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REVIEW

Therapeutic Effects of Serelaxin in Acute Heart Failure

– Necessity for Bilateral Research Translation –

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Over the past few decades, research on the peptide hormone, relaxin, has significantly improved our understanding of its biological actions under physiological and diseased conditions. This has facilitated the conducting of clinical trials to explore the use of serelaxin (human recombinant relaxin). Acute heart failure (AHF) is a very difficult to treat clinical entity, with limited success so far in developing new drugs to combat it. A recent phase-III RELAX-AHF trial using serelaxin therapy given during hospitalization revealed acute (ameliorated dyspnea) and chronic (improved 180-day survival) effects. Although these findings support a substantial improvement by serelaxin therapy over currently available therapies for AHF, they also raise key questions and stimulate new hypotheses. To facilitate the development of serelaxin as a new drug for heart disease, joint efforts of clinicians, research scientists and pharmacological industries are necessary to study these questions and hypotheses. In this review, after providing a brief summary of clinical findings and the pathophysiology of AHF, we present a working hypothesis of the mechanisms responsible for the observed efficacy of serelaxin in AHF patients. The existing clinical and preclinical data supporting our hypotheses are summarized and discussed. The development of serelaxin as a drug provides an excellent example of the bilateral nature of translational research. (*Circ J* 2014; **78:** 542–552)

Key Words: Acute heart failure; Biomarkers; RELAX-AHF; Relaxin; RXFP1

Acute heart failure (AHF or acutely decompensated HF) refers to new or worsening of signs and symptoms of HF that usually requires hospitalization. In fact, AHF is the leading cause of hospitalization for patients >65 years of age with cardiovascular diseases with extremely poor short- and long-term prognosis.[1–3] AHF represents a great social and economic burden. Owing to improved diagnosis and therapy in the recent decades, there has been a significant increase in the lifespan of patients with cardiovascular diseases. Furthermore, compared with the situation 20 years ago, patients suffering from acute myocardial infarction (MI), critical hypertension or severe arrhythmias, now have a much better chance of being discharged. Together with a rapid aging population, these factors have led to an increasing population of patients at risk of AHF. This has resulted in a situation where the prevalence and mortality of major heart diseases such as coronary artery disease, have declined in recent decades, while the incidence of AHF has progressively increased with its associated mortality remaining high.[2,4]

In the past 20 years, therapy for AHF has remained largely unchanged. During this period, a number of drugs, such as nesiritide, levosimendan, milrinone, rolofylline, tezosentan and ularitide, have been studied in phase-III trials but with unsuccessful outcomes.[5–8] None of these drugs given by intravenous infusion was effective in improving dyspnea or HF in the acute phase, or reducing re-hospitalization or mortality within 30- or 60-day follow-up. In contrast, the recent RELAX-AHF trial using serelaxin (human recombinant relaxin) has shown beneficial effects in patients with AHF during both the acute and chronic phases.[9,10] In this review, we will summarize key observations from the recent clinical studies on serelaxin therapy of AHF. We will then discuss the pathophysiology of AHF, and present a mechanistic working hypothesis to explain why serelaxin therapy is effective in patients with AHF. Experimental findings that support this working hypothesis are summarized and discussed.

Key Findings From the Clinical Trials on Serelaxin

Studies in healthy or hypertensive rodents and humans have shown that serelaxin induces systemic vasodilation with reduced systemic vascular resistance (SVR), accompanied by an increase in cardiac index (CI), global arterial compliance and stroke volume;[11–13] vasodilation of both afferent and efferent renal arterioles to increase renal plasma flow and glomerular filtration;[9,14–16] and decreases arterial stiffness through vascular re-

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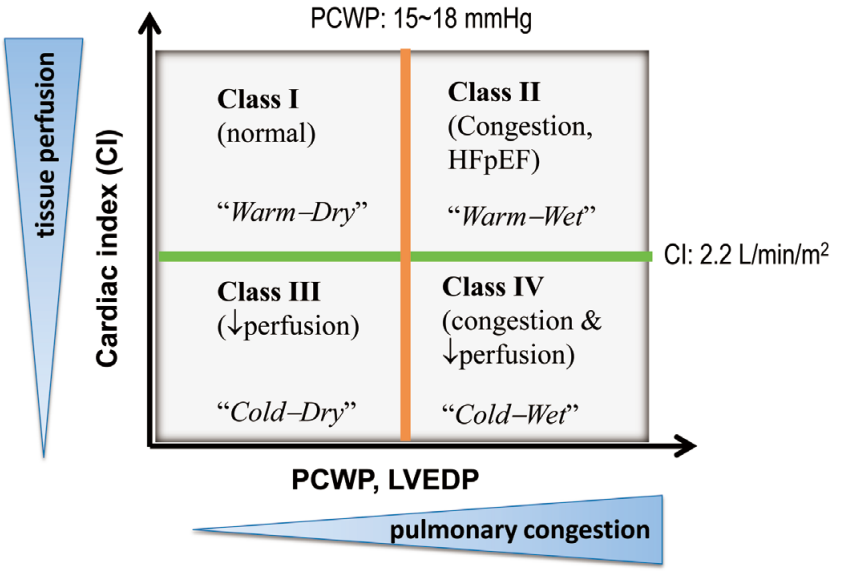

Figure 1. Classification of acute heart failure. Level of cardiac index determines tissue perfusion and body temperature (warm or cold) while the severity of venous congestion is in proportion to the degree of edema in pulmonary (measured by pulmonary capillary wedge pressure, PCWP, or left ventricular end-diastolic pressure, LVEDP) or whole body (dry or wet). HFpEF, heart failure with preserved ejection fraction.

modeling and passive mechanical properties.[12,17,18]

The ability of serelaxin to benefit patients with AHF by inducing similar changes was recently evaluated in phase-II and phase-III clinical trials.[9,11,19] A safety and tolerability phase-I trial assessed the effects of sequential dose levels of serelaxin in 16 patients with chronic HF,[7,11] and demonstrated that serelaxin (10–960$\mu g \cdot kg^{-1} \cdot day^{-1}$ IV for 24 h) was well-tolerated, produced hemodynamic effects consistent with vasodilation and improved markers of renal function without adverse consequences. A follow-up phase-IIb trial evaluated hemodynamic and symptom-relieving effects of serelaxin in 234 AHF patients with systolic blood pressure (SBP) >125 mmHg, where placebo (n=62) or serelaxin was administered for 48 h at 4 dosages (from 10 to 250$\mu g \cdot kg^{-1} \cdot day^{-1}$ IV for 48 h).[11,19] This trial demonstrated that serelaxin was associated with acceptable safety, favorably improved dyspnea, and tended to reduce the number of cardiovascular-related hospital re-admissions and deaths due to heart or renal failure at day-60 post-treatment. A dose of 30$\mu g \cdot kg^{-1} \cdot day^{-1}$ was identified as optimal.

