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Themed Section: Recent Progress in the Understanding of Relaxin Family Peptides and their Receptors

REVIEW ARTICLE

Serelaxin in clinical development: past, present and future

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The availability of highly purified recombinant human relaxin, serelaxin, has allowed clinical trials to be conducted in several indications and the elucidation of its pharmacology in human subjects. These studies have demonstrated that serelaxin has unique haemodynamic properties that are likely to contribute to organ protection and long-term outcome benefits in acute heart failure. Clinical observations support its consideration for therapeutic use in other patient populations, including those with chronic heart failure, coronary artery disease, portal hypertension and acute renal failure.

LINKED ARTICLES

This article is part of a themed section on Recent Progress in the Understanding of Relaxin Family Peptides and their Receptors. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v174.10/issuetoc

Abbreviations

AHF, acute heart failure; Ang II, angiotensin II; BUN, blood urea nitrogen; ET-1, endothelin-1; FF, filtration fraction; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; MRSS, modified Rodnan skin score; PCWP, pulmonary capillary wedge pressure; RAAS, renin-angiotensin-aldosterone system; RAP, right atrial pressure; RBF, renal blood flow; RPF, renal plasma flow; SBP, systolic blood pressure; SSc, systemic sclerosis; SVR, systemic vascular resistance; WHF, worsening heart failure

DOI:10.1111/bph.13695

Tables of Links

TARGETS
GPCRs
ET_A receptors
ET_B receptors
RXFP1 receptors

LIGANDS	
Ang II, angiotensin II	Relaxin 2
Cinaciguat	Rolofylline
Dopamine	TGF-β
ET-1, endothelin 1	TNF-α
Nesiritide	Ularitide
NO	

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015).

Introduction

Relaxin-2 is a naturally occurring peptide hormone which was first investigated clinically in the 1950s as a partially purified extract of porcine ovaries (Casten and Boucek, 1958; Casten *et al.*, 1960). In the intervening decades, large strides have been made in relaxin research, including the ability to produce highly purified recombinant human relaxin, known as serelaxin. The availability of serelaxin has enabled the study of its human pharmacology and unique mechanism of action in randomized, placebo-controlled trials.

As is the case with other peptide hormones, serelaxin is believed to have temporal- and spatial-specific effects, which require expression of the relaxin RXFP1 receptor, in relevant cell types (see Bathgate *et al.*, 2013; Halls *et al.*, 2015). In addition, the determination of which downstream signalling pathways are elicited in target cells and the magnitude of effect size appear to be highly dependent upon the status of other ligand–receptor systems, which are themselves tightly regulated, such as those for endothelin (Danielson *et al.*, 2000; Dschietzig *et al.*, 2003), angiotensin II (Ang II) (Chow *et al.*, 2014) and TGF-β (Heeg *et al.*, 2005; Mookerjee *et al.*, 2009). These relationships are likely to lead to a physiologically complex integrated system regulating both the vasculature and the interstitium. From a clinical development perspective, these interrelationships provide the potential for serelaxin to intervene in the progression of diseases in which dysregulation of these systems is part of the underlying pathology.

In women, endogenous relaxin-2 is released into the circulation by the corpus luteum, initially rising to a peak approximating 1 $ng{\cdot}mL^{-1}$ during the first trimester of pregnancy and subsequently decreasing slightly over the course of gestation (Szlachter *et al.*, 1982). In contrast, relaxin in the systemic circulation in rodents first rises mid-pregnancy and demonstrates a pre-partum surge in circulating concentrations (Sherwood, 1994). This difference in expression pattern may form the basis for the finding that certain aspects of the physiology of relaxin, such as peri-partum cervical ripening, are clearly relaxin-dependent in rodents (see Sherwood, 1994) but apparently not significantly affected by serelaxin in humans (Weiss *et al.*, 2016). Because species-specificity may be relevant with regard to other pharmacological responses to serelaxin, it is useful to take note of the responses that have been observed in completed human clinical studies using serelaxin, including those trials in which the primary endpoints have not been met. This review will describe responses observed in past clinical studies, current indications being pursued and indications that could prove fruitful in the future.

Observations in completed clinical trials

To date, serelaxin has been administered systemically in completed trials to more than 1500 human subjects, including healthy volunteers (Chen *et al.*, 1993; Smith *et al.*, 2006; Dahlke *et al.*, 2015b), patients with systemic sclerosis (SSc) (Seibold *et al.*, 1998; Seibold *et al.*, 2000; Khanna *et al.*, 2009), patients with acute (Teerlink *et al.*, 2009; Teerlink *et al.*, 2013; Ponikowski *et al.*, 2014; Sato *et al.*, 2015) and chronic (Dschietzig *et al.*, 2009; Voors *et al.*, 2014) heart failure (HF), women with post-date pregnancies (Weiss *et al.*, 2016), patients with hepatic impairment (Kobalava *et al.*, 2015) and renal impairment (Dahlke *et al.*, 2015a), and patients with compensated cirrhosis (Lachlan *et al.*, 2015a,b) (Table 1). The pharmacokinetics of serelaxin in humans are well studied (Chen *et al.*, 1993; Seibold *et al.*, 1998; Dahlke *et al.*, 2015a,b; Kobalava *et al.*, 2015). Following i.v. infusion, steady state concentrations are attained by 4 h, the AUC is dose-proportional over a wide range of doses (10–100 $\mu g{\cdot}kg^{-1}{\cdot}day^{-1}$), with terminal half-lives from 7–16 h (Dahlke *et al.*, 2015a).

