Relaxin: New Pathophysiological Aspects and Pharmacological Perspectives for an Old Protein

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Abstract: Human relaxin-2 (hereafter simply defined as “relaxin”) is a 6-kDa peptidic hormone best known for the physiological role played during pregnancy in the growth and differentiation of the reproductive tract and in the renal and systemic hemodynamic changes. This factor can also be involved in the pathophysiology of arterial hypertension and heart failure, in the molecular pathways of fibrosis and cancer, and in angiogenesis and bone remodeling. It belongs to the relaxin peptide family, whose members comprehensively exert numerous effects through interaction with different types of receptors, classified as relaxin family peptide (RXFP) receptors (RXFP1, RXFP2, RXFP3, RXFP4). Research looks toward the in-depth examination and complete understanding of relaxin in its various pleiotropic actions. The intent is to evaluate the likelihood of employing this substance for therapeutic purposes, for instance in diseases where a deficit could be part of the underlying pathophysiological mechanisms, also avoiding any adverse effect. Relaxin is already being considered as a promising drug, especially in acute heart failure. A careful study of the different RXFPs and their receptors and the comprehension of all biological activities of these hormones will probably provide new drugs with a potential wide range of therapeutic applications in the near future.

Key words: relaxin; relaxin family peptide (RXFP) receptors; pleiotropic hormone; new drugs

1. RELAXIN’S HISTORY

A. Introduction: Relaxin’s Structure and Receptors

Human relaxin-2 (H2 relaxin, henceforth simply referred to as relaxin) is a 6-kDa peptidic hormone made up of 53 amino acids and characterized by a structure similar to that of insulin (Fig. 1): in fact, it consists of two separate polypeptide chains (A and B) that are cross-linked by two disulfide bonds; a third disulfide bond is located in chain A (intrachain bond). Its gene is localized on chromosome 9, at a locus shared with relaxin-1.

According to literature, relaxin is best known for its important physiological role in the growth and differentiation of the reproductive tract during gestation. This led to its name and most of the early research on this hormone, mainly regarding the renal and systemic hemodynamic changes that occur during pregnancy.

Many data show that this factor can also be involved in the pathophysiology of arterial hypertension and heart failure, in the molecular pathways underlying fibrosis and cancer, in angiogenesis and in bone remodeling. It belongs to the so-called relaxin peptide family, the members of which comprehensively exert numerous effects binding to different kinds of receptors, classified as relaxin family peptide (RXFP) receptors (RXFP1 [LGR7], RXFP2 [LGR8], RXFP3 [GPCR135], and RXFP4 [GPCR142]) (Fig. 2).

RXFP1 (relaxin/insulin-like peptide family receptor 1, originally indicated with the abbreviation LGR7) is the receptor for relaxin; it is given by the repetition of a rich in leucine residues peptide, containing a G-protein-coupled receptor (GPCR) and characterized by an unusually large ectodomain.

In some species, relaxin can also activate RXFP2 [LGR8], the native leucine-rich repeat-containing GPCR for the insulin-like peptide 3 (INSL3); this suggests that a potential cross-reactivity might be associated with relaxin’s various biological activities, even though there is currently no evidence of a physiological role for relaxin acting through RXFP2. It is important to underline that INSL3 only activates its cognate receptor RXFP2.

RXFP3 [GPCR135] is the type-1 GPCR that binds human relaxin-3. Relaxin-3 is a highly conserved neuropeptide in mammals and lower species, which is expressed within gamma-aminobutyric acid (GABA) neurons of the brainstem. The relaxin-3 neural network constitutes an ascending arousal system able to modulate a number of neurological functions, such as responses to stress, spatial and emotional memory, feeding and metabolism, motivation and reward, and circadian rhythm and sleep/wake states.

Finally, RXFP4 [GPCR142] is the cognate GPCR for insulin-like peptide 5 (INSL5), a member of insulin/relaxin superfamily of peptides, which appears to both stimulate appetite and activate colon motility. It has been demonstrated that relaxin-3 is a potent agonist for RXFP1, RXFP3, and RXFP4, while INSL5 is a selective RXFP4 agonist.

There is also evidence of an interaction of relaxin with the human glucocorticoid receptor, which may partly explain its pleiotropic actions, such as involvement in maternal immunotolerance to fetal allograft during implantation and early pregnancy; ability to

![Figure 1. Amino acid sequence of H2 relaxin.](https://example.com/figure1.png)
Figure 2. Molecular pathways triggered by the interaction of relaxin with its different receptors. Activation of RXFP1: the interaction of relaxin, relaxin-1 or relaxin-3 with RXFP1 is followed by coupling to Gαs and Gαoβ that in turn activate and inhibit adenylate cyclase, respectively, with overall accumulation of cAMP; with time, RXFP1 recruits coupling to Gαi3 to further increase cAMP levels through the cascade Gβγ-PI3K-PKCζ. The activation of RXFP1 also leads to NO production. Activation of RXFP2: INSL3 and relaxin bind to RXFP2 so determining coupling to Gαs and Gαoβ in the same way as RXFP1; this finally results in cAMP accumulation but, unlike RXFP1, there is no late coupling of RXFP2 to Gαi3. Activation of RXFP3 and RXFP4: relaxin-3, binding to RXFP3, acts as an agonist and inhibits cAMP production via Gαi/o; INSL5 behaves as an antagonist of the relaxin-3 inhibition of cAMP production. Through internalization or movement into lipid-rich signaling complexes, RXFP3 is able to trigger two pathways inducing phosphorylation of ERK-1/2 via Raf and MEK: the main path occurs via PKC, the other one by means of PI3K and Src. Both relaxin-3 and INSL5 can bind to RXFP4, and this receptor inhibits cAMP accumulation via Gαi/o. Gαs, stimulatory Gα subunit of Gs; cAMP, cyclic adenosine monophosphate; PI3K, phosphoinositide-3-kinase; PKCζ, protein kinase Cζ; NOS, nitric oxide synthase; PKA, cAMP-dependent protein kinase; NF-κB, nuclear factor κB; IκB, inhibitor of NF-κB, NO, nitric oxide; ERK-1/2, extracellular-regulated kinase 1 and 2; Raf, family of three serine/threonine-specific protein kinases; MEK, family of related serine-threonine protein kinases; Src, tyrosine kinase.

suppress experimentally induced asthmoid reactions and cardiac anaphylaxis; promotion of differentiation of human activated T cells; influence on pituitary release of oxytocin and on the regulation of the central vasopressin system, as described for glucocorticoids; therapeutic potential in the treatment of scleroderma; immunoregulation (relaxin can blunt the endotoxin-induced production of inflammatory cytokines [IL-1, IL-6, TNF-α] by human macrophages; this effect is suppressed by the glucocorticoid receptor antagonist RU-486). Receptors are not yet known for two other members of relaxin family: INSL4 and INSL6. INSL4, also called placentin or early placental insulin-like protein (EPIL), shows a predominant placenta-specific expression; it is also produced, to a much lesser extent, by the maternal decidua at term. INSL4 would take part in the regulation of fetal and placental growth, where it has an inhibitory effect by causing apoptosis and loss of cell viability.

