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RESEARCH ARTICLE

ROLES OF RELAXIN ON CARDIOVASCULAR SYSTEM AND ITS POSSIBLE ROLE IN CARDIOVASCULAR DISEASES

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ABSTRACT

For the first half a century since its discovery, the peptide hormone Relaxin (RLX) encountered little attention as it was considered a pregnancy hormone only producing interpubic ligament elongation and uterine quiescence. Recently, however, RLX has emerged as an intriguing biological and pharmacological agent a very potent and intriguing bioactive agent and new promising pharmacological tool. It resembles insulin and insulin-like growth factors, its receptors have been well characterized as has as its main mechanism of action through AMP and nitric oxide (NO). The hormone is secreted into blood by the corpus luteum in pregnancy and during ovulatory cycles, although paracrine secretion has been found in several organs of both sexes. As it is active in heart, brain, uterus, lungs, kidneys, mammary gland, etc., RLX deserves to be called a 'pleiotropic hormone' and its widespread activities are consistent with the concept that it acts as the general manager of pregnancy, devoted to controlling and adjusting the body's response to the obligatory increase in foeto-maternal needs that occur. In addition, there is evidence that RLX is active outside of pregnancy and in both sexes, particularly on the cardiovascular system (CVS). Based on the potent effects of RLX on the heart, vessels and blood, the results of experimental studies and the recent data from clinical trial in acute heart failure (AHF)(5) and ischemic-cardiovascular diseases (iCVD)(6,7,8) RLX is now recognized as a physiologic cardiovascular hormone it's role as a novel therapy treating cardiovascular disease is currently an area of intense investigation.

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INTRODUCTION

Relaxin: short presentation and pathophysiology on cardiovscular system: For the first half a century since its discovery, the peptide hormone Relaxin (RLX) encountered little attention as it was considered a pregnancy hormone only producing interpubic ligament elongation and uterine quiescence (Fig 1). Recently, however, RLX has emerged as an intriguing biological and pharmacological agent a very potent and intriguing bioactive agent and new promising pharmacological tool. It resembles insulin and insulin-like growth factors, its receptors have been well characterized as has as its main mechanism of action through AMP and nitric oxide (NO). The hormone is secreted into blood by the corpus luteum in pregnancy and during ovulatory cycles, although paracrine secretion has been found in several organs of both sexes. As it is active in heart, brain, uterus, lungs, kidneys, mammary gland, etc. (Bani, 1997), RLX deserves to be called a '*pleiotropic hormone'* and its widespread activities are consistent with the concept that it acts as the general manager of pregnancy, devoted to controlling and adjusting the body's response to the obligatory increase in foeto-maternal needs that occur (Bigazzi, 2001). In addition, there is evidence that RLX is active outside of pregnancy and in both sexes, particularly on the cardiovascular system (CVS). Based on the potent effects of RLX on *the heart, vessels and blood*, the results of experimental studies (Masini, 1997; Nistri, 2007) and the recent data from clinical trial in acute heart failure (AHF)(5) and ischemic-cardiovascular diseases (iCVD) (Bigazzi, 2012; Sonaglia, 2013; Milia, 2013) RLX is now recognized as a physiologic cardiovascular hormone it's role as a novel therapy treating cardiovascular disease is currently an area of intense investigation.

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Physiopathology of Relaxin on The Cardiovascular System

Mechanism Of Action*:* A prompt and sustained release of NO is the main mechanism of action of RLX in the CVS, as demonstrated by our studies on isolated hearts (Bani Sacchi,

1995) and cultures of bovine aortic smooth muscle cells and rat coronary artery and human umbilical vein endothelial cells (Bani, 1998). In these studies RLX induced a dose-dependent biological cascade, increasing NO production and then intracellular levels of cGMP, which inhibited cytosolic Ca2+ concentration increases and resulted in cell relaxation in both cell types. As depicted in Figure 2, NO biosynthesis is activated by increasing the expression and activity of inducible NO synthase. This NO-stimulating effect of RLX can result from both a direct receptor-mediated intracellular signaling mechanism (Nistri, 2003) and an indirect pathway involving RLX-mediated release of matrix metalloprotease, which in turn activate endothelin and stimulate endothelin B receptors (Jeyabalan, 2003).

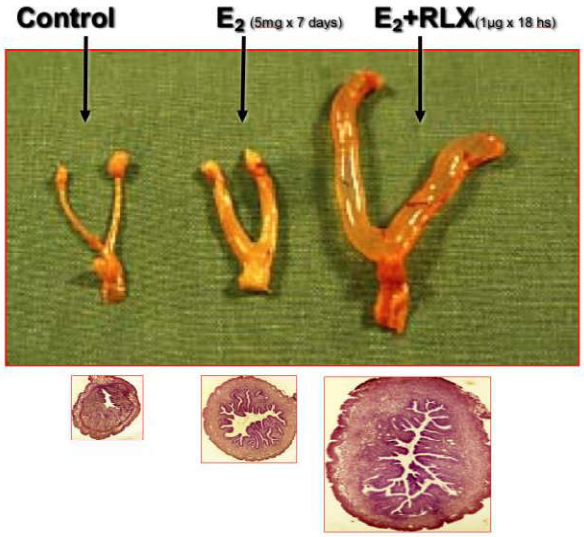

Fig. 1. Potency of the relaxin's effects: uterine growth and vasodilation in virgin prepubertal mice pretreated with E2 for 7 days and then receiving relaxin for 18 hours