The success of these trials led to the phase-III multicenter, randomized and placebo-controlled (RELAX-AHF) trial in 1,161 AHF patients; 581 patients treated with serelaxin and 580 patients receiving placebo.[9] Recruitment criteria included SBP >125 mmHg, presence of dyspnea and renal failure. This trial excluded patients with suspected acute coronary syndrome based on troponin ≥3-fold the level indicative of MI.[9] Randomization was completed within 16 h (average <8 h) followed by commencement of serelaxin treatment (30$\mu g \cdot kg^{-1} \cdot day^{-1}$ IV for 48 h). Serelaxin again improved dyspnea as assessed by the visual analog scale (P=0.007) and improved signs/symptoms of pulmonary congestion.[9] Although the Likert Scale, another primary endpoint for dyspnea relief, did not differ between the 2 groups by 6- to 24 h, further data analysis indicated significant improvement in day-5 Likert Scale (P<0.002). Serelaxin also reduced HF worsening through to day 5 (6.5% vs. 12%, P<0.001) and the dose of diuretic use (P=0.0057). Of further interest, serelaxin administered over 48 h during hospitalization was associated with a 37% reduction in the 180-day cardiovascular or total mortality (7.3% vs. 11.3%, P=0.019).[9] A trend for a better 180-day survival was also seen in the pre-RELAX-AHF trial (8.7% vs. 15.8%).[19] Notably, however, the frequency of death events was low in the RELAX-AHF trial and this finding needs to be interpreted with caution. Nevertheless, the association of short-term therapy and long-term benefits is intriguing and hypothesis-generating.

Another important finding from the RELAX-AHF trial was the elevation of circulating levels of biomarkers associated with cardiac (troponin T), renal (creatinine, cystatin-C) and hepatic (aspartate transaminase, alanine transaminase) damage, in addition to increased N-terminal pro-brain natriuretic peptide (BNP).[9] This finding is in keeping with other reports showing that biomarkers that reflect organ injury or dysfunction bear prognostic value in patients with AHF or chronic HF.[20,21] Further increase of each of the biomarkers studied at day 2 over admission reference level was associated with poor 180-day survival.[10] Importantly, serelaxin administration significantly reduced further elevation of these biomarkers.[10]

A separate study addressed the hemodynamic effects of serelaxin in patients with AHF (NYHA III-IV class) and SBP >115 mmHg.[22] Serelaxin was administered at 30$\mu g \cdot kg^{-1} \cdot day^{-1}$ for 20 h and hemodynamic parameters were monitored for 24 h and blood pressures for 44 h. Compared with the placebo-treated patients (n=37) receiving standard therapy, treatment with serelaxin exerted a rapid (<30 min) hemodynamic improvement measured by a reduction in pulmonary capillary wedge-pressure (PCWP), pulmonary artery pressure (PAP), SVR and pulmonary vascular resistance (PVR) without significant change in CI. Meanwhile, serelaxin administration improved renal function and reduced both SBP and diastolic blood pressure by 7–10 mmHg. Some hemodynamic effects persisted from 4 h up to 20 h after serelaxin infusion was ceased.[22]

Pathophysiology and Classifications of AHF

The clinical presentation of AHF (excluding those caused by acute MI) is new onset or rapid worsening (within hours or days) of signs and symptoms of HF, mainly dyspnea, that usually require urgent treatment and hospitalization. The clinical outcomes during the acute and chronic phases after onset of AHF remain very poor. The in-hospital and 30-day mortality rates