Conversely, INSL6 is predominantly expressed in male germ cells. Data from studies in INSL6 mutant mice, obtained by targeted disruption of the gene, show that this factor is not essential for embryogenesis and gonad differentiation but is required for the progression of spermatogenesis and modulates sperm motility. It seems that INSL6 exerts autocrine actions on the germ cells themselves and probably, it might have some influence on the Sertoli cells through paracrine mechanisms.
B. Relaxin Through Literature

The data available in literature trace back to 1940s the earliest works on relaxin; in that period, this peptidic hormone, discovered and extracted for the first time in 1926 in an impure form by the corpus luteum of a pregnant sow, began to be studied as a factor involved in the physiologic changes of pregnancy. So, its effects on histology and relaxation of the pubic symphysis, the influence on the development of the mammary gland, and the action on the uterine motility were identified in various animal models; the effects of its experimental administration in humans were also investigated and the first methods for purification and evaluation of its concentration in serum, urine, and tissue were developed.

In the following decades, research mainly continued to focus on the relaxin’s functions in the female and also male reproductive system, with rare examples of studies that tended instead to identify for this hormone roles in different fields, such as wound healing, ulcerative ischemic vascular disease, therapy of pulmonary fibrosis, interaction with fibroblasts, and the action on collagen metabolism, where relaxin has demonstrated to exert a collagenolytic activity participating in the collagenases activation.

Moreover, in the 1980s the first findings were published about relaxin’s ability to induce, if administered, a decrease in blood pressure, also reducing the vascular response to the action of vasoconstrictive substances. Bani et al. showed that relaxin is able to regulate vascular tone by activation of the metabolic pathway that leads to nitric oxide (NO) production in the smooth muscle cells of vessel walls. Other authors highlighted the effects of its administration on the heart, including the action on heart rate and the ability to improve myocardial perfusion so determining an increase in coronary blood flow through the stimulation of NO production. Moreover, Taylor and Clark, using an experimental rat model, speculated that atrial cardiomyocytes themselves could represent the source of the relaxin molecules interacting with the specific receptors located on cell surface; therefore, this hormone would regulate the cardiovascular structure and function through autocrine and/or paracrine mechanisms.

Besides the studies on relaxin’s hemodynamic and cardiac effects, evidences of the possible involvement of this hormone in tumors began to be published. Studies were mainly conducted on the possible correlation with breast cancer and, concerning this, a work of Bigazzi et al. is interesting; these authors evaluated the effects of different relaxin’s concentrations on the proliferation of cultured human breast adenocarcinoma cells and observed that low concentrations of relaxin stimulated cell growth while higher concentrations inhibited it.

Today, research looks toward the in-depth examination of all these aspects, always adding new information in an attempt to reach a complete understanding of relaxin in its numerous pleiotropic actions. The main intent is to establish the potential likelihood of employing this substance for therapeutic purposes in diseases where a deficit could be part of the underlying pathophysiologic mechanisms, also avoiding any adverse effect. This first requires a careful study of the different receptors and molecular pathways that mediate its biological activities.

2. ROLE IN THE PREGNANCY’S RENAL AND SYSTEMIC HEMODYNAMIC CHANGES

During pregnancy, the kidney undergoes both anatomical and functional variations with return to baseline characteristics within a few weeks after the delivery. Its longitudinal diameter increases by about 1 cm as a result of glomerular hypertrophy and increased intravascular and collector system volume. There is also an enhanced synthesis of prostaglandin E2 (PGE2), which is responsible for the ureteral hypomotility and distension typical of the pregnant woman. These changes in the anatomy of the kidney begin during the first trimester of pregnancy and may persist for up to 12 weeks postpartum.
Figure 3. Schematic representation of relaxin’s pleiotropic actions on different organs and systems.

From the hemodynamic perspective, a peripheral vasodilatation occurs, with consequent reduction in vascular resistances and blood pressure. The cardiac output tends to increase reaching the maximum value at the 24th week of gestation; despite this, blood pressure is reduced due to decreased vascular resistance. Blood volume increases by about 50% following a greater renal Na⁺ retention.
In the kidney, a significant rise in blood flow takes place early; it is caused by the increased cardiac output and, to a greater extent, by the reduction in renal vascular resistances, secondary to the profound decrease in both afferent and efferent arteriolar resistances. The most important consequence is the rise in the glomerular filtrate by a value of about 45%. This results in a proportional reabsorption of $Na^+$ by the renal tubules, expression of a glomerulus-tubular balance that is necessary during pregnancy to prevent substantial losses of $Na^+$. Relaxin plays a central role in the mechanisms responsible for the renal and systemic hemodynamic changes that occur during pregnancy.

Relaxin is typically secreted by the corpus luteum as well as in the ovary, a local expression of this hormone and its primary receptor, RXFP1, is observed by different cell types of the female reproductive system (nonpregnant endometrium, decidual cells in the pregnant endometrium), and by cytotrophoblast and syncytiotrophoblast. Moreover, the production of relaxin and its receptor has been described in the blood vessels. Therefore, relaxin seems to carry out also autocrine and/or paracrine besides endocrine actions. In pregnant women, it increases the activity of the vascular gelatinase, thereby converting the big endothelin (ET) into ET(1–32); this lastly induces renal vasodilatation, hyperfiltration, and reduced myogenic reactivity of the small caliber renal arteries through the activation of the ET endothelial-B receptor and the synthesis of NO.

The importance of these mechanisms is demonstrated by the fact that the administration of recombinant human relaxin in specimens of rat virgin females causes a significant reduction in systemic vascular resistances and an increase in cardiac output by about 20%, modifications of magnitude similar to the one of the changes observed in mid-gestation rat females. The same applies to the variations in renal hemodynamics.

Finally, hemodynamic adaptations that reproduce the pattern observed in pregnancy also occur in the luteal phase of the menstrual cycle when, compared to the follicular phase, systemic vascular resistances decrease and cardiac output increases, although to a lesser extent than pregnancy. Probably it is not a coincidence that relaxin is also secreted by the corpus luteum, resulting in an increase in its quantifiable (although lower than the levels achieved during gestation) concentration quite during the luteal phase of menstrual cycle.

Indeed, in concepitive cycles, relaxin significantly rises 9–10 days following ovulation and its plasma levels rapidly increase over the next days over 800 pg/mL in women; similarly, a small but measurable augment in plasma relaxin occurs throughout the late luteal phase, approximately reaching the value of 50 pg/mL.