The clinical trials of serelaxin mentioned above provide good evidence for serelaxin-induced, receptor-mediated, systemic and renovascular dilation. Additional but limited evidence, which will not be discussed in this review, has suggested that serelaxin can induce endometrial angiogenesis that may be related to local stimulation of VEGF (Unemori *et al.*, 1999). Based on these and other observations (Unemori *et al.*, 2009; Conrad and Shroff, 2011) two clinical trials were initiated in preeclampsia (clinicaltrials.gov identifiers NCT00333307 and NCT01566630), but enrolling patients into these trials proved difficult and the studies were terminated. Additionally, a modest natriuretic effect has been

Table 1

Completed clinical trials using systemically administered serelaxin

Study population	clinicaltrials.gov or EudraCT identifier	Phase of study	Number of subjects	Serelaxin dose ($\mu g \cdot kg^{-1} \cdot day^{-1}$)	Duration/route of dosing	References
AHF patients	NCT00520806	Ph II (Pre-RELAX-AHF)	234	0, 10, 30, 100, 250	48 h/i.v.	Teerlink *et al.*, 2009
	NCT01870778	Ph III (RELAX-AHF-1)	1161	0, 30	48 h/i.v.	Metra *et al.*, 2013; Teerlink *et al.*, 2013; Filippatos *et al.*, 2014
	NCT01543854	Ph II	71	0, 30	20 h/i.v.	Ponikowski *et al.*, 2014
	NCT02002702	Ph II	46	0, 10, 30	≤48 h/i.v.	Sato *et al.*, 2015
Chronic HF patients	NCT00259116	Ph II	16	Group A: 10, 30, 100; Group B: 240, 480, 960; Group C: 960	Groups A and B: 8 h sequential infusions of increasing doses; Group C: 24 h/i.v.	Dschietzig *et al.*, 2009
	NCT01546532	Ph II	65	0, 30	24 h/i.v.	Voors *et al.*, 2014
	NCT01982292	Ph II	320	0, 30	3 sequential 48 h/i.v.	Teerlink *et al.*, 2016
Systemic sclerosis patients	NA	Ph I	30	0, 6, 12, 50, 100, 200	28 d/s.c.	Seibold *et al.*, 1998
	NA	Ph II	68	0, 25, 100	24 wk/s.c.	Seibold *et al.*, 2000
	NCT00704665	Ph III	231	0, 10, 25	24 wk/s.c.	Khanna *et al.*, 2009; Teichman *et al.*, 2009
Cirrhosis patients	NCT01640964	Ph II	46	0, 80 followed by 30	1 h/i.v. for each dose	Lachlan *et al.*, 2015a,b
Healthy volunteers	NA	Ph I	25	10	Bolus / i.v.	Chen *et al.*, 1993
	NA	Ph II	11	0.2 μg/kg bolus followed by 0.5 μg/kg/h	Bolus/i.v. followed by 5 h/i.v.	Smith *et al.*, 2006
	EudraCT #2010–022528-58	Ph II	40	0, 10, 30, 100	48 h/i.v.	Dahlke *et al.*, 2015b
Healthy volunteers and subjects with hepatic impairment	NCT01433458	Ph II	49	30	24 h/i.v.	Kobalava *et al.*, 2015
Subjects with renal impairment	NCT01875523	Ph I	36	10	4 h/i.v.	Dahlke *et al.*, 2015a
Post-date pregnant women	NCT00259103	Ph II	72	0, 7.5, 25,75	24 h/i.v.	Weiss *et al.*, 2016

Listed are all completed Phase (Ph) I, II, and III clinical trials in which serelaxin was systemically administered. clinicaltrials.gov or EudraCT identifiers are listed except when not available (NA). Data include type of subjects enrolled, number of subjects treated in each trial, doses administered, duration of treatment in hours (h), days (d) or weeks (wk), route of administration (i.v. or s.c.) and publications in which data are summarized.

observed in some (Smith *et al.,* 2006) but not all (Voors *et al.,* 2014) subjects.

