ORIGINAL ARTICLE

Early Anti-inflammatory and Pro-angiogenic Myocardial Effects of Intravenous Serelaxin Infusion for 72 H in an Experimental Rat Model of Acute Myocardial Infarction

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Abstract Sprague Dawley rats were subjected to acute myocardial infarction (AMI) by permanent ligation of the left anterior descending coronary artery. At the time of AMI, a subcutaneous mini-osmotic pump was implanted and animals were randomized into three groups, according to the intravenous therapy received during the first 72 h: placebo-treated (saline), serelaxin10-treated (SRLX10 = 10 μg/kg/day), or serelaxin30-treated (SRLX30 = 30 μg/kg/day). Treatment with SRLX30 reduced the expression of inflammatory cytokines and chemokines, as well as the infiltration of macrophages, and increased the expression of pro-angiogenic markers and vessel density in the infarcted myocardium after 7 days. SRLX30 did not reduce early myocardial fibrosis but reduced myocardial levels of sST2 and galectin-3. No significant effects were observed with SRLX10 treatment. A significant correlation was observed between plasma levels of serelaxin and effect measures. The results suggest serelaxin has a protective effect in early processes of cardiac remodeling after AMI.

Keywords Myocardial infarction · Left ventricular systolic dysfunction · Serelaxin · Remodeling · Fibrosis

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Abbreviations

AMI	Acute myocardial infarction
α-SMA	Alpha smooth muscle actin
GADPH	Glyceraldehyde 3-phosphate dehydrogenase
IL	Interleukin
LVEF	Left ventricle ejection fraction
MCP-1	Monocyte chemoattractant protein 1
MMP	Matrix metalloproteinase
RT-PCR	Reverse transcription polymerase chain reaction
sST2	Soluble isoform of suppression of tumorigenicity 2
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

Introduction

Acute myocardial infarction (AMI) remains a therapeutic challenge. New therapies are necessary to improve the reparative processes involved in early phases after AMI, in order to prevent myocardium cell loss and progressive adverse left ventricular (LV) remodeling, which may lead to heart failure (HF). The naturally circulating hormone relaxin-2 (relaxin) is a member of the insulin-like peptide family [1], which is believed to participate in the adaptive hemodynamic changes

Table 1 Descriptive parameters in the anatomical and echocardiographic study

	Sham	AMI (placebo)	AMI + SRLX10	AMI + SRLX30
n	10	8	9	9
Mortality rate (%)	0	33	25	25
Anatomy				
BW_s (g)	238 ± 6	253 ± 4	260 ± 8	259 ± 5
BW_d (g)	245 ± 9	234 ± 4	250 ± 10	244 ± 5
ΔBW (g)	6.72 ± 5.21	−18.80 ± 3.22**	−9.84 ± 3.68	−15.88 ± 2.51
HW/BW (mg/g)	3.12 ± 0.11	3.31 ± 0.12	3.20 ± 0.09	3.19 ± 0.08
KW/BW (mg/g)	3.90 ± 0.19	3.72 ± 0.10	3.90 ± 0.20	3.81 ± 0.20
LiW/BW (mg/g)	39.23 ± 0.84	38.69 ± 1.19	39.05 ± 0.75	39.12 ± 1.73
LuW/BW (mg/g)	7.91 ± 0.51	9.94 ± 0.76*	8.34 ± 0.59	8.31 ± 0.55
Echocardiography				
AMI size (%)	–	24 ± 16	21 ± 5	23 ± 7
LVEF (%)	52 ± 4	38 ± 13*	42 ± 13	44 ± 9
LVEDV (μl)	271 ± 60	347 ± 93	309 ± 51	317 ± 80
LVESV (μl)	129 ± 67	220 ± 91*	178 ± 40	181 ± 70
E/A	2.88 ± 1.63	4.74 ± 1.79*	3.46 ± 1.52	3.35 ± 2.12
E/e′	23 ± 8	30 ± 6*	24 ± 6	26 ± 11
MPI	0.55 ± 0.22	0.49 ± 0.19	0.54 ± 0.14	0.48 ± 0.20

Anatomical and echocardiographic data are expressed as mean ± SEM and mean ± SD, respectively. *n* = surviving rats; E/A = the ratio of the early (E) to late (A) ventricular filling velocities; E/e = the ratio between early mitral inflow velocity and mitral annular early diastolic velocity; AMI size = measured by echocardiography as percentage of akinetic or dyskinetic segments

BW_s body weight the day of the surgery, *BW_d* body weight at death, *ΔBW* increase of body weight ($\Delta BW = BW_d - BW_s$), *HW* heart weight, *LiW* liver weight, *LuW* lung weight, *KW* kidney weight, *LVEF* left ventricle ejection fraction, *LVEDV* LV end-diastolic volume, *LVESV* LV end-systolic volume, *LVEDD* LV end-diastolic diameter, *LVESV* LV end-systolic diameter, *MPI* myocardial performance index

*$p < 0.05$, **$p < 0.01$, AMI vs. sham; #$p < 0.05$, for serelaxin (SRLX) groups vs. AMI

that occur during pregnancy and parturition. Since the mid-1990s, basic and preclinical research has proposed relaxin as a pleiotropic and cardioprotective hormone in the setting of cardiovascular diseases [2]. Serelaxin is a recombinant form of relaxin developed as a novel therapeutic agent. The intravenous administration of serelaxin during the first 48 h following admission for acute HF was initially associated with encouraging favorable clinical outcomes [3, 4], but a recently ended clinical trial has failed to find a significant clinical benefit in this population [5].