Taking into account relaxin's serum concentrations, it has been observed that the mean value for eumenorrheic young, healthy, nulligravidae elite female athletes with ovulatory cycles is $4.32 \pm 7.56$ pg/mL; in normal singleton pregnancy, relaxin serum concentration varies from $1240 \pm 40$ pg/mL during the first trimester to $970 \pm 2$ pg/mL throughout the third trimester.

### A. Relaxin and Preeclampsia

The functions performed by relaxin during physiological pregnancy require a consideration about the possibility that a deficiency of this hormone may in some way be involved in the pathogenesis of preeclampsia, a pathological condition defined by the development of arterial hypertension associated with proteinuria or edema or both, due to pregnancy or to the influence of a recent pregnancy. It does not occur before the 20th week of pregnancy, in the absence of gestational trophoblastic disease, and it is predominantly a disease of the primigravida.

In 5–10% of severe cases acute renal failure is observed, and this is always a negative prognostic factor with a predictive mortality of 10%. The histological features are those of an acute tubular necrosis associated with endotheliosis, which consists of a glomerular
endothelium inflammation. After the acute phase, the recovery of renal function is usually complete.53

The pathophysiological mechanisms underlying the onset of preeclampsia are not yet fully known and defined; this has earned it the appellation of “disease of theories.”65 Among the various hypotheses, it has been postulated that a reduction in relaxin’s production may play a role. A decrease in the course of the first stage of the disease would lead to a reduction in the molecular signals necessary for the facilitation of trophoblast invasion by the uterine spiral arteries or to impaired decidualization, trophoblast invasion, and vasodilatation in the early phases of pregnancy. If the reduced relaxin’s production occurs in the second stage, it likely contributes to the decrease in uterine blood flow typical of this period. Finally, in the third stage, a deficit of relaxin’s activity would be directly associated to the clinical manifestations of preeclampsia.55

An indirect evidence for the likely role of relaxin’s deficiency in the pathophysiology of preeclampsia comes from studies conducted on women undergoing assisted reproductive technologies and conceiving through donor eggs. These patients seem to have increased risk for adverse pregnancy outcomes; the reason for this might lie in the lack of corpus luteum and circulating relaxin, with resulting reduction in hemodynamic changes during early gestation compared to normal pregnancy.66

Furthermore, it is known that age predisposes to an abnormal vascular adaptation and, as a consequence, to the gestational hypertensive disease; in parallel, a decrease in the vascular response to relaxin has been observed with age in a rat model, despite its levels being comparable in animals of different ages.67

Recent data suggest that antiangiogenic factors (soluble fms-like tyrosine kinase receptor and soluble endoglin) may alter relaxin-mediated mechanisms so determining a reduction in NO synthesis through the production of asymmetric dimethylarginine (ADMA): circulating levels of ADMA are in fact high in preeclamptic women, and this can be expression of the generalized endothelial dysfunction that characterizes such condition.68 The soluble fms-like tyrosine kinase receptor, specific for growth factors, would be able to inactivate the vascular endothelial derived growth factor (VEGF) and the human placental growth factor (hPGF). Given also that there is an increased placental expression and secretion of this factor in preeclampsia, it might play a causal role in the pathogenesis of the disease. Moreover, there may be the formation of autoantibodies that activate the receptors for angiotensin.69 Since relaxin exerts a vasodilator action and its circulating levels result to be reduced in preeclamptic women, we can suppose a future therapeutic use.

Wang et al.71 have also studied the localization and the levels of relaxin’s receptor in placental tissue in both preeclampsia and physiological pregnancy; employing immunohistochemical methods for the detection of the receptor and the RT-PCR (real-time polymerase chain reaction) technique to highlight the expression of the corresponding mRNA, these authors have documented the presence of the receptor in the cytotrophoblast and syncytiotrophoblast cell membranes, demonstrating a statistically significant difference between the two groups of patients with a reduced placental expression of relaxin’s receptor in preeclamptic women compared to controls. In light of these data and the recent work by Lafayette et al.,72 according to which there would not be a significant difference between relaxin’s levels in normal pregnancy and in preeclampsia in the last weeks of gestation, and given that immunoreactivity does not always correspond to bioactivity, we can hypothesize an impaired relaxin’s action secondary not to its deficit but to other mechanisms: an increase in circulating levels of a hypothetical soluble RXFP1 receptor, a reduction in RXFP1 receptors or an increased expression of truncated RXFP1 receptors73,74 on blood vessels, which could affect the signaling pathway stimulated by relaxin and induce relative vasoconstriction.
The understanding of the pathophysiological mechanisms underlying preeclampsia is needed to detect early markers of the onset of the clinical condition and to identify new therapeutic strategies, in order to limit the consequences during pregnancy and the possible long-term complications; as a matter of fact, numerous evidences from literature show that women with preeclampsia are particularly prone to develop cardiovascular diseases, especially ischemia, and preeclampsia and atherosclerosis seem to share the same risk factors. Finally, it is assumed that also the other organs involved in the natural history of preeclampsia (kidney, liver, brain) can undergo dysfunction in the long run.

3. POSSIBLE THERAPEUTIC APPLICATIONS IN HYPERTENSION AND HEART FAILURE

The chronic infusion of relaxin in spontaneously hypertensive rat virgin females mimics the vasodilatation phenomena typical of pregnancy. It carries out its potent vasodilator action in different species, and the mechanism seems to involve endothelial Gα (i/o) protein coupling to PI3K (phosphoinositide-3-kinase), Akt, and eNOS (endothelial NO synthase). Relaxin also acts antagonizing the vasoconstriction induced by angiotensin-II, endothelin-1, and catecholamines; in fact, its circulating levels are significantly lower in subjects affected by arterial hypertension. In particular, relaxin is able to upregulate the endothelial and epithelial endothelin type-B receptor (ETB), via a Ras-independent Raf-1–MEK-1–ERK-1/2 pathway that activates NF-κB (nuclear factor κB); the final outcome of relaxin's action is that the stimulation of ETB induces endothelin-1 clearance and endothelial release of NO. This ETB receptor-mediated mechanism also explains relaxin's ability of antagonizing angiotensin-II-induced endothelin-1 stimulation.

A very recent work has evaluated the effects of relaxin's administration in rats affected by salt-sensitive hypertension. In particular, the authors observed a significant attenuation of the increase in blood pressure induced by diet with a high salt content and an increased NOS production in the kidney with a short-term treatment; the long-term therapy also determined a reduction in glomerular and tubulointerstitial modifications and in the expression of TGF-β1 (transforming growth factor β1). These data suggest that relaxin may be a potential therapeutic agent for salt-sensitive hypertension.