Systemic vasodilation

The vasodilatory effects of serelaxin have been measured invasively in clinical studies as a decrease in cardiac filling pressures, such as pulmonary capillary wedge pressure (PCWP) and right atrial pressure (RAP), and non-invasively as a decrease in systolic blood pressure (SBP). In a double-blind haemodynamic study in male and female patients with acute heart failure (AHF) with an entry PCWP ≥18 mmHg and SBP ≥115 mmHg, serelaxin administration caused a significant reduction in systemic vascular resistance, RAP, PCWP, pulmonary vascular resistance and pulmonary artery pressure with most of the changes evident 30 min after the start of the i.v. infusion and maintained throughout the 20 h infusion period (Ponikowski *et al.,* 2014). No significant change in cardiac index was observed, consistent with the interpretation that a reduction in preload accompanied the decrease in afterload. A serelaxin-dependent decrease in SBP was statistically significant at several time points from 30 min following the start of the infusion through to 24 h after the infusion was terminated. Despite differences in experimental design and study subject, these data are generally consistent with those suggesting serelaxin-mediated systemic vasodilation at comparable doses in an earlier open-label haemodynamic study in patients with chronic HF (Dschietzig *et al.,* 2009). Unlike nitrate vasodilators (Munzel *et al.,* 2014), no tolerance developed. The results from these invasive haemodynamic studies suggest that, at least in HF patients, serelaxin reduces preload and afterload via rapid systemic arterial and venous vasodilation with potential effects on the pre-capillary pulmonary bed, as well.

In the Phase III RELAX-AHF-1 study, which enrolled AHF patients of the 'vascular failure' type (Gheorghiade *et al.,* 2005) with a mean baseline SBP of 142 mmHg, 48 h treatment with serelaxin resulted in greater decreases in SBP from baseline compared with placebo during the infusion period through to 24 h after the infusion was terminated (Teerlink *et al.,* 2013). In the Phase II Pre-RELAX-AHF study of similar AHF patients (mean baseline SBP of 147 mmHg), 48 h serelaxin treatment was also associated with a drop in SBP and in a *post hoc* analysis, the magnitude of the decrease in SBP was dependent upon the baseline SBP measurement (Teerlink *et al.,* 2009). In patients with a starting SBP ≤140 mmHg, a non-significant serelaxin treatment effect of −0.7 mmHg relative to placebo was observed. In contrast, the treatment difference was −4.9 mmHg (P = 0.04 by ANOVA) in patients with a starting SBP >140 mmHg.

The SBP-lowering effect of serelaxin was also evident in patients with SSc, with the effect persisting across the 6 month continuous treatment period (Teichman *et al.,* 2009). The magnitude of the change in these patients was also dependent upon SBP at baseline as subjects with a SBP of ≥140 mmHg at baseline had a much greater fall in SBP than the patient population as a whole. These studies suggest that, as has been observed in nonclinical studies (Debrah *et al.,* 2005), serelaxin may induce more potent systemic receptor-mediated vasodilation in the setting of pre-constricted vessels.

Because of serelaxin's potential to interact with multiple pathways known to regulate vascular tone, including endothelin-1 (ET-1), Ang II (see Baccari and Bani, 2008; Du *et al.,* 2014; Halls *et al.,* 2015) and bradykinin (see Leo *et al.,* 2016), the precise mechanisms underlying the observed vasodilation in these trials remain speculative. However, based on the importance of ET-1 in the vasoconstrictive pathophysiology of HF and SSc, an attractive hypothesis is that serelaxin antagonizes the actions of this potent vasoconstrictor. Circulating ET-1 is elevated in HF and is believed to be at least partly responsible for the vasoconstriction and associated symptoms manifested in HF patients (Kelly and Whitworth, 1999). Patients with SSc also have elevated circulating levels of ET-1, which are believed to contribute to hypertension and renal crisis in these patients (Vancheeswaran *et al.,* 1994; Penn *et al.,* 2013). In non-clinical studies, serelaxin up-regulated the expression of the endothelial endothelin ET_B receptor (Dschietzig *et al.,* 2003) and/or increased local enzymic conversion of Big ET to bioactive ET-1, thus enabling its binding to the endothelial ET_B receptor (Jeyabalan *et al.,* 2003). As ET-1 modulates vascular tone through a balance between ET-1-mediated constriction via vascular smooth muscle cell ET_A and ET_B receptors and dilation and clearance transduced by the endothelial ET_B receptor (Schneider *et al.,* 2007), a serelaxin-mediated preferential increase in the latter receptor would favour ET-1-mediated vasodilation, via both NO production and sequestration of ET-1.

Of further interest is the concept that this pathway may be relevant to the observed reliance of the magnitude of systemic haemodynamic response to serelaxin on baseline haemodynamic status. Because the degree of vasodilation transduced by the endothelial ET_B receptor would theoretically depend not only on the extent of target cell expression of the endothelial ET_B receptor but also on the availability of its ligand (ET-1), serelaxin effect size (magnitude of SBP decrease) could conceptually be more marked in subjects with elevated ET-1 levels (vessel preconstriction) at baseline.

The straightforward clinical readout of a decrease in SBP, however,probably belies a complex and interesting series of serelaxin-induced changes that may involve systemic and local ET-1 concentrations and differential expression of the ET-1 receptors in different vascular beds, as well as contributions by other vasodilator pathways, such as bradykinin (Leo *et al.,* 2016). In addition, the observations of a serelaxin-associated decrease in aldosterone levels, both in AHF (Teerlink *et al.,* 2016) and chronic HF patients (Serelaxin Briefing Document, 2014), hint at additional interactions between serelaxin and components of the renin-angiotensin-aldosterone system (RAAS).