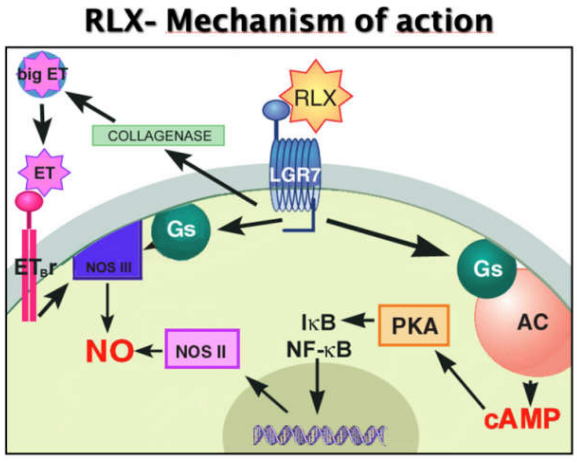

Fig. 2. Main mechanisms of action of relaxin on target cells

Relaxin and Vessels

Vasodilation: Vasodilatation has emerged as a primary effect of RLX. It appears to be physiologic since it is manifest at the nanomolar concentrations that are seen in the blood during human pregnancy and it is particularly evident in the microcirculation, e.g. arterioles, capillaries and venules (Bigazzi, 1986; Bani, 1988). Relaxin induced vasodilation occurs regardless of gender and is accompanied by a prompt increase in blood flow (Bani Sacchi, 1995). In isolated, perfused hearts the increase in coronary flow was significantly higher than that obtained with similar doses of acetylcholin or sodium nitroprusside (respectively 100 and 1000-fold) (Bani Sacchi, 1995); in human penile artery, intracavernousa administration of 10 ng RLX induced a blood flow increase similar to10µg prostaglandin 2α and 10 mg papaverine (Bigazzi, 2001); in the rat mesocaecum RLX counteracted similar concentrations of norepinephrine (Nistri, 2003), in the rat kidney RLX blunted the vasoconstrictive response to angiotensin II (Bigazzi, 1986). It appears that the vasodilatory effects of RLX are more prominent in vasoconstricted than in normal blood vessels (Bani, 1988), suggesting that this hormone could be a natural regulator of vascular tone during physiologic and pathophysiologic conditions and that it could be used as a treatment for hypertension.

Relaxin and Angiogenesis*:* While RLX does not show direct angiogeneic effects under normal conditions, in the presence of ischemia or inflammation, it increases the production of potent angiogenic molecules such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) (Unemori, 1999). Furthermore, in a rat model of myocardial infarction (MI), systemic infusion of RLX potentiates bFGF mRNA expression by both cardiomyocytes and fibroblasts in the peri-infarct region. Similarly, in a post-MI swine model, local production of RLX by *RLN2* gene-transfected myoblasts grafted into the post-ischemic myocardium significantly increased microvessel density and expression of VEGF mRNA by host cardiac cells (Formigli, 2007; Perna, 2005). These findings support a possible role of RLX in augmenting perfusion of ischemic tissue through increased vasodilation and angiogenesis in some conditions.

Relaxin and the Heart**:** Considering the high levels of RLX receptor expression in the heart, this organ has emerged as one of the principal targets for RLX actions (21). In fact, experimental and clinical studies support the concept that RLX potently stimulates functions of the heart and circulation. In isolated perfused hearts, infusion of RLX (30 ng/ml) induced a prompt and highly significant increase of the coronary blood flow and a parallel rise of NO metabolites in the perfusate, which was paralleled by a decrease of histamine (Bani Sacchi, 1995). Several reports indicate that RLX exerts positive chronotropic and inotropic effects on isolated right heart atrium (Nistri, 2007), supporting a role of the hormone in the well-known elevation of the cardiac output in pregnancy. Whereas a positive inotropic effect was not clearly demonstrated in isolated ventricular tissue, a prompt increase of the left ventricular ejection fraction, accompanied by evident clinical amelioration, can be commonly found in patients receiving RLX for severe chronic heart failure (M.B. unpublished data), that could be related to RLX effects on myocardium function as well as indirect effects resulting from vasodilation and a reduction in peripheral resistance that facilitates an increase in cardiac output. The increase in cardiac index with RLX observed in the post-MI swine model is consistent with our clinical observations (Perna, 2005). An additional potential benefit of RLX is that systemic administration induces a prompt release of atrial natriuretic peptide (ANP) from atrial cardiac myocytes (Toth, 1996). which further increases the blood flow and vasodilation and may contribute to the decrease of the serum Na that is commonly observed in normal pregnancy. Finally, paracrine secretion of RLX has been described from atrial cardiac myocytes with a possible role in the physiologic local

regulation of heart and lung function and growth (Taylor, 1994).

Rlx and Blood

Together with vasodilation RLX acts at various levels on blood cells and hemostasis to reduce thrombosis and coagulation. Preincubation of isolated human and rabbit platelets with RLX, ADP- and thrombin-induced aggregation as wells thromboxane release in a dose dependent manner (Bani, 1995) (Fig. 3) and chronic administration of RLX to male rats causes a marked reduction of circulating platelets and thrombogenesis arrest in spleen megacariocytes (Bani, 2005). RLX also acts on hemostasis factors. Chronic subcutaneous administration of porcine RLX to rats reduces plasma fibrinogen level and increases fibrinolytic factors, tissue plasminogen activator (TPA) and plasminogen activator inhibitor-1(PAI-1), reduces serum cholesterol levels and decreases blood concentration of hemoglobin and Na+, probably as a result of blood dilution such as occurs in pregnant females (Bigazzi, 1995). Such effects on clotting and hemostasis may explain the increased menstrual bleeding occasionally observed in young female patients as the only negative side effect reported in chronic RLX treatment for scleroderma (Seibold, 2000). Moreover RLX strongly counteracts the inflammatory activation of neutrophils, reducing oxidative burst reactive oxygen species (ROS) generation and chemotaxis and it inhibits the release of histamine from basophils and mast cells in a dose dependent manner (Masini, 2004).

