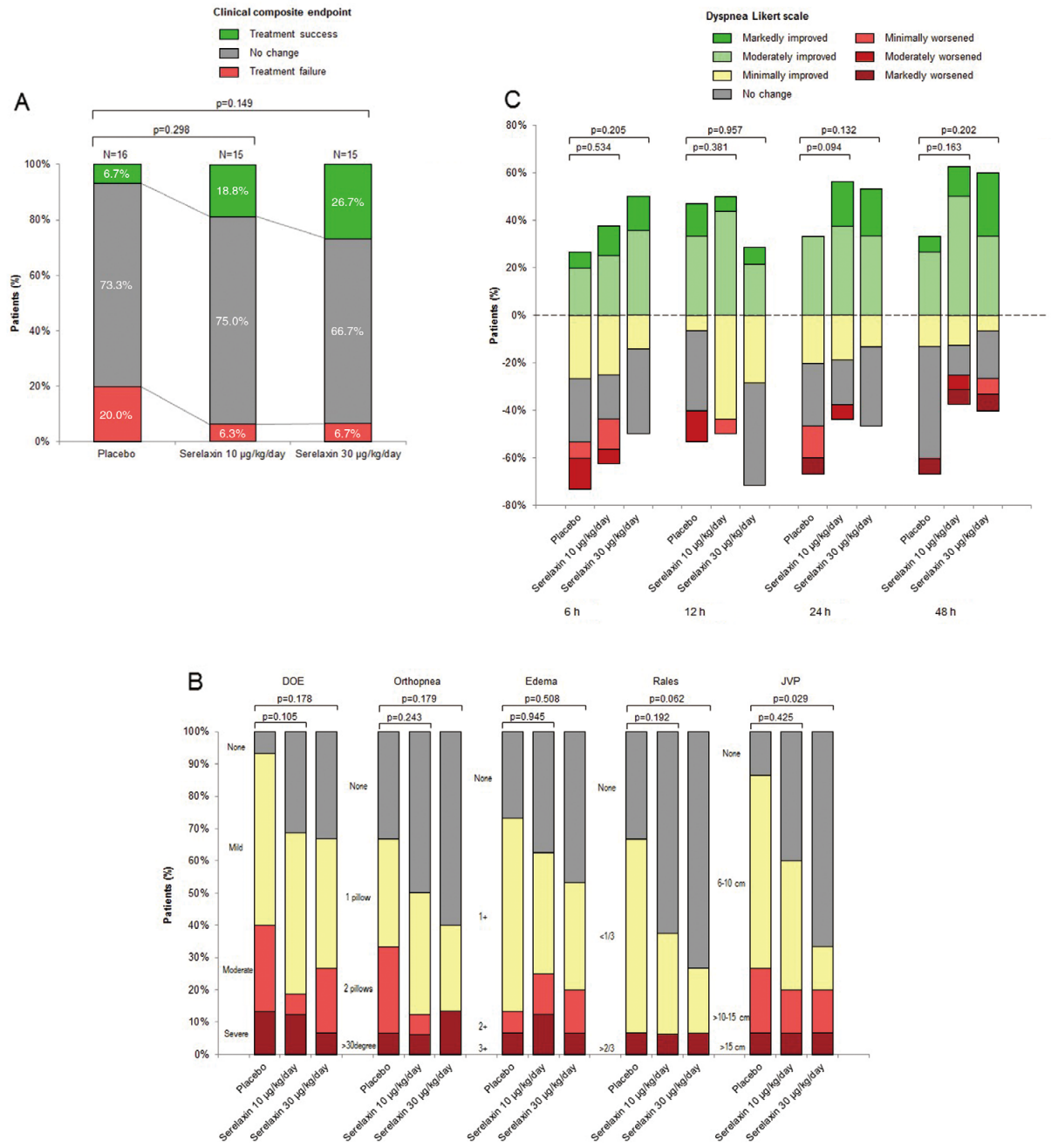

Figure 4. Exploratory efficacy results. (**A**) Clinical composite endpoint: the proportions of "treatment success" (green), "no change" (grey), and "treatment failure" (red) at Day 2. (**B**) Severities of physician assessments of heart failure signs and symptoms (dyspnea on exertion [DOE], orthopnea, edema, rales and jugular venous pulse [JVP]) at Day 2. (**C**) Patient-reported changes in dyspnea relative to baseline during the initial 48 h using the Likert scale are shown with a 7-point scale. P values are for the Wilcoxon test for treatments comparison vs. placebo.

to the higher concentration (ie, $30\,\mu g \cdot kg^{-1} \cdot day^{-1}$) of serelaxin.