Renovascular dilation

Clinical studies that measured renal function indirectly by assessing serum markers such as cystatin C, serum creatinine, blood urea nitrogen (BUN) or uric acid suggest that serelaxin improves GFR. (i) In healthy individuals, an increase from baseline in estimated creatinine clearance was observed following 48 h serelaxin infusion (10, 30 and 100 $\mu g \cdot kg^{-1} \cdot day^{-1}$) compared with the placebo group (Dahlke *et al.,* 2015b). (ii) In hospitalized AHF patients, serelaxin treatment prevented renal worsening (Metra *et al.,* 2013), which is highly predictive of adverse long-term outcomes in these patients

(Gottlieb *et al.,* 2002). In accordance with the decline in renal function in hospitalized AHF patients reported in other studies (Gottlieb *et al.,* 2002; Klein *et al.,* 2008; Lassus *et al.,* 2010), the placebo treatment group in the RELAX-AHF-1 trial showed increases from baseline in mean serum creatinine, BUN and uric acid by 24 h after the start of infusion of placebo and in cystatin C by the earliest time point tested (48 h) (Metra *et al.,* 2013). Serelaxin significantly attenuated the elevation in these serum markers to at least Day 5 and if assessed by cystatin C concentrations, to Day 14 (12 days following termination of infusion). This renal benefit occurred concomitantly with a larger mean decrease in BP in the serelaxin group compared with the placebo group (Teerlink *et al.,* 2013). (iii) Similar creatinine results were found following 20 h serelaxin infusion in AHF patients (Ponikowski *et al.,* 2014). Creatinine clearance increased by 20% by the end of the infusion period in the serelaxin treatment group compared with a 24% decrease in the placebo group (39% treatment difference, $P = 0.0143$ by ANCOVA) (Ponikowski *et al.,* 2014). (iv) Serum creatinine and BUN were also decreased following short-term i.v. administration of 30 and 100 $\mu g \cdot kg^{-1} \cdot day^{-1}$ doses of serelaxin in a small, open-label study in stable HF patients (Dschietzig *et al.,* 2009). (v) In patients with SSc, estimated creatinine clearance showed persistent increases during 6 months of continuous serelaxin administration (Khanna *et al.,* 2009; Teichman *et al.,* 2009), concomitant with small decreases in SBP (Teichman *et al.,* 2009). (vi) In the recently completed RELAX-REPEAT study conducted in chronic HF patients (Teerlink *et al.,* 2016), plasma cystatin C levels were lower and estimated GFR was higher for the serelaxin group compared with placebo group after each of the three sequential infusions administered 4 weeks apart.

In these clinical studies, it is possible that the reported changes in serum renal markers could be due to factors other than the proposed serelaxin-mediated augmentation of glomerular filtration. For example, changes in uric acid depend on status of the xanthine oxidase pathway (Doehner *et al.,* 2007), and creatinine is subject to changes in tubular secretion (Perrone *et al.,* 1992) and extrarenal influences (Sandilands *et al.,* 2013). However, cystatin C is believed to be an accurate measure of GFR (Taub *et al.,* 2012), and the data in aggregate strongly suggest that serelaxin improves GFR. Unlike the observations regarding serelaxin-mediated decreases in SBP, a reliance of effect size on baseline renal status has not been apparent in human studies conducted to date.

The renal vasodilatory effect of serelaxin has been demonstrated in three clinical trials that measured either renal plasma flow (RPF) using clearance of *para*-aminohippurate or renal artery flow in response to serelaxin infusion. Firstly, in a small, open-label study of healthy volunteers (Smith *et al.,* 2006), a 47% increase from baseline in RPF, with no change in GFR (measured using clearance of inulin), was recorded following a 5 h infusion of serelaxin. Filtration fraction (FF), the ratio of GFR/RPF, was reduced at the end of the infusion period relative to baseline. Secondly, in a placebo-controlled study of stable chronic HF patients, a 13% increase in RPF, relative to placebo, with no change in GFR (measured using clearance of iothalamate) was observed following serelaxin administration (Voors *et al.,* 2014). Creatinine clearance was unchanged. FF was reduced 16% in the serelaxin group compared to placebo. Finally, in an open-label study of patients with stable alcohol-related cirrhosis, renal artery flow measured using magnetic resonance imaging increased 65% relative to baseline following a 2 h serelaxin infusion (30 and 80 $\mu g \cdot kg^{-1} \cdot day^{-1}$); GFR was not measured in this study (Lachlan *et al.,* 2015b).

In general, these data demonstrating a serelaxin-mediated enhancement of RPF inform the underlying basis of the increases in GFR reported in clinical studies. Reasons for the dissociation of RPF and GFR in the Smith *et al.* (2006) and Voors *et al.* (2014) studies remain speculative. During early pregnancy, both afferent and efferent glomerular arterioles dilate, resulting in a RPF increase greater than the increase in GFR and a reduction in FF (Helal *et al.,* 2012). In the two trials studying healthy volunteers (Smith *et al.,* 2006; Dahlke *et al.,* 2015b), it is possible that the discordant results (no increase in GFR in the former, improvement in estimated GFR in the latter) reveal a difference in sensitivity of the afferent and efferent arterioles to serelaxin such that the magnitude of serelaxin exposure (dose X duration of treatment) studied by Smith *et al.* (2006) did not enable sufficient dilation of afferent vessels and a GFR rise while the larger exposure studied by Dahlke *et al.* (2015b) did. In the HF patients (Voors *et al.,* 2014), it is possible that the relatively small increase in RPF was insufficient to enable GFR augmentation.