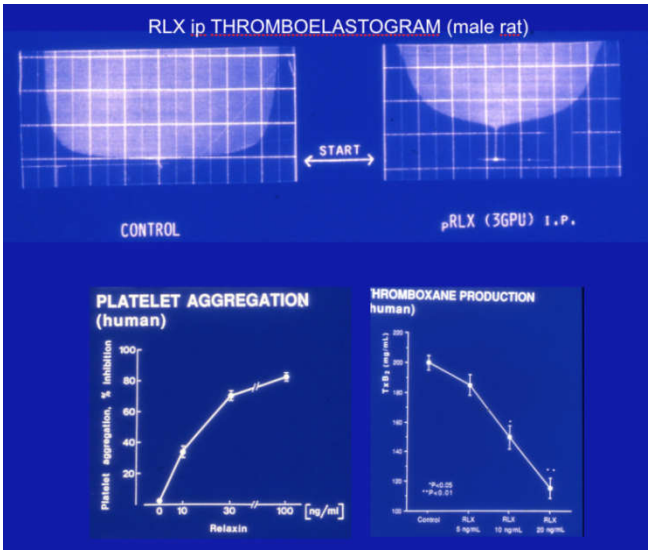

Fig. 3 Upper panels: thromboelastograms showing the reduction of blood clotting in male rats receiving i.p. injection of relaxin Lower panels: dose-related inhibition of in vitro human platelet aggregation and thromboxane release

Relaxin and Ischemic cardiovascular diseases

Vasospasm, thrombosis and ischemia on one hand and recruitment and activation of inflammatory leukocytes and platelets on the other are recognized as playing a role in the initiation and evolution of atherosclerosis and ischemic CVD (Napoli, 2006). The effects of RLX described are well suited to counteract the pathogeneic steps of ischemic CVD as well as the consequent tissue damages. Therefore a potential role of relaxin against ischemic CVD (Nistri, 2007) can be postulated. Support for the concept that RXL can act as a potent new tool for the treatment, prevention and rehabilitation comes from epidemiological studies, animal experiments and human clinical observations.

Therapy

***Rlx and Icvd: Animal Experiments*:** We performed studies on isolated and perfused guinea pig hearts, and on intact rats and pigs using the Ischemia-Reperfusion model (IR) to induce myocardial infarction (Lefer, 1991). Results of these experiments provided evidence that RLX counteracts the primary damage to the coronary endothelium and opposes the resulting impairment in NO production that initiates the cascade of events leading to myocardial contractile dysfunction and irreversible tissue injury (Masini, 1997). In fact when RLX was given preventatively as a single bolus (100 ng), 20 min before ischemia, or (in the attempt to replicate the clinical course of patients ongoing acute MI treated with percutaneous coronary interventions) administered, at varying doses at the time of reperfusion after a 30-min period of ischemia, there were highly significant beneficial effects including maintaining coronary flow and reducing the necrotic myocardial area as well as of the occurrence of severe ventricular tachyarrhythmias and hence reduced the overall mortality at reperfusion of the animals from 81% in the controls to 25% in the RLX-treated ones. In addition, RLX prevented neutrophil extravasation into the myocardial tissue and produced an highly significant decrease of the main tissue markers of leukocyte accumulation, mast cell activation and myocardial injury and biomarkers coming from myocytes and other cells. Relaxin strongly reduced histopathological signs of irreversible cardiomyocyte and endothelial injury and maintained the ultrastructure of the myocardial tissue (Bani, 1998). Finally, RLX improved ventricular performance and increased salvage of myocardium in this model (Perna, 2005; Osheroff , 1993; Toth, 1996; Taylor, 1994; Bani, 1995; Bani, 1995; Bigazzi, 1995; Seibold, 2000; Masini, 2004; Napoli, 2006; Lefer, 1991 and Bani, 1998) (Fig.4). These findings are consistent with the possibility that RLX could be used not only as a preventative tool, but also as adjunctive therapeutic agent at the time of MI (Fig 4-5). Evidence that RLX induces angiogenesis in the peri-infarct area in both rat and swine models of chronic MI (Formigli, 2007; Perna , 2005) raises the possibility of a positive influence of RLX in chronic vascular artheriosclerosis. In support of this possibility is a study on rats with isoproterenol-induced cardiac ischemia, in which the assayed parameters of myocardial damage and dysfunction improved markedly upon treatment with exogenous RLX (Zhang, 2005). In this study, there was also evidence that the injured heart also appears to up-regulate the production and release of endogenous RLX, supporting the notion that RLX is a cardiac hormone that could through paracrine effects help protect against cardiac ischemic injury and dysfunction (Dschietzig, 2001). Interestingly, RLX also appears to have protective effects against IR-induced injury in the brain and other organs (Casten, 1960).

RLX and ICVD: Human Observations

As far back as 50 years ago, Casten, using partially purified porcine-RLX (pRLX), noted impressive amelioration of circulatory symptoms and ischemic ulcers in patients. The results were attributed to a passive dilation of the vessels induced by a loosening effect of RLX on connective tissue. Moreover subjects also suffering from ischemic heart disease had a reduced need for daily nitrovasodilator therapy (Casten, 1960).

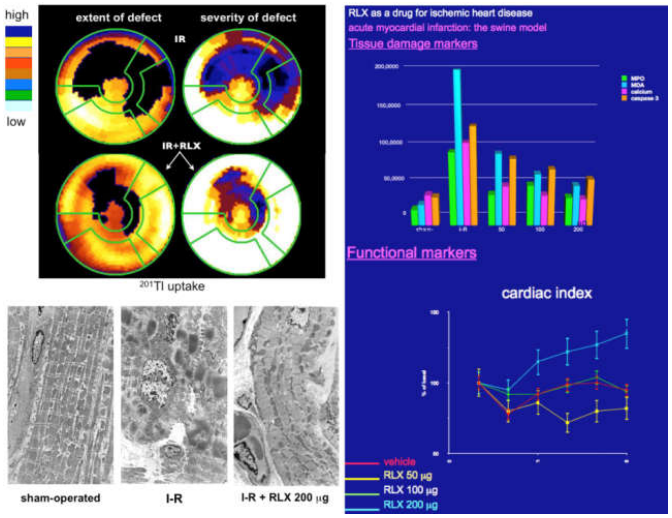

Fig. 4. Experimental myocardial infarction in pigs: clock wise from upperless, Relaxin, given at reperfusion, reduces the extension and severity of myocardial tissue injury and the markers of myocardial tissue injury and inflammation, produces the functional improvement of cardiac index and the myocardial salvage