There were more patients receiving serelaxin $30\,\mu g \cdot kg^{-1} \cdot day^{-1}$ who experienced a BP decrease that met protocol-defined BP-related study drug adjustment or discontinuation than those receiving $10\,\mu g \cdot kg^{-1} \cdot day^{-1}$ or placebo. The higher incidence observed with serelaxin $30\,\mu g \cdot kg^{-1} \cdot day^{-1}$ might be in part explained by the fact that there were more patients in this group with a lower SBP at baseline (ie, 137±10 mmHg vs. 145±20 mmHg and 148±18 mmHg for the serelaxin $10\,\mu g \cdot kg^{-1} \cdot day^{-1}$ and placebo groups, respectively). However, the majority of BP decreases that met protocol-defined BP-related study drug adjustment or discontinuation were asymptomatic and resolved spontaneously without requiring concomitant medications or non-drug therapy. The higher incidence of BP-

Table 4. Summary of Improvement in Physician-Assessed Signs and Symptoms of HF and Patient-Reported Dyspnea by Likert Scale During the Initial 48 h in a Phase II Study of Serelaxin in Japanese Patients With AHF

	Placebo (n=15)	Serelaxin 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ (n=16)	Serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ (n=15)
Dyspnea on exertion	M=14	M=11	M=11
Hour 6	3 (21.4)	1 (9.1)	1/10* (10.0)
Hour 12	2 (14.3)	2 (18.2)	2/10* (20.0)
Hour 24	2 (14.3)	4 (36.4)	1 (9.1)
Hour 48	2 (14.3)	5 (45.5)	4 (36.4)
Orthopnea	M=9	M=9	M=9
Hour 6	2 (22.2)	1 (11.1)	3/8* (37.5)
Hour 12	3 (33.3)	4 (44.4)	4/8* (50.0)
Hour 24	2 (22.2)	4 (44.4)	6 (66.7)
Hour 48	3 (33.3)	6 (66.7)	7 (77.8)
Edema	M=9	M=10	M=5
Hour 6	0	1 (10.0)	0*
Hour 12	1 (11.1)	2 (20.0)	0*
Hour 24	1 (11.1)	0	1 (20.0)
Hour 48	1 (11.1)	2 (20.0)	2 (40.0)
Rales	M=7	M=5	M=5
Hour 6	1 (14.3)	0	0*
Hour 12	1 (14.3)	1 (20.0)	1/4* (25.0)
Hour 24	2 (28.6)	2 (40.0)	2 (40.0)
Hour 48	3 (42.9)	3 (60.0)	4 (80.0)
Jugular venous pulse	M=6	M=5	M=8
Hour 6	1 (16.7)	0	1/7* (14.3)
Hour 12	1 (16.7)	1 (20.0)	1/7* (14.3)
Hour 24	0	1 (20.0)	2 (25.0)
Hour 48	2 (33.3)	1 (20.0)	5 (62.5)
Patient-reported dyspnea by Likert scale			
Hour 6	4 (26.7)	6 (37.5)	7/14* (50.0)
Hour 12	7 (46.7)	8 (50.0)	4/14* (28.6)
Hour 24	5 (33.3)	9 (56.3)	8 (53.3)
Hour 48	5 (33.3)	10 (62.5)	9 (60.0)

Data are n (%) who experienced ≥2 points improvement for each physician-assessed sign and symptom of HF; or markedly or moderately better for patient-reported dyspnea by Likert scale. *One patient in the serelaxin 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ group had missing assessment results at 6 and 12 h. M, total number of patients with moderate or severe dyspnea on exertion; requiring 2 pillows or >30 degree incline for orthopnea; 2+ or 3+ edema; ≥1/3 in rales or >10 cm for JVP at baseline, respectively. Abbreviations as in Table 1.

decrease events in patients treated with 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ serelaxin than in those with placebo is consistent with what was observed in RELAX-AHF, in which 29% patients on 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ serelaxin vs. 18% patients on placebo had protocol-defined BP-decrease events.[19] All protocol-defined BP-decrease events were mild and clinically manageable. The incidence of discontinuation in patients treated with 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ serelaxin (ie, 33.3%) seems a little higher than in RELAX-AHF (21.7%), but there were no AE of concern in these patients. The required treatment incidence was, however, 6.7% in the present study in contrast to 12% in the RELAX-AHF study. Taken together, the present protocol was designed to prevent serious AEs and the study results showed that serelaxin at 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ was safe.