In AHF, a decline in renal function, measured as an increase in serum creatinine, occurs in 20–40% of hospitalized AHF patients (Metra *et al.,* 2012) and is a greater predictor of adverse outcomes than measurements of intrinsic cardiac function, such as left ventricular ejection fraction (Fonarow *et al.,* 2005). While the primary mechanism underlying the serelaxin-mediated improvement of renal function in the AHF studies cited above is likely to reflect a direct effect on intrarenal haemodynamics (improved RPF), other serelaxin-mediated haemodynamic effects may also have contributed. A reduction in preload, which is associated with renal venous pressure, and a reduction in afterload without hypotension are known to positively affect renal perfusion and GFR (Verbrugge *et al.,* 2014) and may also have contributed to the observed serelaxin effect.

The fact that a two day infusion of serelaxin was associated with improved intermediate to long-term outcomes in AHF patients (Teerlink *et al.,* 2009; Teerlink *et al.,* 2013) is in keeping with the hypothesis that effectively preventing the acute decline in renal perfusion during hospitalization attenuates the progressive deterioration in renal function described by the cardiorenal syndrome (Gheorghiade *et al.,* 2005). It may be worth noting that while the detoxification aspect of improved glomerular filtration is beneficial, increased renal perfusion in the absence of an elevation in GFR could help to preserve tubular function, including volume homeostasis, in patients with AHF (Verbrugge *et al.,* 2014). In addition, the observed serelaxin-induced reduction in FF may also have salutary effects on renal function (Mullens and Nijst, 2016). Complete understanding of the protective effect of serelaxin in AHF is partly constrained by limitations in understanding the pathophysiological mechanisms tethering renal worsening during hospitalization to long-term adverse outcomes in AHF. The precise molecular mechanisms underlying the increase in renal function

conferred by serelaxin in AHF patients, as well as in other human subjects, have yet to be defined but may involve antagonism of the renal vasoconstrictive effects of ET-1 and Ang II via up-regulation of NO (see Conrad and Shroff, 2011).

That serelaxin mediates an improvement in renal function is notable in the context of clinical experience with other vasodilators tested in AHF. In completed Phase II and III, placebo-controlled, randomized trials, the vasodilators cinaciguat (Erdmann *et al.,* 2013), tezosentan (McMurray *et al.,* 2007), nesiritide (O'Connor *et al.,* 2011) and ularitide (Mitrovic *et al.,* 2006) have had, at best, a neutral effect on renal function (no different from placebo). In addition, agents in other pharmacological classes that have been tested in AHF, such as rolofylline (Massie *et al.,* 2010) and dopamine (Chen *et al.,* 2013), have also failed to show a favourable effect on renal function.

The observed coincidence of serelaxin-mediated systemic and renal vasodilation in human subjects appears to be pharmacologically unique as the state of arterial underfilling that occurs as a consequence of peripheral vasodilation typically stimulates sympathetic and RAAS activation, which can lead to renal vasoconstriction (Schrier and Briner, 1991). Indeed, these untoward compensatory mechanisms have been associated with the use of other systemic vasodilators, such as nitrates, in HF (Elkayam *et al.,* 2004; Munzel *et al.,* 2011).

Anti-fibrosis

To date, evidence suggestive of a serelaxin effect on modification of the extracellular matrix in humans, a robust aspect of its pharmacology in rodent models (reviewed in Samuel *et al.,* 2007), is lacking when studied following systemic serelaxin administration in trials of SSc (Seibold *et al.,* 1998; Seibold *et al.,* 2000; Khanna *et al.,* 2009) and cervical ripening (Weiss *et al.,* 2016). The cervical ripening trial was the first in this indication to test the efficacy of serelaxin systemically administered at the end of pregnancy to advance ripening and labour (Weiss *et al.,* 2016). Unlike the potent activity observed in rodents and pigs, serelaxin failed to accelerate cervical ripening in post-date pregnant women, a difference that may be related to the dissimilarity in the secretion patterns of endogenous relaxin (see Sherwood, 1994) and its role during pregnancy among these species.