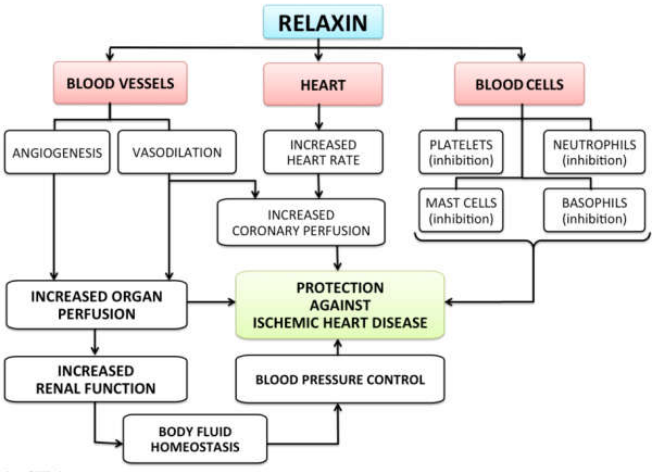

Fig. 5. Multiple levels at which relaxin counteract ischemic cardiovascular damage, in the heart

These results, however, were criticized for the use of the impure RLX preparation. This concern can now be countered by our recent clinical observation (Bigazzi, 2012) in a 30 years old male with severe occlusive PAD of the popliteal artery of left lower limb. After an unsuccessful attempt at surgical reconstruction of the femoral-popliteal arteries, the patient was scheduled for leg amputation at the thigh level because of the rapid progression of severe ischemia and ulcers. Compassionate use treatment with RLX was attempted and the patient was treated with subcutaneous injections of human recombinant RLX (Connetics, USA), 20 µg./kg bw, x day. Within the first few hours after the first RLX injection there was evidence of improvement and it was decided to continue treatment for four months. This resulted in progressive improvements in clinical symptoms as well as evidence of increased blood flow to the leg and foot which was demonstrated by the presence of a well developed collateral circulation. Therapy was continued using purified oral pRLX, (kindly given by Dr Yue, USA), 20 µg twice a day for 3 months cycles, alternating with 3 months pauses. After 4 years of treatment, repeat study revealed the presence of a newly formed elicoidal artery connecting the deep femoral artery to the tibial arteries, producing an efficient by-pass that delivered an adequate blood flow to the foot (Fig. 6). The patient is being maintained on periodical cycles of oral pRLX, and he has resumed a normal life style. No side effects of prolonged RLX administration have been observed.

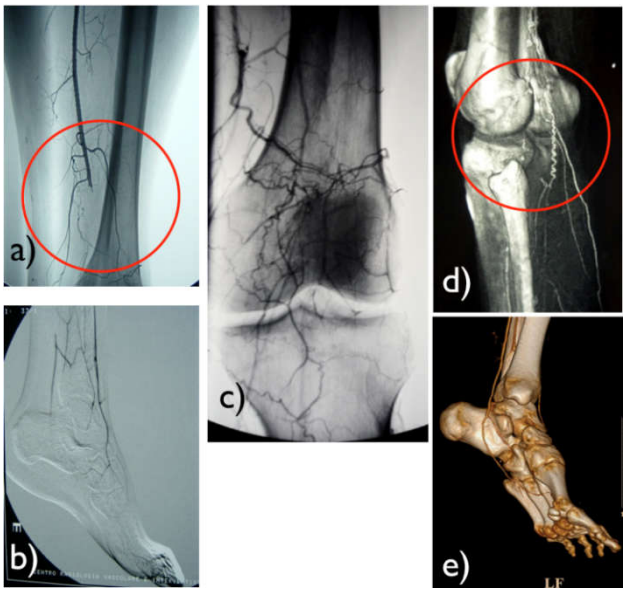

Fig. 6. Immediate and long-term therapeutic effects of relaxin treatment in young patient with PAD of the left leg: a-b) superficial femoral artery occlusion at first admission; c) well-developed collateral circulation upon 4 months treatment; d-e) newly formed helicoidal artery producing an efficient natural by-pass between the deep femoral and tibial arteries, with efficient restoration of downstream blood flow

This experience of re-vascularization in human has been anecdotically confirmed in another male patient, 56 years old, with severe and diffuse coronary atherosclerosis who was treated with oral pRLX administration (20µg, twice daily for 2 years) and who reported relief from angina, while repeat angiogram after 2 years of treatment suggested neo-formation of new coronary arteries (Fig 7) .

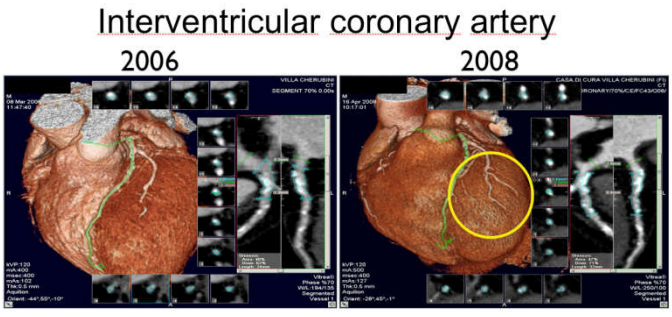

Fig. 7. Angio-TC scans of coronary arteries in a 56-years old male patient suffering for angina pectoris, taken before and 2 years after oral pRelaxin treatment. The objective improvement was accompanied by remission of symptoms

Rlx and ICVD :Prevention It has been postulated that cyclic secretion of RLX in blood from the ovulatory corpus luteum is likely to be the yet unidentified endogenous factor responsible for protection against ischemic CVD in women until menopause (3). Indeed, clinical observations and epidemiological studies clearly indicate that men and women differ substantially in terms of onset and outcome of CVD which is very low in women during fertile life, while after menopause it increases to match that of men, albeit with a 10-20-year delay (35). Until recently the cause of such loss of