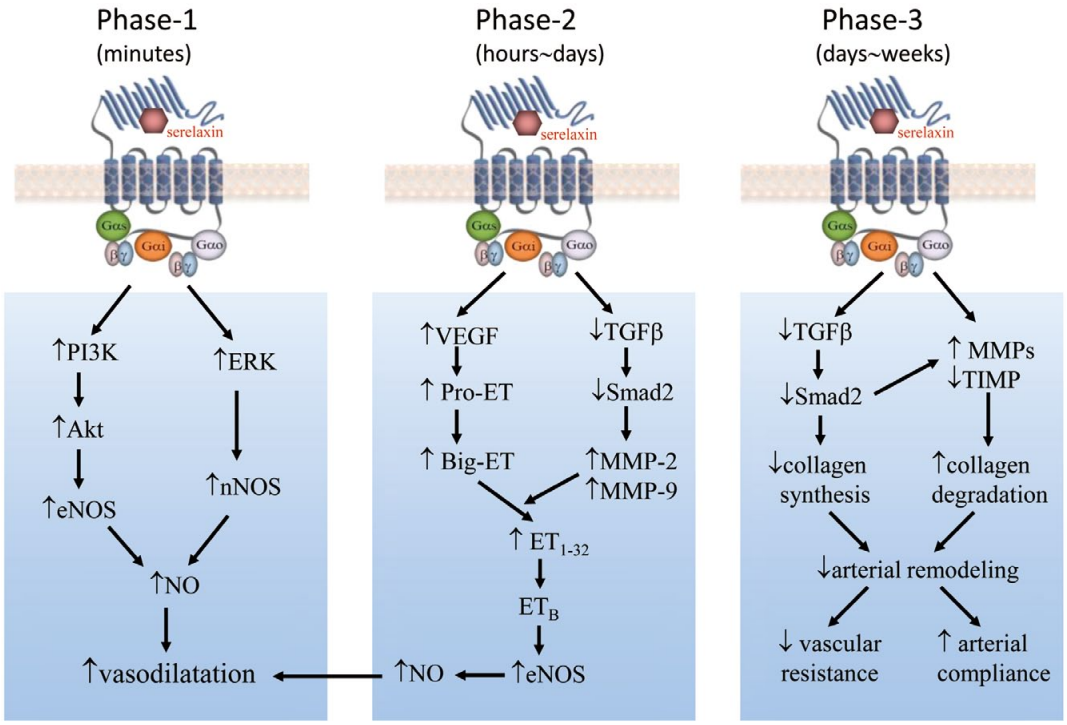

Figure 2. Serelaxin treatment induces time-dependent vasodilatation and reduces arterial vascular resistance or large artery stiffness. Serelaxin indices rapid vasodilatation via non-genomic mechanism by phosphorylation of nitric oxide synthases (NOS) and generation of nitric oxide (NO).[13,72] Treatment with serelaxin for hours or a few days induces persistent vasodilatory effect mediated by genomic mechanism involving upregulation of vascular endothelial growth factor (VEGF), pro-endothelin (pro-ET) and matrix metalloproteinases (MMP), as well as increased synthesis of ET_{1-32} as a ligand for ET_B that mediates vasodilatation. This is associated with simultaneously reduced conversion of endothelin-1, ET_{1-21}, a potent agonist for ET_A mediating vasoconstriction.[13] Serelaxin therapy for days or weeks also reduces arterial stiffness that involves remodeling of resistant and conduit arteries.[29–31]

are approximately 7% and 10%, respectively.[1–3] After discharge, 180-day and 1-year mortality is 15% and 35–40%, respectively.[1,3] It is estimated that approximately 25% patients die with each episode of AHF and that readmission rate within 12 months is approximately 50%. Thus, AHF represents an enormous socioeconomic burden and adversely affects the public health of the elderly.

Several clinical features characterize AHF patients. Firstly, AHF includes patients with diverse clinical presentations from sudden onset of HF symptoms with preserved ejection fraction (EF) and normal or higher SBP to those with severely suppressed systolic function and CI. Due to more frequent onset of left HF, patients usually present with dyspnea as the major clinical presentation. Secondly, AHF patients are usually old with an average age of >70 years. Thirdly, comorbidities such as hypertension, ischemic heart disease, metabolic syndrome, hyperlipidemia and atrial fibrillation (AF) are common in patients with AHF. Hypertension occurs in 60–70% of AHF patients, while other comorbidities present in 50% of patients. In addition, 30–40% of cases have renal failure and mitral regurgitation.[1,2] Fourthly, patients usually have previous multiple episodes of HF, with an average of 1–3 episodes in the preceding year. Finally, we need to remember that AHF can be triggered by factors with a cardiovascular or noncardiovascular origin. Cardiovascular factors that precipitate AHF usually involve acute coronary syndrome, arrhythmias or uncontrolled hypertension. Noncardiovascular facilitating factors consist of a failure to maintain a fluid or salt restriction, dietary or medication noncompliance, acute infection, sepsis or anemia.

The major pathophysiology of AHF involves 2 aspects: organ congestion and edema with resultant symptoms particularly dyspnea, and a reduced CO or EF leading to symptoms because of organ underperfusion. The clinical pathophysiology of AHF is highly heterogeneous and hence there has been a number of different ways to classify AHF, based on different aspects of AHF including location (left or right heart), history of HF (acute onset, rapid worsening of chronically compensatory HF or advanced HF with progressively worsening), Killip class, hemodynamics (CI and PCWP), and clinical severity. AHF has traditionally been classified according to the extent of pulmonary congestion (or PCWP) and organ perfusion (or CI) (**Figure 1**). Another classification currently in common use is based on whether EF is reduced (HFrEF) or preserved (HFpEF). This classification emphasizes the fact that >50% HF patients maintain cardiac systolic function (ie, diastolic HF). It is worth noting that the majority of patients with AHF suffer from hypertension (60–70%) with BP higher or within the normal range at the time of HF decompensation. In a clinical registry of 187,565 cases of AHF, at the time of admission, approximately 50% of patients had SBP >140 mmHg and only 2% of patients with SBP <90 mmHg.[2]

Although such varied ways of classifying AHF signify the diversity of its clinicopathophysiology, a number of the clinical trials in AHF over the last few decades have used unselected