Furthermore, relaxin exerts a protective action on the cardiovascular system. For instance, Debrah et al. have demonstrated that its administration in rat models induced a vascular remodeling both in the geometric sense (increased unstressed wall area and wall-to-lumen area ratio) and in terms of the molecular composition of vessel walls, determining an increase in smooth muscle cell density and a reduction in collagen/total proteins ratio. As evidence of this, relaxin knockout mice showed an exactly opposite pattern. Because of its hemodynamic properties and probable involvement in cardiovascular pathology, relaxin has been suggested as a potential biomarker with prognostic significance in cardiovascular morbidity and mortality.

This hormone also seems to possess the ability to reduce myocardial fibrosis and promote its regeneration, thus proposing itself as a possible therapeutic agent in many cardiovascular diseases that cause abnormal tissue remodeling with myocardial hypertrophy, loss of heart muscle cells and fibrosis.

Several studies have been performed to evaluate the potential therapeutic effects of its administration in the treatment of arterial hypertension and heart failure. In a hypertensive rat model, relaxin determined a significant reduction in arterial blood pressure, albuminuria and oxidative stress markers, and preserved the glomerular structure if angiotensin-II had been previously administered; on the contrary, relaxin's administration did not produce the same effects if rats had been treated with the NOS inhibitor N(ω)-nitro-L-arginine methyl ester.
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Then, the vasodilator action of relaxin depends on the NOS system activity and on the increased NO bioavailability probably because of a reduction in oxidative stress.

In another study conducted on hypertensive rats as well, relaxin's administration determined an antifibrotic effect normalizing the collagen content of left ventricle and renal tissue; hence, it has a therapeutic potential that could be used in the treatment of hypertensive disease also to reduce or prevent organ damage. In the Pre-RELAX-AHF study, a multicenter-randomized trial recently published in *Lancet*, the authors have evaluated the effects of the administration of different doses of relaxin versus placebo in a cohort of patients with acute heart failure (AHF), dyspnoea, radiographic evidence of pulmonary congestion, increased BNP, mild-to-moderate chronic renal failure, and systolic blood pressure greater than 125 mmHg. The results obtained are encouraging: improvement of dyspnoea, decrease in days of hospitalization, reduction in cardiovascular mortality, and in readmissions in hospitals for heart or kidney failure at 60 days, all associated with an acceptable safety profile. The just published randomized, placebo-controlled trial RELAX-AHF has further confirmed the positive effects of the administration of serelaxin, recombinant human relaxin-2, in patients affected by AHF.

Another relevant biological effect of relaxin on cardiovascular system is represented by its ability to act as a positive inotrope in nonfailing and failing human atria. Dschietzig et al. have recently investigated this aspect, finding that the relaxin-induced inotropic response is preserved in end-stage failing atrial myocardium and does not apply to ventricles; it depends on protein kinase A activation and inhibition of the transient potassium outward current, with an increasing role for PI3K in failing atrial myocardium. Therefore, these and other preliminary data allow to foresee the possibility that relaxin might be a new piece in the clinical management of cardiovascular diseases in the next future.

A. Relaxin’s Action in Cardiovascular Medicine Seems to be Influenced by Gender

Relaxin is a pregnancy-associated hormone, so its actions are expected to be different in males and females. Indeed, a number of studies have revealed such an influence by gender on the effects of this hormone, particularly at the cardiovascular level. For example, male relaxin knockout mice develop cardiac alterations (increased left ventricular collagen synthesis and impaired left ventricular function) that are not observed in relaxin knockout females.

Moreover, Hocher et al. have demonstrated that relaxin is an independent risk factor predicting death in male patients with end-stage renal disease on chronic hemodialysis, while in female patients it is not. Actually, the increase in plasma relaxin levels could be a compensatory mechanism to cardiac dysfunction and coronary disease, and probably female patients are more sensitive to relaxin than male subjects and then the formers have more cardiac protection from this hormone. This important effect of gender on cardiovascular disease involves relaxin in the same way of other gender-related factors in cardiovascular function as well as in cardiac and renal fibrosis in mice.

4. RELAXIN IN FIBROSIS AND CANCER: A NEW ISSUE TO BE FOCUSED

A. Antifibrotic Effect

It has been long since known that relaxin is able to interact with fibroblasts, so interfering with the synthesis of collagen and other components of extracellular matrix. In 1992, Unemori et al. observed a significant reduction in collagen’s expression by cultured fibroblasts coming from patients affected by scleroderma, after addition of relaxin alone or in association with...
interferon-γ. The same group of authors has demonstrated that human relaxin is able to reduce the in vivo accumulation of collagen in two rodent models of fibrosis, halt the deposition of extracellular matrix by human lung fibroblasts in vitro (by inhibiting the hyperexpression of types 1 and 3 interstitial collagen and the upregulation of fibronectin, both mediated by TGF-β), and stop pulmonary fibrosis in an in vivo murine model of bleomycin-induced alveolar thickening.

As observed in the previous paragraph, relaxin also induces a reduction in myocardial fibrosis; through this ability, it might interfere with the tissue remodeling secondary to numerous cardiovascular diseases. Furthermore, relaxin has demonstrated to determine a decrease in interstitial fibrosis at the renal level and delay the progression of kidney disease. In a work published in 2001, Garber et al. employed an animal model of interstitial fibrosis induced by bromoethylamine; relaxin’s administration determined a decrease in albuminuria, serum creatinine, interstitial fibrosis at the corticomedullary junction, macrophage infiltration, and immunohistochemical staining for TGF-β. It is remarkable that the antifibrotic effect of relaxin did not appear to be mediated by systemic hemodynamic changes, since the mean arterial pressure did not differ in a statistically significant way compared to the control group.