This study specifically enrolled patients with normal-to-elevated BP, which represents 60–80% of the general AHF population. The number of patients in each treatment arm was small, and the baseline characteristics (in particular age [for the baseline comparability of treatment groups, P=0.061], SBP [P=0.176], and HF history [P=0.077]), were not well-balanced across the 3 treatment groups. This has to be taken into account when interpreting the clinical outcomes of this study. Patients were randomized to study treatment as early as possible after presentation, a mean (SD) time of 8.3 (4.6) h. This is notably earlier than in previous randomized trials of AHF with other drugs, such as OPTIME (mean: 15.3 h)[23] or VERITAS (median: 11 h),[24] but almost same as in other studies of serelaxin: Pre-RELAX-AHF 8.4 (5.4) h[18] and RELAX-AHF 7.9 (4.6) h.[19]

There were observationally greater reductions in NT-proBNP in the 2 serelaxin groups compared with the placebo, at both Day 2 (end of infusion) and Day 5 in even this small number of patients. The reductions were similar in the serelaxin 10 and 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ groups. The trend of the change was consistent with the findings of serelaxin treatment in the RELAX-AHF Phase III study, indicating potential benefits of serelaxin.

Interestingly, despite the small numbers in the treatment

groups, the exploratory efficacy endpoints still showed trends of beneficial effects of serelaxin. In the clinical composite endpoint, the proportion of patients with "treatment success" at Day 2 was numerically greater in the 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ group (26.7%) than that in the 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$ group (18.8%), both of which were more than the placebo group (6.7%). In-hospital WHF, which is associated with higher readmission and death rates and could be an indicator of short-term risk and treatment efficacy in AHF, is also of note.[25–27] The cumulative proportion of patients with WHF through Day 5 was lower in the serelaxin groups (10 $\mu g \cdot kg^{-1} \cdot day^{-1}$, 6.3%; 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$, 6.7%) compared with the placebo group (20.0%). In addition, greater improvements in patient-reported dyspnea by VAS and Likert scale were observed in the serelaxin groups. The findings of dyspnea improvement measured by VAS and Likert in this small trial were in line with those reported in the large Phase III RELAX-AHF trial. Of the 2 doses studied, 48-h intravenous serelaxin at 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ also provided greater numerical beneficial effects on improvement of HF signs and symptoms at Day 2 (dyspnea on exertion, orthopnea, edema, rales and JVP).

Although P values are provided for comparisons, the results must be interpreted in the context of the small sample size. The study was not sized to provide conclusive evidence for a benefit-risk assessment of serelaxin. Although the trends noted in the exploratory efficacy endpoints between serelaxin and placebo may suggest possible benefits of serelaxin in improving the signs and symptoms of congestion and reducing in-hospital WHF in Japanese patients, these require confirmation in an appropriately sized clinical trial, which will be provided by the ongoing Phase III RELAX-AHF-Asia study.

Conclusions

This was the first study to examine serelaxin therapy in Japanese AHF patients. The study demonstrated that serelaxin was well tolerated by Japanese AHF patients, with no AEs of concern, dose-dependent increases in serum concentration, and favorable numerically beneficial trends on exploratory efficacy. These findings support the continued investigation of serelaxin treatment in Japanese AHF patients in the ongoing Phase III study, RELAX-AHF-ASIA trial designed to confirm the clinical efficacy, safety and tolerability of serelaxin administered at a dose rate of 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ for up to 48 h in Asian AHF patients.

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Supplementary Files

Supplementary File 1

Table S1. Study sites and investigators

Table S2. Patient eligibility criteria

Exploratory efficacy endpoints

Please find supplementary file(s);
http://dx.doi.org/10.1253/circj.CJ-15-0227