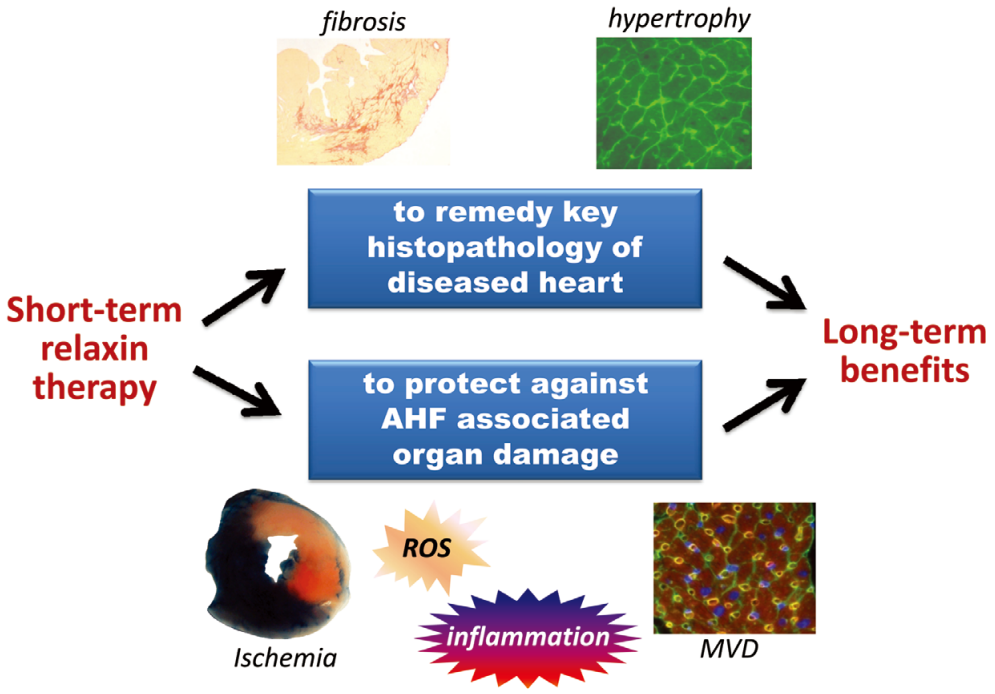

Figure 3. Working hypothesis for mechanisms by which a short-term relaxin therapy yield long-term benefits in patients with acute heart failure (AHF). The working hypothesis is inspired by the finding from the RELAX-AHF trial in patients with AHF that relaxin administration for 48 h during hospitalization was associated with 37% reduction in the 180-day mortality relative to the placebo control group. Based on currently existing preclinical findings, relaxin therapy might reverse established histopathology of failing heart and/or protect against organ damage because of a variety of factors, leading to persistency of its therapeutic benefits. ROS, reactive oxygen species; MVD, microvascular dysfunction.

AHF patients. Not surprisingly, this in itself is regarded as a major reason for the failure to demonstrate therapeutic efficacy in these studies.[5,6] In this regard, the RELAX-AHF trial differed in that the patients were selected based on SBP >125 mmHg,[9] yielding a cohort of patients with the prevalence of hypertension higher than that seen in the general patient population (86% vs. 60–70%).

Vascular Actions of Serelaxin

Vasoconstriction following neurohormonal activation contributes to organ congestion and poor perfusion as well as resultant symptoms in patients with AHF. The adaptive changes of hemodynamics and renovascular dilatation during mammalian pregnancy can be well mimicked by serelaxin infusion.[14,23] The vasodilatory mechanisms of serelaxin are primarily mediated via the cognate receptor for H2 relaxin, relaxin-like peptide receptor 1 (RXFP1)[24,25] and are time-dependent (Figure 2). Rapid vasodilation was reported to involve GTP-binding proteins ($G_{\alpha i}$ or $G_{\alpha o}$) coupling to phosphatidylinositol-3 kinase (PI3K)/Akt (protein kinase B)-dependent phosphorylation and activation of nitric oxide synthases (NOS).[23,24,26,27] The more persistent vasodilatory actions of serelaxin are mediated by vascular endothelial growth factor (VEGF), placental growth factor, and increases in arterial gelatinase activity (Figure 2).[23,24,27,28] MMP-9 and MMP-2 are demonstrated to play central roles in the short-term (hours) vs. longer-term (days) vasodilation following serelaxin administration in hydrolyzing big endothelin (ET) to form $ET_{1–32}$, and activating the endothelin ET_B/nitric oxide (NO)-induced vasodilatory pathway.[23] Serelaxin also enhances bradykinin-mediated relaxation.[29] The density of RXFP1 varies greatly in different vascular beds as well as arteries and veins,[29] which has led to speculation that unlike nitrides such as NO donors, vasodilatation mediated by serelaxin varies in different organs or vessels. The clinical significance of the features of serelaxin/RXFP1-mediated vasodilation has not been investigated. Reduced resistance of the arterial system at a later phase following serelaxin therapy for 1–2-weeks may involve remodeling of resistant and conduit arteries (Figure 2).[13,30,31]

Serelaxin also has several additional actions, and hence is advantageous to currently used vasodilators, such as nitrides (see below). These actions may contribute to the efficacy of serelaxin in AHF patients in addition to inducing vasodilation. These combined findings point to serelaxin being more than just a simple vasodilator.

Hypothesis for the Mechanisms Responsible for Serelaxin's Long-Term Efficacy in RELAX-AHF Trial

Two findings from the RELAX-AHF trial are intriguing. Foremost, short-term serelaxin therapy is associated with improved 180-day survival. Better long-term outcomes by a short-term therapy in AHF patients has not been achieved in previous drug trials and therefore the focus has been shifted to short-term benefits such as symptom relief. In fact, long-term endpoints have consistently been neglected in AHF trials.[5,32] Furthermore, patients with AHF exhibit multiple organ damage at the early phase of AHF that correlate with the long-term survival. For