Relaxin’s action on connective tissue remodeling is performed through a change in the expression of matrix molecules. In particular, it seems to specifically inhibit a microfibril component, named fibrillin 2 (FBN2), without interfering with the synthesis of fibrillin 1 (FBN1). It is important to emphasize, among other things, how the regulation of FBN2 production is most likely associated with the functional changes in elastic tissues occurring during development and growth. Relaxin also reduces the production of fibrillar collagen and, at the same time, increases the activity of a fibrillar collagenase in a rat model of liver fibrosis. The consequence is a reduction in levels of total collagen and type-1 collagen, and the inhibition of the synthesis of new collagen. As demonstrated in normal cultured human dermal fibroblasts, relaxin regulates collagen deposition at multiple levels with resulting increase in collagen matrix turnover and decrease in overall tissue collagen content. It stimulates the expression of the metalloproteinase procollagenase at the level of mRNA; it also reduces the expression and secretion of tissue inhibitor of metalloproteinases (TIMPs); finally, it downregulates the secretion of interstitial (specifically types 1 and 3) collagens induced by TGF-β in a dose-dependent manner. These findings suggest that relaxin’s administration might exert beneficial effects in the treatment of diseases characterized by excessive production of fibrotic tissue, such as systemic sclerosis (where relaxin would repress fibroblast proliferation and transdifferentiation into myofibroblasts, as demonstrated in vitro employing fibroblasts deriving from skin lesions of patients affected) liver fibrosis (for instance, a decrease in collagen deposition by cultured hepatic stellate cells and in rat liver fibrosis in vivo have been observed), pulmonary and airway fibrosis, renal fibrosis (in this case, relaxin would induce a nephroprotection secondary both to the reduction in arterial pressure and to a probable direct action on kidney function). An increasing amount of evidence even proposes that kidney might be not only a possible therapeutic target but also a potential source of relaxin. Although its renal expression is low, endogenous relaxin appears to be important in the homeostasis of kidney’s connective tissue, and exogenous relaxin has demonstrated to hamper both early and established phases of tubulointerstitial fibrosis through different actions, mainly interfering with TGF-β1 activity. In particular, relaxin binds to its primary receptor (RXFP1) and inhibits the phosphorylation of Smad2 through a nNOS-NO-cGMP-dependent pathway, thereby interfering with TGF-β1-mediated renal myofibroblast differentiation and collagen production. As already highlighted, relaxin’s vasodilator and angiogenic properties add to this, which concur to improve renal function and to suggest its therapeutic use in kidney disease.
B. Relaxin and TGF-β1: Opposite Significances

TGF-β1 is the key factor in the regulation of the fibrotic process. H3-relaxin, the latest member of the relaxin peptide family that has been discovered, can potentially bind all previously defined relaxin's receptors. In particular, its interaction with RXFP1 is followed by antifibrotic effects, as demonstrated in rats in both ventricular fibroblasts in vitro and myocardial fibrosis in vivo. In these experimental models, H3-relaxin almost completely abolishes the deposition of collagen and significantly inhibits the differentiation of cardiac myofibroblasts, processes both stimulated by TGF-β1; it also inhibits TIMP-1 and TIMP-2. These effects are obtained with H3-relaxin's doses similar to those of relaxin necessary to observe the same results.

Even in the context of renal interstitial fibrosis, relaxin inhibits myofibroblasts’ differentiation in vitro; this outcome is partly due to its ability to interfere with the TGF-β1's path through the inhibition of Smad2 phosphorylation and translocation.

Furthermore, a new antifibrotic peptide, called CGEN25009, has been developed; it possesses relaxin-like activity and has the receptor RXFP1/LGR7 as its target. As well as relaxin, also this peptide exerts an inhibitory action on the collagen deposition induced by TGF-β1 in fibroblasts of humans’ dermis and stimulates MMP-2 expression. These effects have in particular been observed in human cell cultures and in a murine model of pulmonary fibrosis induced by bleomycin, in terms of both prevention and therapeutic purpose.

Relaxin also induces a direct inhibition of TGF-β1 production by human vaginal fibroblasts in vitro; the levels of the two substances are in fact inversely related to each other. Conversely, TGF-β1 has shown to suppress basal and stimulated relaxin’s release in a model of cultured-in monolayer porcine luteal cells; such inhibition was dose-dependent. Based on this evidence, relaxin might be administered to reduce fibrosis, and then loss of function, in various clinical situations, at the renal level or that of other organs.

C. Relaxin is a Growth Factor, Just Like Erythropoietin

The antifibrotic, and therefore regenerative, action of relaxin brings to mind another hormone that, although different for other aspects, has these same properties: erythropoietin. Erythropoietin stimulates erythropoiesis and induces vasoconstriction, whereas relaxin does not exert any action on bone marrow erythroid cells and produces vasodilatation. But, as for erythropoietin, able to stimulate neoangiogenesis and cellular proliferation, also for relaxin numerous data exist in favor of its aptitude to behave as a growth factor and so to facilitate the processes of tissue regeneration.

It is well known that relaxin plays a major role as an endocrine growth factor acting on the female reproductive tract during pregnancy. Its regenerative action has been also demonstrated in myocardial and skeletal muscle remodeling and repair; in the skeletal muscle, it had previously been documented that relaxin may prevent the formation of fibrotic tissue. In a recent work by Mu et al., relaxin has shown to promote myogenic differentiation and matrix metalloproteinases (MMPs) migration and activation in both murine and human-cultured myoblasts. Moreover, relaxin’s intramuscular administration in the wound site in a murine model on the second day after the injury has been followed by the activation of Pax7-positive skeletal muscle satellite cells and by an increase in its cellular population in a significant way compared to nontreated mice. This was associated to a rise in angiogenesis and tissue revascularization, with repression of the inflammatory response. The same results were observed in the repair of aged mice’s muscle tissue.

Even relaxin, as well as erythropoietin, stimulates the formation of new blood vessels at the sites of ischemic injury, by means of the specific induction of VEGF (vascular endothelial...
growth factor) and bFGF (basic fibroblast growth factor) at the level of the cells present in the wound's site, presumably including macrophages; as evidence of the local involvement of such cells, macrophages resident in different sites from that of the lesion do not show changes in the expression of these cytokines after relaxin's administration.\textsuperscript{128}

In addition, the angiogenic action of relaxin intervenes during pregnancy. This hormone, in fact, also regulates the PI3K/Akt B-dependent NO production and the number and function of bone marrow derived circulating endothelial cells, which tend to increase in the course of gestation and have the task of facilitating angiogenesis and contributing to the repair of the vascular endothelium. These relaxin's effects on vascular remodeling, mediated by the interaction with the receptor RXFP1, might have a therapeutic potential in vascular diseases.\textsuperscript{129}

Moreover, several angiogenic growth factors, such as VEGF, appear to be essential intermediaries in the mechanisms by which relaxin exerts its vasodilator action in rats, mice and humans.\textsuperscript{130} This also applies to erythropoietin,\textsuperscript{131} even though the latter is able to stimulate angiogenesis also directly.