The two efficacy studies conducted in patients with SSc tested the ability of serelaxin to reverse, halt or delay progressive fibrosis using serial measurements of skin thickening, a metric that is predictive of visceral fibrotic involvement, and is the key clinical criterion used in SSc diagnosis and is correlated with decreased survival (Krieg and Takehara, 2009). A continuous 6 month systemic treatment regimen with serelaxin was considered necessary to allow manifestation of the putative anti-fibrotic effects of serelaxin, including inhibition of interstitial collagen deposition and increased collagen degradation by MMPs, in patients with early diffuse SSc (Unemori and Amento, 1990; Unemori *et al.,* 1992). While the small Phase II study was encouraging (Seibold *et al.,* 2000), the larger pivotal trial failed to meet its primary endpoint, a clinically meaningful reduction in the Modified Rodnan Skin Score (MRSS), a palpation-based measurement of skin thickness, compared to placebo (Khanna *et al.,* 2009). Subgroup analysis by duration of disease ($\leq$2.5 and >2.5 years) yielded no difference in MRSS results. While limitations of the MRSS, including susceptibility to intra- and inter-observer variability, are acknowledged, assessors were routinely trained and the MRSS still remains the gold standard for quantitation of cutaneous fibrosis. Serelaxin-associated improvements in lung function tests, such as forced vital capacity, which would be suggestive of pulmonary fibrosis amelioration, were also not observed. Serial pharmacokinetic measurements confirming the presence of serum serelaxin and observations of serelaxin-associated menorrhagia and changes in estimated creatinine clearance and blood pressure confirmed continuous systemic exposure to serelaxin. For these reasons, the data in the pivotal study are a reasonable basis upon which to conclude that chronic exposure to a pharmacological dose of serelaxin sufficient to cause systemic and renal haemodynamic and uterine effects does not produce a measurable reduction in cutaneous fibrosis in patients with early diffuse SSc.

While these negative data comprise an important component of the database on human experience with serelaxin, they do not preclude the potential utility of serelaxin in other clinical indications in which fibrosis plays a pathophysiological role. A complex and varied array of cell types and soluble mediators can shape the dysregulated wound healing process that culminates in fibrosis (Wynn and Ramalingam, 2013), and it seems unlikely that serelaxin would be equipotent as an anti-fibrotic in all circumstances. Translational rodent studies, while they may or may not faithfully represent serelaxin's anti-fibrotic pharmacology in humans, serve to highlight the importance of the underlying basis of fibrosis in determining susceptibility to amelioration by serelaxin (Wong *et al.,* 2013; Dschietzig *et al.,* 2015).

Current and future trials

Acute heart failure

The clinical development programme in AHF is currently continuing with the 6800-patient randomized, placebo-controlled RELAX-AHF-2 trial (clinicaltrials.gov identifier NCT01870778). This study is designed to demonstrate an improvement in cardiovascular and all-cause mortality, consistent with observations in the Phase III RELAX-AHF-1 (Teerlink *et al.,* 2013) and Phase II Pre-RELAX-AHF trials (Teerlink *et al.,* 2009) (Metra *et al.,* 2013). It will also examine serelaxin's effect on worsening heart failure (WHF), which is strongly associated with increased mortality (Torre-Amione *et al.,* 2009; Metra *et al.,* 2010); serelaxin reduced WHF in the Phase III RELAX-AHF-1 study (Teerlink *et al.,* 2013) and showed similar trends in the Phase II Pre-RELAX-AHF study (Teerlink *et al.,* 2009). RELAX-AHF-2 should also be instructive regarding causes of death in this AHF subpopulation in general and should also inform mechanisms specifically pertaining to serelaxin's apparent effect on mortality, including those related to stroke (Felker *et al.,* 2014). The AHF programme has also expanded to Asia with the completion of a Phase II (Sato *et al.,* 2015) and initiation of a Phase III AHF study (clinicaltrials.gov identifier NCT02007720). A Phase II study is also currently recruiting to assess the safety and pharmacokinetics of serelaxin in paediatric heart failure patients (clinicaltrials.gov identifier NCT02151383).

As AHF is an episodic disease with patients repeatedly hospitalized following decompensation, sequential dosing with serelaxin over time in these patients is envisioned. Although serelaxin is identical to the naturally occurring hormone relaxin-2, antibody development to human proteins is known to occur following repeated or extended dosing (Schellekens, 2008). An additional study (RELAX-REPEAT) has been completed, therefore, to examine the safety and antibody response to three sequential 48 h i.v. infusions of serelaxin 4 weeks apart in chronic HF patients (clinicaltrials.gov identifier NCT01982292). Results indicated that the repeat doses of serelaxin were safe and well tolerated with a favourable immunogenicity profile, including no confirmed hypersensitivity or infusion-related reactions and only one patient of the 200 receiving serelaxin transiently developing antibodies (Teerlink *et al.,* 2016). The antibodies were non-neutralizing and not associated with adverse events.

Chronic heart failure

Fifty per cent of HF patients are in the subset of heart failure with preserved ejection fraction (HFpEF) whose 3 year mortality prognosis approximates to 50% (Loffredo *et al.,* 2014). While patients with reduced ejection fraction have been successfully treated, HFpEF patients have not shown similar benefit in clinical trials after treatment with ACE inhibitors, Ang II receptor blockers, β-blockers, digoxin, aldosterone antagonists or sildenafil (Jeong and Dudley, 2015). A *post hoc* analysis of data from RELAX-AHF-1 suggested that the serelaxin-mediated clinical responses and favourable changes in HF-relevant biomarkers of organ damage were similar in the HFpEF subgroup (approximating 25% of the patient cohort) compared with that observed in patients with reduced ejection fraction (Filippatos *et al.,* 2014).