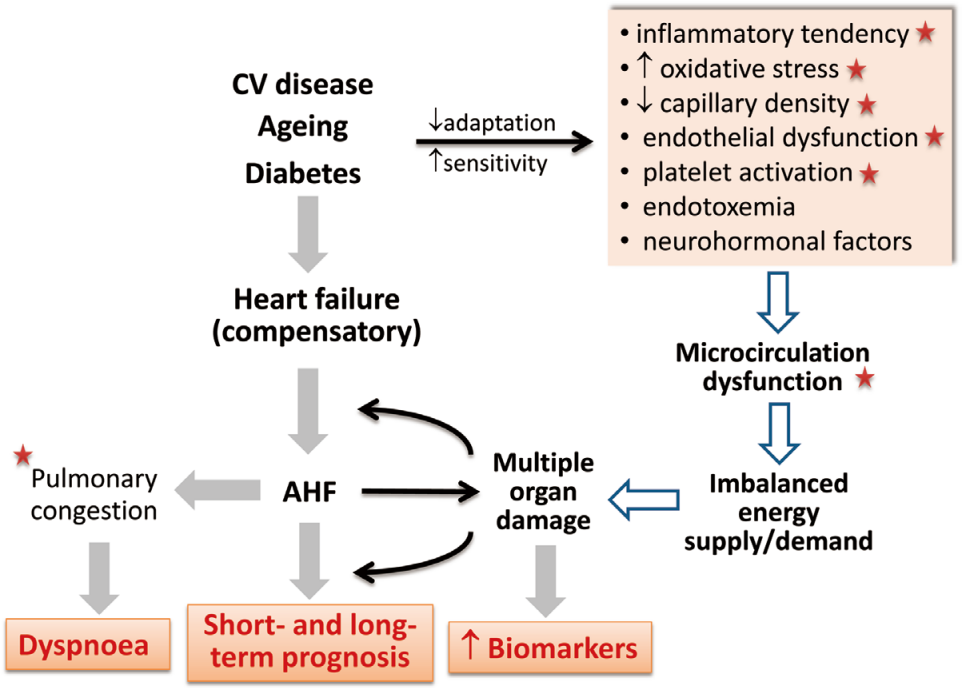

Figure 4. Proposed central role of microvascular dysfunction in the pathophysiology of acute heart failure (AHF), particularly in mediating multi-organ damage. Presence of cardiovascular diseases will eventually result in HF. In settings of comorbidities (particularly diabetes) and aging, factors that are associated with increased vulnerability or reduction in adaptation to pathological stressors, microvascular dysfunction may occur owing to a number of possible mechanisms, leading to diminished energy supply to whole body and hence onset of multi-organ damage with elevated biomarkers. Organ damage acts as a facilitating event for acute decompensation of HF and/or factor that affects long-term prognosis. Stars indicate possible targets of serelaxin therapy.

selected biomarkers of organ damage including the heart, kidney and liver, it is not the initial level of biomarkers, but rather the increment within the first 2 days after hospitalization, that is, associated with 180-day prognosis.[10] This finding implies that the onset of multi-organ damage in AHF patients negatively affects post-discharge outcomes.

Based on our current understanding of the pathophysiology of AHF, and the known cardiovascular actions of serelaxin from preclinical studies, we propose a working hypothesis to explain serelaxin's potential long-term therapeutic effects (**Figure 3**). In the setting of AHF, short-term serelaxin therapy is able to (a) effectively remedy certain fundamental pathologies in heart disease so that functional benefits are maintained for relatively long-term with ongoing standard medications, and (b) protect against multi-organ damage, including the heart, that trigger acute decompensation of HF or are consequences of AHF. Both would be expected to affect long-term outcomes. Thus, therapies targeting AHF pathology and reducing organ damage would be expected to yield long-term benefits.

AHF represents multi-organ pathology and dysfunction and there is experimental evidence that serelaxin reverses existing pathology (fibrosis, hypertrophy) and/or provides protection against further injury (**Figure 4**). Notably, serelaxin is a pleiotropic drug with experimentally proven actions of antioxidative stress, regulation of inflammation, anti-ischemic injury, anti-apoptotic, platelet inhibition, angiogenesis, protection of endothelial cells, improvement of endothelial cell function (ie, NO synthesis) and antifibrotic.[33] Thus, in the setting of AHF, its complex pathophysiology might allow for drugs such as serelaxin to exert simultaneous multiple actions with its efficacy additively beneficial. This pleiotropic pharmacological property of serelaxin is unique compared with other commonly used drugs. One may argue that other drugs, such as angiotensin II, antagonizing drugs also have multiple beneficial actions in patients with cardiovascular diseases, particularly in suppressing the extent of cardiac remodeling. However, there is insufficient evidence for this class of drugs to exert such actions after a brief period of use. Relatively early initiation of serelaxin treatment because of the prompt randomization process in the RELAX-AHF trial is also potentially important considering the presence of multi-organ damage within the first few days after hospitalization.

Experimental Evidence for Serelaxin Therapy to Remedy Cardiac Histopathology

Cardiac remodeling is the fundamental pathophysiology of heart disease and failure. Several key histopathological components are common in the remodeled heart, regardless of etiology. These include hypertrophy, inflammation, interstitial fibrosis, and continuous drop-out of cardiomyocytes. These components orchestrate functional decline and development of complications, thereby influencing short and long-term prognosis of AHF. In theory, remedy of some of these key components would be expected to have a beneficial influence on the post-discharge outcome of patients with AHF.

In addition to preventing de novo onset of cardiac fibrosis (**Table**),[33] there is strong experimental evidence for serelaxin's

Table. Summary of in Vivo Experimental (Se)relaxin Therapy Showing Multi-Organ Protection