\section*{D. Relaxin and Bone Tissue}

Relaxin is able to regulate osteoclasts' activity and then to play a role in bone physiology, diseases, and metastasis. Mature osteoclasts are multinucleated cells derived from hematopoietic progenitors; both express the relaxin receptor RXFP1 in humans.\textsuperscript{132} Moreover, patients with mutations in the RXFP2 gene show a greater risk of reduced bone mass and osteoporosis.\textsuperscript{133} RXFP2 is the receptor for INSL3, whose actions are quite different from those of relaxin; indeed, the INSL3/RXFP2 signaling pathway intervenes in bone metabolism by acting on the mitogen-activated protein kinase (MAPK) cascade and stimulating the expression of important genes involved in osteoblast maturation/differentiation and osteoclastogenesis. Another link between relaxin and bone tissue is that this hormone is involved in growth, differentiation, local invasion, and metastasis of different tumors, mainly those that give bone metastasis, such as prostate, breast, and thyroid cancer, and myeloma.\textsuperscript{132}

The influence exerted by relaxin on bone metabolism may be consequent to its modulating action on the RANKL (receptor activator of NF-\kappa B ligand)/OPG (osteoprotegerin) system through RXFP1 receptor, as recently shown in a rat adjuvant induced arthritis model. Combined administration of relaxin and estrogens increased circulating OPG more than estrogens alone and lowered the RANKL/OPG protein ratio in the affected rats relative to controls. This combination also decreased local RANKL transcripts, increased OPG mRNA and reduced the RANKL/OPG mRNA ratio in joints of the arthritic rats when compared to controls.\textsuperscript{134}

Even though the results of the studies concerning relaxin's influence on osteoclasts' activity are not univocal, these observations might have important clinical consequences: the modulation of relaxin's signaling in osteoclasts could become a new pharmacological approach to bone diseases.

\section*{E. Relaxin's Receptor Blockage: A New Therapeutic Strategy for Cancer?}

Relaxin acts as a growth factor and there are several evidences in literature in favor of its possible involvement in cancer biology. In particular, the expression of relaxin has been observed in reproductive organs’ and endocrine tumors. Specimens of male mice deficient in relaxin (RLX−/−) show growth delay and a reduction in the development and function of reproductive system, with a decrease in prostatic epithelial proliferation. The observed histological changes (reduced sperm maturation in the testis, reduced epithelial proliferation, and increased
collagen in the prostate) correlate with an increase in cellular apoptosis rate. This is probably related to the fact that the expression of relaxin's and its LGR7 receptor's mRNA has been observed in the prostate gland and in the testis in the wild-type mouse (RLX+/+); these data suggest that relaxin behaves as an antiapoptotic factor essential for prostate's growth and male fertility's maintenance. Because of such actions, this hormone is potentially involved in the development and progression of prostate cancer.

In a canine model of breast cancer, Lamp et al., studying the tumor microenvironment by RT-PCR, have identified a strong correlation between relaxin and RXFP-1 and between RXFP-1 and MMP-2 and the independence of their local expression compared to relaxin's plasmatic levels; the latter, apart from anything else, did not correlate with benign or malignant nature of the tumor, metastases' development, recurrence or survival at 12 months. Therefore, it appears that the local relaxin's production might take a part in the pathogenesis of this form of cancer by acting as an inducer of connective tissue remodeling.

An action of relaxin in tumor biology is supposable also in humans. In an in vitro model of thyroid carcinoma, relaxin induces an increase in cellular motility and invasiveness. In particular, relaxin is believed to upregulate the calcium-binding protein S100A4 (metastasin) at the transcriptional level and to increase the cytosolic 10-kDa monomeric and the 20-kDa dimeric form of S100A4 in human thyroid carcinoma cells, thus leading to a rise in cell motility. This protein is likely to promote also xenograft angiogenesis. Hence, S100A4 is a potential mediator of relaxin's actions in the pathophysiology of thyroid cancer.

Prostate cancer is the tumor in which the action of relaxin has been studied more extensively. Prostate is the main site of relaxin expression in men. Relaxin production is significantly higher in recurrent prostate cancer samples compared with the normal tissues, and a significant association exists between relaxin staining index and extracapsular extension and seminal vesicle invasion, the latters being related with greater aggressiveness of the disease. In fact, relaxin stimulates proliferation, invasiveness, and adhesion of human prostate cancer LNCaP and PC3 cell lines in vitro. Moreover, it can produce a dysregulation of androgen-regulated genes and an increase in cell motility; both actions appear to be mediated in part by Wnt11 pathway. It is also noteworthy that prostate cancer cells express both relaxin and its receptor; so, relaxin exerts an autocrine/paracrine action which probably is more important than its endocrine functions.

The observation that an enhanced relaxin's expression in prostate cancer correlates with the aggressiveness of the disease has prompted Feng et al. to study the effects of the downregulation of RXFP1 (expressed in prostate carcinomas both positive and negative for the androgen receptor) induced by administration of small interfering RNAs (siRNAs) in a mouse model in vivo. These authors have globally observed an extensive tumor necrosis, a drop in cell proliferation rate and an increased apoptosis, with a marked reduction in the metastatic process. A therapeutic potential for relaxin's antagonists has been hypothesized even in breast cancer. They might in fact prevent metastases development since relaxin stimulates the invasiveness of the neoplastic disease by upregulating MMPs production and remodeling extracellular matrix in in vitro cell lines of human breast carcinoma.

Hence, the increasing evidence that relaxin is produced by cancer cells and can act in an autocrine manner on their RXFP1 receptors promoting the growth and invasiveness of several types of neoplasias (endometrial, mammary, thyroid, and prostate tumors) is leading to the attempt to chemically synthesize human relaxin-2 analogs that may act as RXFP1 antagonists and impair tumor growth.

First, Silvertown et al. have created a lentivirus in order to express an H2 pro-relaxin peptide, characterized by the arginine residues at positions 13 and 17 on the B-chain, which are essential for relaxin activity, mutated to lysine. This H2 relaxin analog turned out to be able to decrease prostate cancer xenograft growth. A more recent example is B-R13/17K H2, an
analog that has been demonstrated to inhibit breast cancer cell invasion induced by relaxin, interfering with relaxin signaling through RXFP1 receptors. The attempt to block relaxin is comprehensible since it affects a variety of pathophysiological mechanisms (angiogenesis, blood flow, pressure, extracellular matrix remodeling, cellular proliferation, apoptosis) and might therefore intervene at multiple levels in tumor development and progression.

On the other hand, it is known that the extracellular matrix influences the therapeutic effectiveness of the drugs used in the treatment of solid tumors as it can block their intratumoral spread or mask the target receptors on neoplastic cells. The transfer of the relaxin’s gene to tumor cells in a murine model of breast cancer leads to a reduction in the proteins of extracellular matrix: the controlled degradation of the extracellular matrix facilitates the pharmacological action of trastuzumab, a monoclonal antibody whose target is the tumor-associated antigen Her2/neu, which is quite colocalized with matrix proteins. Thus, a greater effectiveness in the tumor treatment could be obtained by combining trastuzumab administration with the induction of an increased relaxin’s expression.