CVD protection after menopause has been attributed to the decay of ovarian sex steroid hormones. However, replacement therapy with estrogens and progesteron has failed to restore vascular protection; rather the administration of these hormones, as a contraceptive pill, is associated with an increased risk of thrombosis (36). If carefully analyzed, these ostensibly opposite conditions share a common biologic event, i.e. the cessation of ovulation and hence of cyclic RLX secretion, which may cause the interruption of the women's natural protection. Therefore, it is conceivable that RLX administration could restore the natural cardiovascular protection in un-ovulating women (for contraception or menopause) and also be also useful for primary and secondary prevention of iCVD in both men and women (Bigazzi, 2001).

of tests and, in particular the assessment of cognitive function, in these populations. Moreover, the general status of the subjects was maintained even in the following months after RLX administration had been completed (Fig 8). These findings raise the possibility that RXL may be useful in post-stroke rehabilitation.

Concluding remarks on RLX and iCVD: From all the above reported studies and experiences we may speculate that, in the near future, relaxin hormone could represent a new therapeutic and preventative tool to afford reduction of both the incidence and the disability of vascular ischemic diseases which represent the most commune cause of death and disability in the western civilized countries.

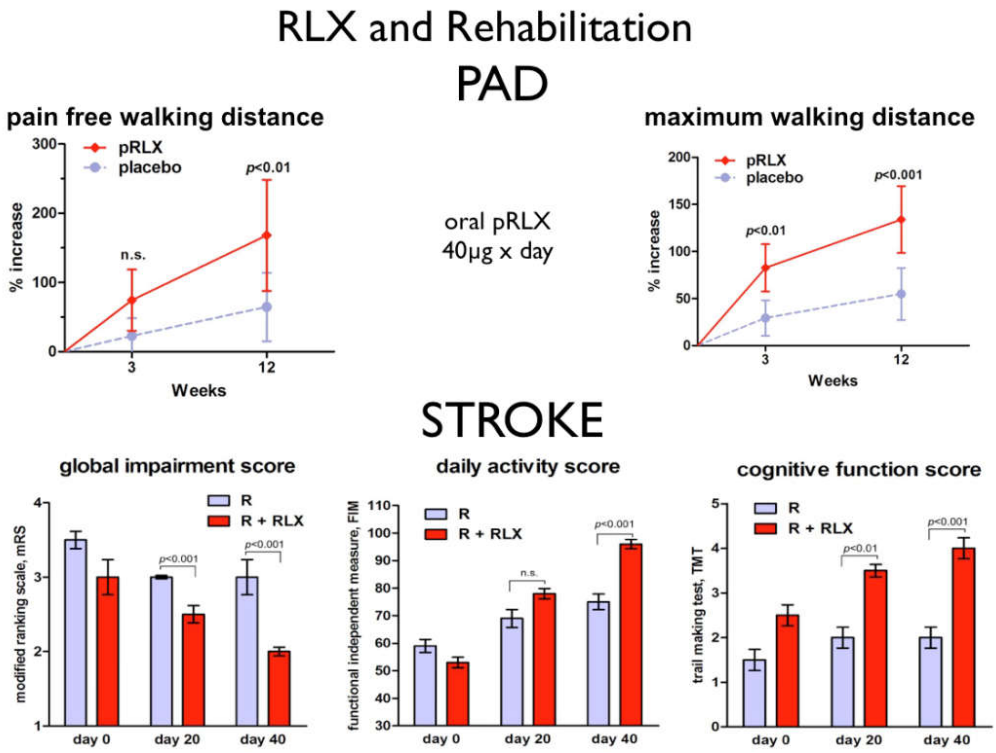

Fig.8. Preliminary results on the use of oral pRelaxin in physical rehabilitation. Upper: the prompt amelioration of the functional status of patient of the PAD; Lower: the prompt, marks improvement of the clinical status in patients recovering with recent brain stroke

RLX and ICVD: Rehabilitation: Preliminary results from recent proof-of-concept, double mind-controlled clinical trials performed at the Prosperius Tiberino Rehabilitation Clinic (Umbertide, Italy) on patients under rehabilitation treatment for Peripheral Arterial Diseases of the lower limbes (PAD) and recent brain stroke have shown the beneficial effects of adjunctive oral porcine RLX (20 µg, twice daily) for 12 weeks or 40 days respectively, without any negative side effects. In PAD patients RLX has produced a prompt, significant amelioration of the primary outcome parameters of physical rehabilitation, as measured by the Pain Free Walking Distance and Maximum Walking Distance tests, associated with subjective health improvement (Sonaglia, 2013) (Fig.8). In stroke patients, RLX treatment was associated with improved psyco-neurologic recovery and increased physical resistance to rehabilitative exercises, as assessed by specific tests (Global Impairments Score, Daily Activity Score and Cognitive Function Score) These improvements, especially the global impairment and the cognitive function, were already appreciable after the first week of treatment and became significant at 20 days of treatment (Milia, 2013). The beneficial effects of RLX were manifest across a broad range

Relaxin and Heart Failure

As already noted, RLX is known to have substantial effects on the cardiovascular system. Recognition that many of these effects might benefit patients with heart failure lead to the assessment of a recombinant form of this hormone, serelaxin (sRLX), in patients with acute heart failure (AHF). Findings from Pre-RELAX-AHF, a multi-dose pilot study, provided initial evidence that sRLX improved the clinical course of patients hospitalized with AHF (37). This study randomized patients with documented AHF within 16 hours of hospital admission. Patients were required to have preserved or increased systolic blood pressure >125 mmHg, impaired renal function with estimated glomerular filtration rate (eGFR) between 30-75 ml/min/1.73m^2 and to have been treated with the equivalent of at least 40 mg of IV furosemide prior to enrollment into the study. Once randomized, the 234 patients were assigned to treatment for 48 hours with either sRLX in doses ranging from 10 to 250 ug/kg/day IV or placebo in addition to their standard therapy. A dose of 30 ug/kg/day appeared to have the most favorable effects across multiple domains including the relief of dyspnea and these findings

were supported by results with the 10- and 100 ug/kg/day dose. Patients treated with the 30 ug/kg/day dose experienced an 87% reduction in the hazard ratio for the combined end-point of death due to cardiovascular causes or readmission due to heart failure or renal failure at day 60 as well as a significant reduction in all-cause mortality at day 180 compared to patients assigned to placebo.