Organ/ Etiology	Experimental model/species	Source of relaxin	Major effect	Mechanism involved
Heart				
Aging	Relaxin KO/mouse	Serelaxin	↓LV collagen content and related stiffness	↓fibroblast differentiation[37]
Hypertension	AngII-injection/rat	Serelaxin	Acute ↑cardiac output, arterial compliance, ↓vascular resistance	↑systemic hemodynamics and arterial vasodilation[73]
	Hypertension/rat (SHR)	Serelaxin	Chronic ↑cardiac output, arterial compliance, ↓vascular resistance	↑heart rate and stroke volume[73]
		Serelaxin	↓LV fibrosis	↓fibroblast proliferation and differentiation, ↑MMP activity[34]
		Serelaxin	↓Hypertrophy	↓ERK1/2[74]
		Serelaxin	↓large artery remodeling, ↑arterial compliance	↓collagen content, ↑elastin/collagen ratio[31]
		Serelaxin	↓atrial fibrillation	↓atrial hypertrophy and fibrosis, ↑Na^+ current density[35]
β-AR overstimulation	β_2-AR overexpression/ mouse	Serelaxin, mouse	↓LV fibrosis	↓fibroblast differentiation, collagen content, ↑MMP activity[37,38]
	Isoproterenol/ mouse	Serelaxin	↓LV collagen content	↓TGF-β1-mediated fibroblast differentiation, ↑MMP activity[75]
Anaphylaxis	Antigen (ovalbumin)-induced inflammation/guineapig	Porcine	↓antigen-induced chronotropic and inotropic effects	↓histamine release and Ca^{2+} accumulation, ↑nitrites, NO synthase and cGMP levels[76]
Toxicity	Chronic AngII infusion/mouse	Mouse	↓LV fibrosis	↓α-SMA, collagen[54]
Ischemic injury	Ischemia-reperfusion/guineapig	Porcine	↓myocardial damage	↓histamine and mast cell release, MDA production, Ca^{2+} overload, ↑NO generation, coronary flow and heart contractility[77]
	Ischemia-reperfusion/rat	Porcine	↓myocardial damage	↓ventricular arrhythmias, inflammatory cell infiltration, Ca^{2+} overload, mortality[49]
	Ischemia-reperfusion/pig	Serelaxin	↓myocardial damage and contractile dysfunction	↓cardiomyocyte apoptosis, oxidative stress [78] ↓plasma/cardiac histamine and mast cell release[79]
		Serelaxin	↓ventricular arrhythmias	↑myoblast-mediated MMP activity and microvessel density (angiogenesis), ↓fibrosis[80]
	Myocardial infarction/pig	H2 relaxin	↑engrafted myoblast viability, tissue repair, cardiac contractility	↑ myoblast-mediated microvessel density and cardiac function, ↓sclerosis and cardiomyocyte apoptosis[81,82]
	Myocardial infarction/rat	H2 relaxin	↑engrafted myoblast viability, cardiac function and contractility	TGF-β1-induced fibroblast differentiation and cardiomyocyte apoptosis, ↑MMP activity and angiogenesis[51]
	Myocardial infarction/mouse	Serelaxin	↓LV fibrosis, infarct size	
Metabolic disease	mRen2 diabetic/rat	Serelaxin	↓LV collagen content and related stiffness	↓fibroblast differentiation and TIMP-1 activity, ↑MMP activity[36]
	High-fat diet-fed/ mouse	Serelaxin	↓insulin resistance	↓collagen content, ↑endothelial-dependent vascular reactivity[83]
Kidney				
Aging	Relaxin KO/mouse	Serelaxin, Mouse	↓collagen content, sclerosis and related dysfunction	↓cortical thickness, interstitial and glomerular matrix deposition[84,85]
	Munich Wistar/rat	Serelaxin	↑renal function	↑eGFR, renal plasma flow, gelatinase activity, ↓collagen content[86]
Toxicity	Renal papillary necrosis/rat	Serelaxin	↓renal fibrosis, ↑renal function	↓macrophage infiltration, TGF-β1 levels[87]
Acute inflammation	Anti-GBM nephritis/ rat	Serelaxin	↓fibronectin, ↑renal function	↑fibronectin degradation, ↓sclerosis, fibrosis and related dysfunction[88]
Renal insufficiency	Sub-total renal ablation/rat	Serelaxin	↓renal injury, ↑renal function	↓sclerosis[89]
	Sub-total infarction/ rat	Serelaxin	↓renal injury, ↑renal function	↓sclerosis, SBP[89]
Hypertension	Hypertension/rat (SHR)	Serelaxin	↓renal fibrosis	↓collagen content[34]
	AngII-injection/rat	Serelaxin	↓hypertension and related injury	↓blood pressure, oxidative stress, sclerosis and excretion of albumin or NO metabolite[90]
	Salt-sensitive Dahl rat	Serelaxin	↓renal injury, fibrosis	↓SBP, TGF-β signaling, glomerular, arterial and tubulointerstitial pathology, ↑NO synthase[91]
Hydronephrosis	Unilateral ureteric obstruction/mouse	Serelaxin	↓renal injury, fibrosis	↓TGF-β, Smad2 phosphorylation, myofibroblast differentiation, collagen deposition, TIMP activity[68]

(Table continued the next page.)

Organ/ Etiology	Experimental model/species	Source of relaxin	Major effect	Mechanism involved
Acute ischemic injury	Renal artery clamping/rat	Serelaxin	↑ renal function, ↓ structural damage	↓ inflammation and apoptosis[92] ↓ lipid peroxidation, oxidative stress, inflammatory cell infiltration, ↑ NO synthase, Akt and ERK1/2[93]
Metabolic disease	mRen2 diabetic/rat	Serelaxin	No change in sclerosis or function	No change in collagen content, glomerulosclerosis or proteinuria[94]
Lung				
Aging	Relaxin KO/mouse	Mouse, serelaxin	↓ lung fibrosis	↓ collagen content, bronchial epithelial thickness, alveolar congestion, lung weight[85,95]
Allergic airways disease	Antigen (ovalbumin)-induced inflammation/mouse	Serelaxin	↓ airway remodeling and related dysfunction	↓ epithelial thickness, subepithelial and total collagen, airway hyperresponsiveness, ↑ MMP activity[96,97]
Interstitial lung disease	Bleomycin-treated/ mouse	Serelaxin	↓ fibrosis	↓ TGF-β, fibroblast differentiation, collagen content, ↑ MMP activity[98] ↓ myofibroblast contractility, MLC_{20} phosphorylation and Rho/ROCK signaling, ↑ NO/cGMP/PKG-1[99]
Hypertension	Hypoxic/rat	Serelaxin	↓ ventricular pressure and fibrosis	↓ TGF-β-induced collagen and fibronectin content[100]
Ischemia-reperfusion	Ischemia-reperfusion in isolated lung/rat	Serelaxin	↑ protection	↓ ET-1, neutrophil elastase, cell damage and oxidative stress[101] ↑ iNOS, ERK1/2, PI3K, FOX phosphorylation[102]
Liver				
Toxicitiy	Carbon tetrachloride-treated/rat	Serelaxin	↓ hepatic fibrosis	↓ collagen content, TIMP activity[103]
	Carbon tetrachloride-treated/mouse	Serelaxin	↓ hepatic fibrosis	↓ TGF-β, nuclear Smad2 levels, myofibroblast differentiation, collagen content, TIMP activity, ↑ MMP activity[104]
Ischemia-reperfusion	Isolated reperfused liver/rat	Serelaxin	↓ hypoxia, ↑ organ preservation	↓ cell damage and oxidative stress[105,106]
Hypertension	Portal hypertensive rat	Serelaxin	↓ portal pressure, ↑ portal blood flow	↑ NO signaling, ↓ myofibroblast contractility and cirrhosis[107]
Metabolic disease	High-fat diet-fed/ mouse	Serelaxin	↓ insulin resistance	↓ collagen content[83]
Intestine				
Ischemia-reperfusion	Splanchnic artery occlusion/rat	Porcine	↓ mortality	↓ inflammation, apoptosis, oxidative stress[108]
Dystrophy	Muscular dystrophy/mouse	Porcine	↓ Ileal spontaneous contractions	↑ iNOS, NO biosynthesis[109]
Pancreas				
Acute Pancreatitis	Biliopancreatic duct exclusion/rat	Human	↓ acute inflammation, ischemia, necrosis	↓ neutrophils, myeloperoxidase, ↑ NO signaling, vasodilation[110]
Skeletal muscle				
Aging	Tibialis (leg) muscle injury/ mouse	Serelaxin	↑ muscle healing and strength	↑ Myogenic differentiation, migration, MMP activity, angiogenesis, revascularization, ↓ inflammation[67]
Traumatic injury	Tibialis (leg) muscle injury/ mouse	Serelaxin	↑ muscle healing and strength	↓ TGF-β, fibroblast proliferation and differentiation, fibrosis[30,111]
	Cremaster muscle/ hamster	Serelaxin	↑ vasodilation	↑ capillary/transient NO and K^+ channel-dependent vasodilation[112]
Metabolic disease	High-fat diet-fed/ mouse	Serelaxin	↓ insulin resistance	↑ skeletal muscle capillarity[83]