5. RELAXIN AND BODY FLUID HOMEOSTASIS: WHAT LINKS?

As a demonstration of the surprising pleiotropic aspects of relaxin, data exist in literature regarding its relationship with body fluid homeostasis and plasmatic osmolality. Yet in 1993, Weisinger et al. observed that relaxin’s administration in rat ovariectomized females determined a significant reduction in plasmatic osmolality compared to nontreated rats, whereas plasmatic AVP (arginine vasopressin) did not change. This exactly reproduced the plasmatic osmolality/AVP ratio trend typical of pregnancy, condition in which a drop in plasmatic osmolality is maintained without suppression of AVP and that corresponds to the time when relaxin is measurable in plasma. This also applies to humans. During pregnancy, the reduction in plasma osmolality is accompanied by a decrease in osmotic threshold for AVP release and by water appetite. Relaxin plays a role in determining such osmotic adaptations, probably through different mechanisms: action in the central nervous system at the level of circumventricular organs; induction of an alteration of the threshold for thirst; local production and action of relaxin on its cerebral receptors; decrease in peripheral vascular resistance, so that AVP release occurs in a nonosmotic way. The last pathway represents an important link between the osmoregulatory and cardiovascular roles of relaxin during pregnancy.

A few years later, it has been observed that relaxin is able to induce a dose-dependent dipsogenic response not only when injected into the cerebral ventricles of rats but also when acutely administered intravenously in the bloodstream. Water intake was almost tripled if nondipsogenic doses of angiotensin-II were added to relaxin.

Angiotensin-II and relaxin, binding to AT1 and LGR7 receptors, respectively, in the subfornical organ and in the organum vasculosum of the lamina terminalis (OVLT), stimulate AVP release. During gestation, relaxin induces vasopressin secretion and thirst, acting through the lamina terminalis in the presence of pregnancy levels of estrogen and progesterone, with consequent hypervolemia and hyponatremia.

Moreover, long-term administration of recombinant human relaxin determines diuresis and natriuresis and then a reduction in plasmatic osmolality and sodium concentration in anesthetized male rats; this condition is also observed in pregnancy. Such situation seems not to depend on changes in GFR or circulating atrial natriuretic peptide (ANP) or aldosterone. Probably, natriuresis is induced by relaxin’s interaction with the ETB receptor, with following inhibition of Na\(^{+}\)-K\(^{+}\)-ATPase activity in the inner medullary collecting duct.

Then, these and other evidences allow us to assert that relaxin may potentially take part in the regulation of water balance.

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6. RXFP3/RELAXIN-3 SIGNALING PATHWAY IN CENTRAL NERVOUS SYSTEM

RXFP3/relaxin-3 signaling pathway is a recently discovered ascending arousal system involved in the regulation of some important neurological functions, including behavioral responses to stress, spatial and emotional memory, feeding and metabolism, arousal/sleep states, motivation and reward.7

Relaxin-3 is a highly conserved hypothalamic neuropeptide expressed within GABA neurons of the nucleus incertus of the brainstem, which has projections to the hypothalamus. Even though it can also bind and activate RXFP1 and RXFP4, relaxin-3 binds RXPF3 with higher affinity.156 This cognate receptor is predominant in the central nervous system, particularly in the hypothalamic paraventricular nucleus (PVN), which plays an essential role in stress response.157

CRF (corticotropin-releasing factor) is expressed in parvocellular neurons of this area and, during stress conditions, stimulates the release of adrenocorticotropic hormone by binding to the CRFR1 receptor located on anterior pituitary corticotrope cells. Through this mechanism, CRF activates the hypothalamic-pituitary-adrenal axis and initiates the acute phase of the stress response. There is another receptor for CRF, called CRFR2, which has a different distribution and is involved in the maintenance and recovery phase of stress response.158 Tanaka et al. have demonstrated that almost all relaxin-3-positive neurons in the nucleus incertus of the rat coexpress CRFR1 and react to intracerebroventricular administration of CRF by expressing c-Fos, so taking part in the regulation of stress response.159

C-Fos is one of the transcription factors that regulate neuronal gene expression and is also involved in the increase in water intake induced by the intracerebroventricular administration of relaxin-3, probably through binding to RXFP1.160 Moreover, administration of human relaxin-3, either intracerebroventricularly or intra-PVN, is able to increase food intake in male Wistar rats through activation of RXFP3.161

Relaxin-3 is also implicated in the regulation of the hypothalamopituitary gonadal axis, as intracerebroventricular and intra-PVN administration of relaxin-3 in adult male rats significantly raises plasma luteinizing hormone concentrations. This occurs because relaxin-3 dose-dependently stimulates GnRH release by hypothalamic GnRH neurons.162

In conclusion, the central distribution and function of RXFP3/relaxin-3 network indicate potential therapeutic use of RXFP3 modulators to treat stress/anxiety, cognitive disorders, and metabolic diseases. An example of this approach is R3/I5, a chimera of the INSL5 A chain and the relaxin-3 B-chain that behaves as a RXFP3-selective agonist.163 In a rat model of intrahypothalamic injections of a recombinant adenoassociated virus 1/2 vector that drives expression and constitutive secretion of bioactive R3/I5, Ganella et al. have observed that R3/I5 is effective in modulating feeding by chronic hypothalamic RXFP3 activation.164 Thence, the administration of RXFP3 agonists might be a path to follow in the future to take advantage of the brain regulatory effects exerted by relaxin-3.

7. CONCLUSIONS

The numerous pleiotropic actions of relaxin make it difficult to completely understand its involvement in human pathophysiology and the real therapeutic potential of the administration of relaxin or its antagonists. Relaxin is already being thought as a promising drug (Table I), especially in patients affected by AHF. Pre-RELAX-AHF results have suggested that infusion of RLX030 (recombinant form of human relaxin-2) may accelerate dyspnoea relief and improve prognosis in patients hospitalized with AHF.89 The just published randomized, placebo-controlled trial RELAX-AHF has further evaluated these effects, demonstrating that
Table I. Clinical Trials and Clinical Experiences Concerning Administration of Recombinant Human Relaxin