Assessment of adverse events did not show a deleterious study drug effect and hypotensive adverse events were similar to placebo. Based on the results of Pre-RELAX-AHF, the pivotal RELAX-AHF study was undertaken to test the hypothesis that sRLX patients would have greater dyspnea relief than patients treated with standard care and placebo (5) (Fig. 9). In RELAX-AHF patients admitted to hospital for AHF were randomly assigned (1:1) to standard care plus 48-h intravenous infusions of placebo or sRLX (30 μg/kg per day) within 16 h from presentation.

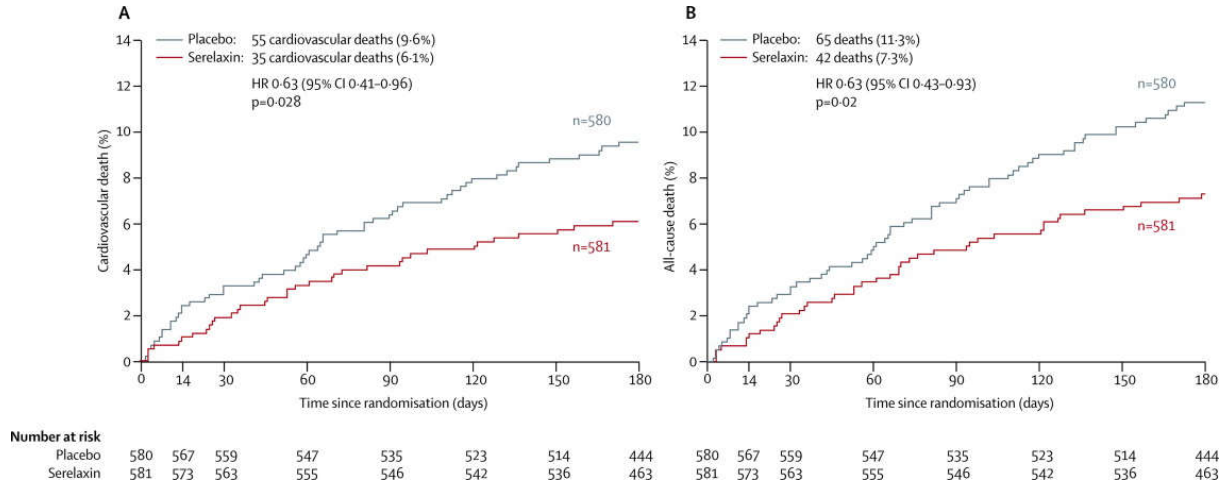

Fig. 9. Kaplan-Meier analysis of death (A) Cardiovascular and (B) all-cause death during 180 days of follow-up in the placebo-treated group compared with the group that received serelaxin in the intention-to-treat (ITT) population. The additional efficacy analysis of cardiovascular death in the ITT population was protocol-specified, whereas all-cause death in the ITT population is a post-hoc sensitivity analysis presented for comparison. A Kaplan-Meier analysis of the protocol-specified, all-cause death up to day 180 in the safety population is shown in the appendix (placebo, 64 deaths [11·3%]; serelaxin, 41 [7·3%]; HR 0·63 [95% CI 0·42–0·93]; p=0·02). HR=hazard ratio