Ang II, angiotensin II; bFGF, basic fibroblast growth factor; eGFR, estimated glomerular filtration rate; ERK1/2, extracellular signal-regulated kinase 1/2; ET-1, endothelin-1; FOX, Forkhead transcription factor; KO, knockout; MDA, malonyldialdehyde; MMP, matrix metalloproteinase; NO, nitric oxide; SHR, spontaneously hypertensive rat; Smad2, Mothers against decapentaplegic homolog-2; TGF-β, transforming growth factor-β; TIMP, tissue inhibitor of metalloproteinase; TNFα, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

antifibrotic actions in several animal models of cardiovascular diseases, including spontaneously hypertensive rats (SHR),[31,34,35] hypertensive rats with streptozotosin-induced diabetes,[36] and transgenic cardiomyopathy because of cardiac overexpression of β_2-adrenergic receptor (β_2AR-TG).[37,38] In these models, serelaxin treatment for 2 weeks reduced cardiac collagen content (**Table**). Using the β_2AR-TG model of established cardiac fibrosis, we recently observed a progressive reduction in cardiac collagen content within 7 days of serelaxin treatment with such efficacy becoming statistically significant at day 3 (unpubl. data). Although the half-life of circulating relaxin is relatively short (1–4 h, depending on species), sustained activation of RXFP1 was observed after binding with serelaxin partly because of the fact that RXFP1 does not undergo internalization.[39,40] Thus, following the regime of a 48-h period of infusion with serelaxin, as applied in the RELAX-AHF trial,[9] it is likely that the effects of serelaxin persists for another 24 h, thereby allowing for such a short-term therapy to partially re-

verse established organ fibrosis with efficacy maintained through the ongoing use of standard medications.

The functional benefits of reversing myocardial fibrosis potentially involve at least 2 aspects. Firstly, improvement of ventricular diastolic filling because of a reduction in chamber stiffness. This is particularly desirable considering that the majority of patients trialed in RELAX-AHF had HFpEF.[9] Secondly, anti-arrhythmic efficacy. Myocardial fibrosis is a pivotal arrhythmogenic substrate, and is well known to interfere with electrical conductance and formation of re-entry.[41] Serelaxin inhibits activation and differentiation of fibroblasts to myofibroblasts, as measured by de novo expression of α-smooth muscle actin (α-SMA).[34,37,42] Recent in vitro studies have revealed an alternative mechanism by which enhanced fibrogenesis contributes to arrhythmogenesis.[43,44] Myofibroblasts form direct electrical coupling with adjacent cardiomyocytes by increased expression of tight junction proteins (connexins) and ion channels or formation of microtubes.[43,45–47] However, both types of cells differ widely in their electrophysiological features and such heterogeneous coupling results in membrane currents at any phase of membrane potentials,[44] resulting in slow conduction and ectopic activity because of delayed after-depolarization.[44,48] Although studies are required to show operation in vivo of this mechanism, it is anticipated that serelaxin as a potent antifibrotic agent may also prevent such a pro-arrhythmic mechanism in addition to reversal of interstitial fibrosis. In this regard, a very recent report showed that in 12-month-old SHR, serelaxin therapy (2 weeks) reduced atrial content of collagen, expression of α-SMA and lowered inducibility of AF ex vivo.[35] Further studies are warranted to test the effects of serelaxin therapy in models with cardiac fibrosis and spontaneous arrhythmias.

Experimental Findings on Organ Protection by Relaxin Therapy

Numerous in vitro and in vivo studies have documented that a brief administration of serelaxin, or upregulation of relaxin expression, is cardioprotective and reduces subsequent fibrosis in experimental models of acute injury because of ischemia or MI,[49–51] oxidative stress[52] and drug toxicity (**Table**).[33,53,54] Damage and dysfunction of other organs, in particular the kidney, is known to accompany cardiac injury in patients with AHF.[4] The RELAX-AHF trial was the first to reveal the association between the magnitude of biomarker changes and 180-day mortality.[10] This finding clearly suggests that damage to these organs, particularly the heart and kidney, during the onset of AHF affects the long-term outcomes of patients. However, there is no effective therapy that has been shown to limit organ damage in patients with AHF. Also, the underlying pathophysiology of organ damage and the likely scale of such damage remain unclear.

There is evidence for widespread organ damage occurring at the whole-body level in AHF patients. For instance, malfunction of the intestine(s) in the settings of heart disease and circulatory translocation of gut bacterial endotoxins and circulating endotoxemia have been reported in patients with HF or renal failure.[55,56] Intestinal ischemia may play an important role in the pathophysiology of AHF in which increased bowel permeability because of abnormality of the microcirculation promotes systemic inflammatory processes and impairs cardiac function, which further diminish intestinal microcirculation leading to a vicious cycle. It is also possible that those patients whose AHF are believed to be triggered by "abdominal discomfort" or sepsis are also likely caused in part by intestinal underperfusion and venous congestion leading to systemic inflammation.[57] Impaired perfusion and oxygen supply to skeletal muscle have also long been known to occur in patients with chronic or acute HF because of elevated regional vascular resistance and endothelial dysfunction,[58] which contributes in part to reduced exercise capacity. Indeed, these findings are in keeping with the view that in the setting of AHF, increased neurohormonal mediators, including angiotensin II, norepinephrine, ET-1 and vasopressin contribute to systemic vasoconstriction that mediates redistribution of blood flow from "non-vital" to vital organs resulting in multiple organ damage and/or dysfunction.