The reduction in mortality observed following short-term serelaxin treatment during hospitalization in Pre-RELAX-AHF and RELAX-AHF-1 has led to the hypothesis that serelaxin may be interrupting pathophysiological cascades of events that, if unmodified, lead to cumulative organ damage and subsequent adverse outcomes (Metra *et al.,* 2013; Diez, 2014; Dschietzig, 2014; Du *et al.,* 2014). This hypothesis could be envisioned as encompassing a beneficial effect of short-term serelaxin infusion on the attenuation of myocardial fibrosis characteristic of HFpEF patients, as well. In these patients, left ventricular diastolic dysfunction is believed to be the result of dysregulated remodelling of myocardial architecture, including cardiomyocyte hypertrophy and interstitial fibrosis (Loffredo *et al.,* 2014). It has been proposed that cardiac fibroblasts, once activated along the pro-fibrotic cascade to become myofibroblasts, are long-lived, surviving for months to years following an inciting insult, and continuously stimulated to produce a collagen matrix in autocrine fashion by the generation of Ang II and TGF-β in a persistent 'secretome' (Weber *et al.,* 2013). Non-clinical studies suggest that short-term serelaxin treatment can block myofibroblast activation induced by both Ang II and TGF-β, (Samuel *et al.,* 2004). Thus, a 48 h infusion of serelaxin during a period of exacerbation of pro-fibrotic stimuli, such as acute decompensation, could theoretically have incremental benefits in preventing progression of ventricular stiffness in the intermediate term.

A recent hypothesis regarding pathophysiology in HFpEF suggests that coronary microvascular inflammation, rather than afterload mismatch, is the primary driver of fibrosis and cardiomyocyte hypertrophy (Paulus and Tschope, 2013). Co-morbidities such as obesity and ageing contribute to a chronic systemic proinflammatory state, resulting in coronary microvascular inflammation, NO dysregulation and oxidative stress. This process ultimately stimulates cardiomyocyte stiffness due to both hypertrophy and titin hypophosphorylation, as well as triggering myofibroblast activation and interstitial fibrosis. Non-clinical studies suggest that serelaxin can potentially interfere at a number of steps in the progression of the disorder, including reduction of oxidative stress and attenuation of Ang II- and TNF-α-induced endothelial inflammation (see Bani, 2008; Conrad and Schroff, 2011; Du *et al.,* 2014; Halls *et al.,* 2015). For these reasons, chronic serelaxin treatment of HFpEF patients following hospital discharge may also have benefits in preventing worsening in these patients. In addition, serelaxin theoretically has the ability to reduce established cardiac fibrosis by stimulating a pro-matrix degrading fibroblast phenotype (see Samuel *et al.,* 2007). Chronic serelaxin treatment could also be surmised to effect a decrease in vascular stiffness (see Conrad and Shroff, 2011), thus potentially improving compliance and cardiac performance in HFpEF patients (Ooi *et al.,* 2008; Butler *et al.,* 2014).

Requirements for a drug development programme in this indication include (i) establishment of a schedule and route of serelaxin administration that is not only efficacious but also amenable to both patients and physicians to facilitate adherence to the chronic treatment regimen; (ii) the identification of quantifiable, clinically meaningful direct and surrogate endpoints; and (iii) demonstration of safety in the setting of the disease and concomitant medications. The safety data from the RELAX-REPEAT study (Teerlink *et al.,* 2016) are a requisite and encouraging component of development in this indication.

Coronary artery disease

In AHF patients, release of cytoplasmic cardiac troponin into the circulation ('troponin leak') reflects myocyte apoptosis, necrosis or lack of membrane integrity. This can occur as a consequence of increased wall stress, myocardial ischaemia, oxygen supply–demand inequity/oxidative stress or exposure to inflammatory cytokines or neurohormones (ET-1 and Ang II) (Kociol *et al.,* 2010; Januzzi *et al.,* 2012). In RELAX-AHF-1, serelaxin, added to standard care, blunted the troponin increase at Day 2, suggesting a cardioprotective effect that may have contributed to increased survival at 180 days (Metra *et al.,* 2013). Because elevated cardiac filling pressures have been associated with increased troponin release in HF (Horwich *et al.,* 2003; Takashio *et al.,* 2013), it is hypothesized that the observed serelaxin-mediated rapid decrease in filling pressures (Dschietzig *et al.,* 2009; Ponikowski *et al.,* 2014) may have played a role in the observed decrease in troponin. In addition to the salutary effect of systemic haemodynamic correction induced by serelaxin, a direct vasodilatory, anti-oxidative stress and anti-inflammatory effect on coronary vessels, as demonstrated in non-clinical studies (see Du *et al.,* 2010), may also have contributed to the potential cardioprotective effect.

An exploratory double-blind, randomized study has been initiated to examine the effect of 48 h infusion of serelaxin (30 $\mu g \cdot kg^{-1} \cdot day^{-1}$) on coronary perfusion reserve and pulse

wave velocity in patients with coronary artery disease (clinicaltrials.gov identifier NCT01979614). Myocardial perfusion will be assessed using adenosine stress cardiac magnetic resonance imaging and augmentation index will be evaluated at several time points during infusion.