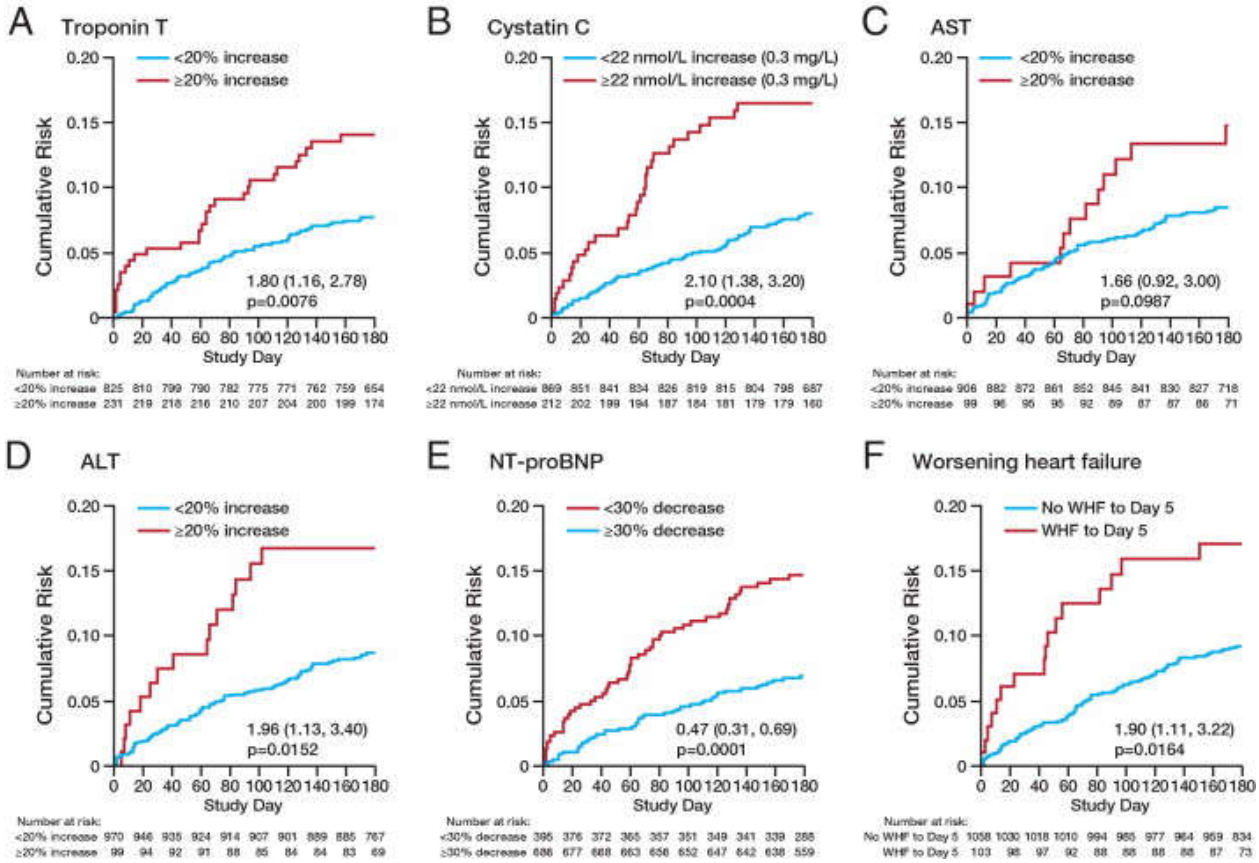

Fig. 10. Risk for Death by Early Changes in Markers of Organ Function, Damage, and Congestion . All-cause death through day 180 in Relaxin in Acute Heart Failure study patients subdivided by percentage change in troponin T from baseline to day 2 (A), absolute change in cystatin-C from baseline to day 2 (B), percentage increases in serum aspartate transaminase (AST) (C) and serum alanine transaminase (ALT) (D) from baseline to day 2, decrease in N-terminal pro–brain natriuretic peptide (NT-proBNP) from baseline to day 2 (E), and worsening heart failure (WHF) by day 5 (F)

Entry criteria were similar to those used in Pre-RELAX-AHF and the characteristics of the 1161 patients are summarized in Table 1.

Table 1. (from reference 5) Baseline characteristics of the patients in the intention-to-treat population

	Placebo (n=580)	Serelaxin (n=581)
Age (years)	72·5 (10·8)	71·6 (11·7)
Men	357 (62%)	368 (63%)
White	552 (95%)	544 (94%)
Weight (kg)	82·8 (18·7)	81·9 (18·5)
Body-mass index (kg/m^2)	29·5 (6·1)	29·1 (5·3)
Region*		
Eastern Europe	282 (49%)	280 (48%)
Western Europe	101 (17%)	103 (18%)
USA	55 (9%)	59 (10%)
Argentina	37 (6%)	34 (6%)
Israel	105 (18%)	105 (18%)
Systolic blood pressure (mm Hg)	142·1 (17·0)	142·2 (16·2)
Diastolic blood pressure (mm Hg)	81·7 (13·2)	82·2 (14·2)
Heart rate (beats per min)	80·4 (14·9)	78·9 (15·0)
Respiratory rate (breaths per min)	22·0 (4·6)	21·8 (4·6)
Admitted to hospital for heart failure in past year	181 (31%)	216 (37%)
Number of admissions for heart failure in past year	1·5 (1·1)	1·7 (1·5)
Most recent ejection fraction (%)	38·6% (14·3)	38·7% (14·8)
Ejection fraction <40%	295 (55%)	303 (55%)
New York Heart Association class 30 days before admission		
Class I	11 (3%)	12 (3%)
Class II	140 (33%)	164 (38%)
Class III	198 (47%)	191 (44%)
Class IV	72 (17%)	63 (14%)
Medical history		
Hypertension	510 (88%)	496 (85%)
Hyperlipidaemia	313 (54%)	304 (52%)
Stroke or other cerebrovascular event	84 (14%)	73 (13%)
Cigarette smoking	81 (14%)	72 (12%)
Peripheral vascular disease	82 (14%)	73 (13%)
Mitral regurgitation	182 (31%)	179 (31%)
Ischaemic heart disease	307 (53%)	296 (51%)
Pacemaker	58 (10%)	63 (11%)
Biventricular pacing	52 (9%)	61 (10%)
Implantable cardiac defibrillator	75 (13%)	79 (14%)
Atrial fibrillation or flutter	305 (53%)	297 (51%)
Atrial fibrillation at screening	246 (42%)	233 (40%)
Asthma, bronchitis, or chronic obstructive pulmonary disease	88 (15%)	96 (16%)
Diabetes mellitus	272 (47%)	279 (48%)
Concomitant heart failure drugs at baseline		
Angiotensin-converting enzyme inhibitors	320 (55%)	313 (54%)
Angiotensin receptor blockers	97 (17%)	88 (15%)
β blocker	407 (70%)	387 (67%)
Aldosterone antagonist	173 (30%)	193 (33%)
Digoxin	108 (19%)	120 (21%)
Intravenous loop diuretic	580 (100%)	578 (99%)
Time from presentation to randomisation (h)	7·9 (4·7)	7·8 (4·6)
Intravenous nitrates at randomisation	42 (7%)	39 (7%)
NT-proBNP (ng/L)	5003 (4633–5404)	5125 (4772–5506)
Troponin T (μg/L)	0·036 (0·034–0·039)	0·034 (0·032–0·037)
eGFR (mL/min per 1·73 m^2)†	53·3 (12·9)	53·7 (13·1)

Data are mean (SD), n (%), or geometric mean (95% CI). NT-proBNP=N-terminal prohormone of brain natriuretic peptide.
*Eastern Europe (Hungary, Poland Romania), western Europe (France, Germany, Italy, Netherlands, Spain).
†Estimated glomerular filtration rate (eGFR) calculated by the simplified Modification of Diet in Renal Disease formula.