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<th>Year</th>
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<th>Design</th>
<th>No. of patients</th>
<th>Status</th>
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| 1993 | Evaluation of the safety of vaginal recombinant human relaxin for cervical ripening<sup>167</sup> | Multicenter, randomized, double-blind placebo-controlled trial          | 40 women        | Completed   | - No important maternal or fetal-neonatal complications  
                                    |                                                                      | studied before induction of labor because of postdates               |                 | - No differences compared to the placebo group                                                   |
| 1997 | Assessment of efficacy and safety of recombinant human relaxin as a cervical ripening agent in women with unfavorable cervix before induction of labor at term<sup>168</sup> | Multicenter, double-blind, placebo-controlled trial                    | 96 women        | Completed   | Recombinant human relaxin 1–4 mg, administered as an intravaginal gel, has no effect             |
| 1998 | Estimation of safety and pharmacokinetics of recombinant human relaxin in systemic sclerosis<sup>169</sup> | Double-blind, sequential panel, dose-escalation study                   | 30 patients     | Completed   | Recombinant human relaxin was safe and well tolerated in the doses used                           |
| 2000 | Evaluation of the efficacy, safety, and dose–response effect of recombinant human relaxin in patients with scleroderma<sup>170</sup> | Multicenter, parallel group, randomized, double-blind, placebo-controlled trial | 68 patients     | Completed   | - Reduced skin thickening  
                                    |                                                                      |                                                             |                 | - Improved mobility  
                                    |                                                                      |                                                             |                 | - Improved function                                                   |
| 2006 | Evaluation of the safety of relaxin in preeclampsia<sup>171</sup>     | Phase I randomized, double-blind, placebo-controlled, dose-escalation study | Estimated enrolment: 18 women | Suspended  | —                                                                                              |
| 2009 | First clinical experience with intravenous recombinant human relaxin in compensated heart failure<sup>172</sup> | Clinical experience                                                   | 16 patients     | Completed   | - No relevant adverse effects  
                                    |                                                                      |                                                             |                 | - Vasodilatation (increase in the cardiac index and decrease in pulmonary wedge pressure, without inducing hypotension) |

*Medicinal Research Reviews DOI 10.1002/med*
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<th>Year</th>
<th>Trial Description</th>
<th>Design</th>
<th>No. of patients</th>
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| 2009 | Use of recombinant human relaxin to attempt *cervical ripening* in postdelivery date pregnancies<sup>173</sup> | Randomized, double-blind, placebo-controlled trial | 68 patients | Completed | - High doses were safe  
- They did not advance cervical ripening or induce labor |
| 2009 | Comparison of placebo with recombinant human relaxin in stable, diffuse, moderate-to-severe *systemic sclerosis*<sup>174</sup> | Large randomized, double-blind, placebo-controlled clinical trial | 231 patients | Completed | - Relaxin not significantly better than placebo  
- Serious renal adverse events, mainly after stopping the infusion |
| 2009 | Determination of safety, tolerability, and pharmacodynamic effects of human relaxin in *stable heart failure*<sup>175</sup> | Clinical experience | 16 patients | Completed | - Relaxin was safe and well tolerated  
- Vasodilatation  
- Improved markers of renal function |
| 2012 | Examination of the effect of relaxin on *tooth movement and stability*<sup>176</sup> | Single-center, blinded, placebo-controlled, randomized clinical trial | 40 patients | Completed | - No differences compared to placebo  
- The local doses might have been too low to affect tooth movement or short-term relapse |
| 2012 | Pre-RELAX-AHF (effect of RLX030 [recombinant form of human relaxin-2] on symptom relief and clinical outcomes in patients with *acute heart failure* [AHF])<sup>18</sup> | Phase II dose-finding, single-center, placebo-controlled, blinded, randomized clinical trial | 234 patients | Completed | Infusion of RLX030 may accelerate dyspnoea relief and improve prognosis with an acceptable safety profile |
| 2012 | RELAX-AHF (evaluation of the efficacy and safety of serelaxin in patients with *AHF*)<sup>90, 165</sup> | Phase II/III, double-blind, multicenter, placebo-controlled study | 1161 patients | Completed | - Treatment with serelaxin is associated with dyspnoea relief and improvement in other clinical outcomes, but has no effect on readmission to hospital.  
- Serelaxin treatment is well tolerated and safe, supported by the reduced 180-day mortality. |
treatment of AHF with serelaxin, recombinant human relaxin-2, is well tolerated and safe and is associated with dyspnoea relief, improvement in other clinical outcomes and reduced 180-day mortality.\textsuperscript{90,165}

The first step that research ought to do is the exact definition of structure and function of all RXFP receptors, in order to aim at stimulating or inhibiting only those implicated in the disease that we will need to threat. Then, all metabolic and signaling pathways underlying the different activities of RXFPs should be clarified. Attention should be especially paid to relaxin’s antifibrotic effects that correspond, on the other hand, to its ability of stimulating cancer progression. The association between these two behaviors, already highlighted for other molecules, appears to be extremely important because of its clinical and therapeutic implications.\textsuperscript{166}

The comprehension of all RXFPs’ biological activities is a challenging issue and will probably provide new drugs with a potential wide range of pharmacological applications in the near future. The results obtained in patients with AHF\textsuperscript{90} are very encouraging, and we reckon that right cardiovascular diseases might be the main field of successful therapeutic use of relaxin analogs.

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Antonio Lacquaniti graduated with vote 110/110 cum laude cum laude in medicine and surgery at the University of Messina (Italy) on July 27, 2006 debating an experimental thesis on title “Stem Cells in Uremia”. In 2007 he has achieved the access at the postgraduate school of Nephrology II of the University of Messina. From 2007 to 2012, during his postgraduate medical training in Nephrology, had several responsibilities in different areas, but in particular in the outpatient management of renal transplant patients (evaluating patients to be inserted on cadaver and living donor transplant waiting lists, follow-ups of transplant recipients), renal biopsy and the histopathological pattern studies of renal disease; and intermittent and continuous dialysis techniques in patients with acute renal failure or with multiorgan failure, in the Intensive Care Unit or in other Units. Lacquaniti also conducted research activities during these years, with the publication on national and international journals of over fifty scientific articles in the nephrology field.

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Davide Bolignano was born on 31 October 1980. He graduated in Medicine from the University of Messina (Italy) in 2004 and obtained the post-graduate specialization in nephrology in 2009, both cum laude. He currently works as clinical researcher of the Italian National Research Council (CNR) at the Institute of Biomedicine and Molecular Immunology (IBIM) in Reggio Calabria, Italy. Dr. Bolignano has participated in several national and international scientific congresses and courses focusing on epidemiology, biostatistics, physiology and pathophysicsology of kidney diseases. In 2012 he has joined the Cochrane Renal Group (Sydney, Australia) as a honorary research fellow, learning skills in systematic reviews, meta-analysis and literature searching. Dr. Bolignano is co-author of several scientific papers published on national and international peer-review journals and official interventions at national and international congresses on nephrology, metabolism, cardiovascular and laboratory medicine. His main fields of research are: the epidemiology and pathophysiology of chronic and acute kidney diseases, renal biomarkers, cardiovascular risk, vasopressin system, medullar and systemic effects of erythropoietin and stem cells in uremia.

Michele Buemi graduated in medicine and surgery in 1973. After graduation in 1974, he became an assistant at the Institute of General Medicine and Medical Care at the University of Messina. He specialized in renal, hematologic diseases, and metabolic disorders in 1976. In 1979, he became university teacher of renal physiology at the School for Specialization in Nephrology, and in 1985, teacher of andrologic urology at the School for Specialization in Diabetology. In 1986, he became
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