What is the common mechanism in the setting of AHF responsible for the multi-organ damage? Clinical studies have revealed by imaging techniques the presence of coronary microvascular dysfunction that is associated with poor long-term prognosis.[59] Several groups have reported abnormalities in microcirculation of the sublingual tissues in AHF patients. Studies using a microscan imaging system observed a significantly reduced density of microvessels (10–50μm), but not larger vessels (51–100μm), with the degree of reduction being associated with prognosis.[60] By determining peripheral tissue oxygen extraction rate (OER, increase indicates poorer tissue microvascular perfusion), Hogan et al reported increased OER in AHF vs. stable HF patients.[51,61] Repeated measurement of these parameters has revealed that the standard therapy, including nitrides, is able to improve organ perfusion, dyspnea symptoms and hemodynamics, as well as reduce elevated biomarkers including norepinephrine, BNP and ET-1.[61–63] These studies strongly suggest that abnormalities in organ microcirculation exist in AHF and likely constitute a fundamental mechanism for observed multi-organ damage.

Preclinical studies conducted in the past decade have documented serelaxin's protection against injury to multiple organs (**Table**). Serelaxin has also been shown to protect vascular endothelial cells against inflammatory changes (such as TNFα) or oxidative stress[33,64] and promoting activity of NOS.[23] Here we propose that in AHF patients, underperfusion occurs at the whole-body level with subsequent organ damage, which can be detected by changes in biomarkers of different organs (**Figure 4**). Additional disease-promoting risk factors such as aging and diabetes are associated with reduced microvessel density and microvasculopathy, and increase susceptibility to microvascular injury and dysfunction.[65,66] Serelaxin may also facilitate subsequent repair (healing) processes post-injury through angiogenesis,[24] mobilization of bone marrow-derived stem cells or promotion of stem cell homing,[24,67] as well as inhibition of aberrant fibrogenesis (**Table**).[51,68]

Limitations of Experimental Evidence

Although serelaxin has now been administered to several thousand patients in clinical trials, most of our mechanistic insight comes from its use in animal models of disease. As always, extrapolation to human disease can be difficult as animal models only approximate the human condition. However in this regard, a large number of animal models have been studied, with great consistency in findings (**Table**). Nevertheless, important differences in relaxin biology exist. Humans have 3 relaxin genes but rodents have only 2. Sheep do not have a functional relaxin hormone,[113] but without deleterious consequences. Interestingly, although the protective efficacy of serelaxin therapy has been demonstrated experimentally in multiple organs (**Table**), there is a lack of head-to-head comparison with the currently best available therapies. Importantly, there has been no animal model of AHF although some models mimic part of the human AHF syndrome.[34,35]

Further Research to Develop Serelaxin as a New Cardiac Drug

The RELAX-AHF trial has clearly indicated the potential significance of conducting drug trials in selected subgroup of AHF patients, in this case with SBP >125 mmHg. This has also been borne out by findings in a few previous studies. The SHIFT trial showed therapeutic benefits of ivabradine in chronic HF patients by recruiting patients with heart rate (HR) >70 beats/min, with results showing even better efficacy if HR >77 beats/min.[69] Levolsmondan as a calcium sensor plus vasodilator was trialed in HF patients (REVIVE-2 trial), with overall results showing lack of benefit or even increased 31-day mortality. Further data analysis of the REVIVE-2 Trial, however, suggested that SBP was critical in the trial outcome; the relative risk of 31-day death was 5.8 for patients with SBP <100 mmHg but was 1.0 for those with SBP >100 mmHg.[6,70]

Although limited in number, clinical trials in AHF have focused on improvement of symptoms during hospitalization such as dyspnea. However, in many cases, dyspnea relief or changes in body weight (a rough estimation of volume load) during the acute phase are not associated with improvement of clinical outcomes post-discharge.[5,71] Indeed, the current guideline-directed therapy for AHF aims to control for dyspnea during hospitalization in a majority of patients without significant effect on long-term prognosis. Thus, the RELAX-AHF trial is unique in that both short- and long-term endpoints are improved in serelaxin vs. placebo groups.[9] A successful new drug should be effective in AHF as estimated not only by short-term but also by the long-term endpoints, including 6–12 month mortalities, which, for many years, has been at unacceptably high levels.

The outcomes of the RELAX-AHF trials inspire future clinical and preclinical investigation. They reveal our limited understanding on the pathophysiology of AHF and clinical classification that are critical for the management of patients as well as the design of drug trials. Furthermore, the intriguing finding of better long-term prognosis is inspiring and hypothesis-stimulating, albeit this needs to be confirmed in a larger-scale phase-III trial. Finally, the characteristics of the patient population of AHF once again urge scientists to conduct preclinical research on laboratory models that better simulate clinical conditions. Thus, future research efforts should be given to establish animal models of human AHF to meet the need of urgent requirement of research in this field.

The development of serelaxin for AHF treatment has provided an excellent case for the bilateral feature of translational medical research. Further clinical studies are also required to explore mechanisms responsible for serelaxin's efficacy. Meanwhile, preclinical studies are essential to address pharmacological features of serelaxin and likelihood of serelaxin use in conjunction with other drugs. With a joint effort from clinicians, research scientists and the pharmaceutical industry, we foresee breakthroughs in the near future not only in research on serelaxin-AHF, but also the development of other new drugs for this important condition.

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Disclosures

Conflict of interest: X.J.D. and C.S.S. serve as members of the Novartis Serelaxin Advisory Panel.

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