The primary endpoints evaluating dyspnea improvement were change from baseline in the visual analogue scale area under the curve (VAS AUC) to day 5 and the proportion of patients with moderate or marked dyspnea improvement measured by Likert scale during the first 24 h, both analysed by intention to treat. Serelaxin improved the VAS AUC primary dyspnea endpoint by 19.4% (p=0.007) compared with placebo. Although sRLX had no significant effect on the other primary endpoint, there were numerical advantages compared to placebo at all time points extending out to day 14. Despite increased use of intravenous diuretic and vasoactive drugs there were significiantly greater reductions in signs and symptoms of congestion (e.g. edema, rales, orthopnea, jugular venous pressure and dyspnea on exertion) in sRLX compared to placebo group patients at day 2. Treatment with serelaxin was also associated a reduced likelihood of worsening heart failure (defined as worsening signs or symptoms of heart failure that necessitated intensification of intravenous or mechanical heart failure treatment) from the time of randomization to day 5 and the average length of hospital stay was significantly reduced in the sRLX group by 0.9 days and

time in the intensive care or coronary care unit was reduced by 0.4 days. While there were no significant improvements with sRLX for the secondary endpoints of cardiovascular death or readmission to hospital for heart failure or renal failure or days alive out of the hospital up to day 60, treatment with sRLX was associated with significant reductions of other pre-specified additional endpoints, including fewer deaths at day 180 (placebo, 65 deaths; sRLX, 42; HR 0.63, 95% CI 0.42–0.93; p=0.019).

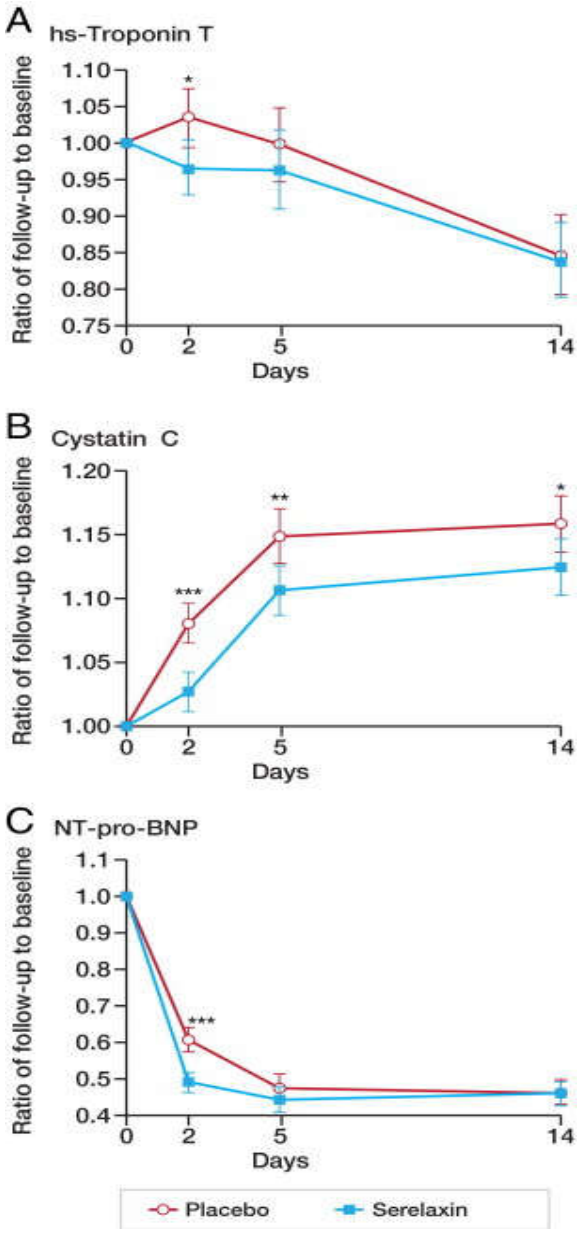

Fig. 11. Biomarker Changes From Baseline in the Placebo and Serelaxin Groups. Changes from baseline to each study day in the Relaxin in Acute Heart Failure study in high-sensitivity (hs) troponin T (A), cystatin-C (B), and N-terminal pro–brain natriuretic peptide (NT-proBNP) (C). *p < 0.05, **p < 0.005, and ***p < 0.001 by repeated-measures analysis of variance with adjustment for baseline value

A similar 37% reduction in the risk of cardiovascular death was also seen in patients treated with sRLX. Further analysis of the results failed to detect subgroups of patients in the study that were more likely to respond to the favorable effects of sRLX (38,39). Since an ejection fraction (EF) cut-off was not mandated for entry into RELAX-AHF, the study included both patients with reduced and preserved EF. For those patients whose EF was known, analysis of response based on whether heart failure was due to either preserved or reduced EF indicated no significant difference between the groups for efficacy of sRLX in dyspnea relief, mortality reduction or renal failure at 60 days (39). The effects of sRLX on 180 cardiovascular and all-cause mortality in both Pre-RELAX-AHF and RELAX-AHF raised the possibility that treatment that was administered for 48 hours shortly after hospitalization for AHF might have sustained benefits on the patients' clinical course. Biomarkers have been shown to be useful in determining risk in heart failure patients. To help provide information about the possible mechanism(s) by which sRLX improved the clinical course of RELAX-AHF patients, a panel of biomarkers was assessed in the study population(40). Findings from this analysis confirmed that changes in high sensitivity troponin T, creatinine and cystatin-C, aspartate transaminase and alanine transaminase and N-terminal pro-brain natriuretic peptide (as indicators of cardiac, renal and liver injury and the extent of congestion, respectively) were all associated with worse 180 survival in the RELAX-AHF population (Fig.10). However, sRLX administration improved these biomarkers, suggesting that this drug both prevented organ damage and lead to more rapid decongestion (Fig.11).These results provide a plausible explanation for how an agent that is administered for only 48h could benefit the long-term course and reduce post-discharge mortality of patients hospitalized with AHF. This possibility is currently being tested in the RELAX-AHF2 study, an international randomized clinical trial that is designed to enroll over 6000 patients hospitalized with AHF. This study has been designed with cardiovascular mortality as the primary endpoint. RELAX-AHF2 is currently underway with a projected completion time in 2015.

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