Portal hypertension

Portal hypertension, which is defined as an increase in the pressure gradient between the portal vein and inferior vena cava, is associated with serious complications, including variceal bleeding and the formation of ascites, and is the leading cause of death or liver transplantation in patients with cirrhosis (Bosch *et al.,* 2015). While structural changes such as fibrosis and nodule formation are in part responsible for the increase in pressure, augmentation of hepatic vascular tone following stimulation by ET-1, Ang II and other vasoconstrictors is believed to account for 30% of the increase in vascular resistance. Therefore, this component is theoretically amenable to rapid modulation (Reynaert *et al.,* 2008). Because portal hypertension can result from increased hepatic artery blood flow, as well as increased intrahepatic vascular resistance, the ideal vasodilator would not contribute to the former and inhibit the latter. The challenge of developing such a therapeutic agent is highlighted by the limitations of simvastatin, which was shown to reduce portal resistance but concomitantly increased hepatic blood flow, resulting in no decrease in portal pressure in patients with cirrhosis (Zafra *et al.,* 2004). NO donors, such as isosorbide 5-mononitrate, reduced portal pressure (Navasa *et al.,* 1989) but can also decrease renal function (Salmeron *et al.,* 1993).

In non-clinical models of cirrhosis, serelaxin administration rapidly decreases portal vein pressure, through mechanisms involving an increase in intrahepatic NO signalling and a reduction in contractile filament expression in hepatic stellate cells (Fallowfield *et al.,* 2014). In RELAX-AHF-1, salutary effects of 48 h serelaxin infusion on serum liver injury markers were noted (Metra *et al.,* 2013), perhaps suggestive of haemodynamic correction of hepatic congestion and/or impaired liver perfusion (Samsky *et al.,* 2013).

A Phase II study of the effects of serelaxin (i.v. infusion of 80 $\mu g \cdot kg^{-1} \cdot day^{-1}$ for 60 min followed by 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ for at least 60 min) on portal hypertension and the hepatic and renal circulation in patients with compensated alcohol-related cirrhosis was recently completed (Lachlan *et al.,* 2015a; Lachlan *et al.,* 2015b). In an open-label portion of the study (n = 6), the mean portal pressure gradient, which measured 8.2 mmHg at baseline, was reduced by 31.3% after 120 min of serelaxin infusion. Portal vein pressure declined 30 min following the start of infusion and dropped progressively over time by 33.9% compared with baseline at 135 min. In another group of similar patients (n = 40), serelaxin increased blood flow in the hepatic artery slightly, while no effect on superior mesenteric artery flow was measured. Serelaxin infusion was associated with a 65% increase from baseline in renal blood flow (RBF). These encouraging results support additional larger studies on the acute effects of serelaxin in reducing portal hypertension in cirrhosis (clinicaltrials.gov identifier NCT02669875). If these studies are successful, studies on the potential of extended dosing of serelaxin on amelioration of the fibrotic component of the disease, as has been observed in non-clinical studies (Williams *et al.,* 2001; Bennett *et al.,* 2014), would be of clinical interest.

Renal failure

Acute injury resulting in renal ischaemia often leads to further renal vasoconstriction (loss of autoregulation), progressing to endothelial damage due to an increase in ROS, a decrease in endothelial NOS and vasodilatory prostaglandins, and an increase in ET-1 (Schrier *et al.,* 2004). An influx of inflammatory cells contributes to tubular structural changes, such as shedding of epithelial cells, loss of polarity, aberrant fibronectin deposition and luminal obstruction, which lead to impaired tubular function. Prolongation of the insult or inflammation, in which ET-1 and TNF-α are implicated, can lead to renal failure, including fibrosis. Theoretically, if vasoconstriction could be reversed or prevented prior to tubular damage, therapy could blunt progression to acute renal failure (Schrier *et al.,* 2004; Singh *et al.,* 2013). Pharmacological agents with the potential to increase RBF that have been tested in acute renal failure include dopamine, fenoldopam and theophylline, but their efficacy remains unproven (Rosner and Okusa, 2006). Because of serelaxin's specific renal vasodilating ability, early therapeutic intervention in diseases of renal failure may be worth considering. From a clinical development perspective, short-term treatment in clinical settings in which the initial ischaemic insult is known, such as in contrast media-induced nephropathy (ten Dam and Wetzels, 2008) or cardiac surgery-associated nephropathy (Mao *et al.,* 2013), would enable the attractive scenario of early or prophylactic intervention.

In conclusion, clinical trials using serelaxin have provided data regarding its systemic and renal vasodilatory ability and organ protective effects in humans, potentially leading to long-term outcome benefits in AHF. This information has led to additional hypotheses regarding its mechanisms of action and its consideration for future use in additional clinical indications. This 'bench to bedside and back again' basis of serelaxin research fosters the continuing evolution of understanding of its pharmacology and will help determine the full potential of this unique molecule in the treatment of human disease.

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Conflict of interest

The author declares no conflicts of interest.

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