To date, there are no data supporting serelaxin-mediated benefits in patients suffering from AMI. However, serelaxin has been shown to be protective in several experimental models of ischemic myocardial damage. For example, in ischemia-reperfusion models, serelaxin administered at reperfusion decreased myocardial damage, improved coronary blood flow and heart contractility, and protected against ventricular arrhythmias [6–9]. In AMI models induced by permanent ischemia, serelaxin improved cardiac function, induced angiogenesis, and decreased fibrosis and apoptosis [10–12]. However, from a translational point of view, serelaxin was not intravenously administered, instead intraperitoneal or subcutaneous injections were used [10–12]. Moreover, the duration of serelaxin administration was significantly prolonged (between 7 and 30 days of treatment), while the dose administered was up to 15-fold higher than that in humans [10–12].

Therefore, the present study aimed to evaluate the myocardial effects of the intravenous administration of serelaxin for 72 h, starting immediately after an experimental AMI, at doses comparable with those used in clinical studies [3, 4].

Methods

This study was approved by the ethics committee of the University of Murcia (A13150105). Expanded methods are provided in the Supplementary material.

Experimental Protocol

A total of 46 male Sprague Dawley rats (weighing 230–250 g, 6 to 8 weeks old) were obtained from the Laboratory Animal Service, University of Murcia, Spain. AMI was induced by permanent ligation of the left anterior descending (LAD) coronary artery as described in the Supplementary materials. Electrocardiography was used to demonstrate ST segment

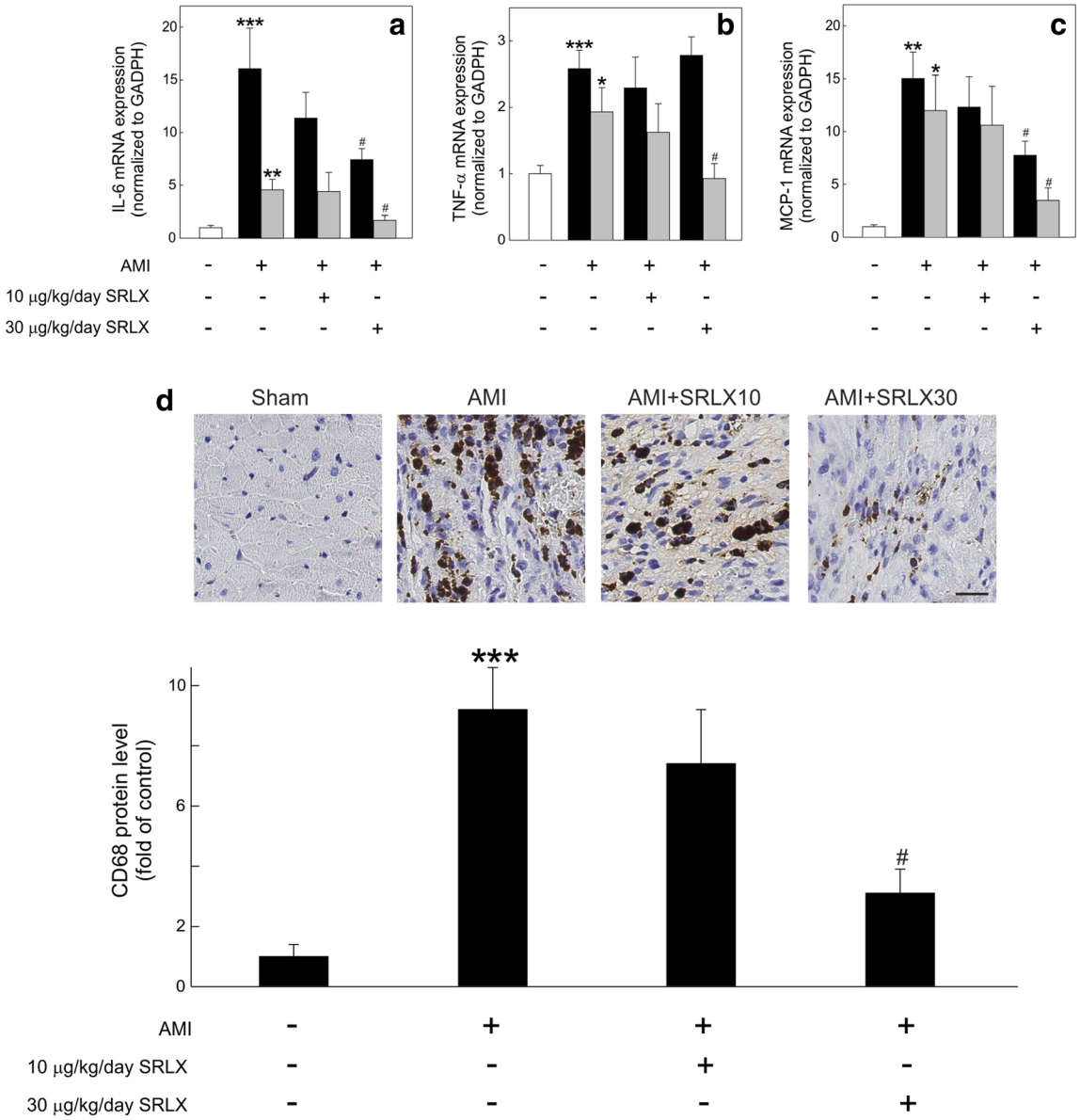
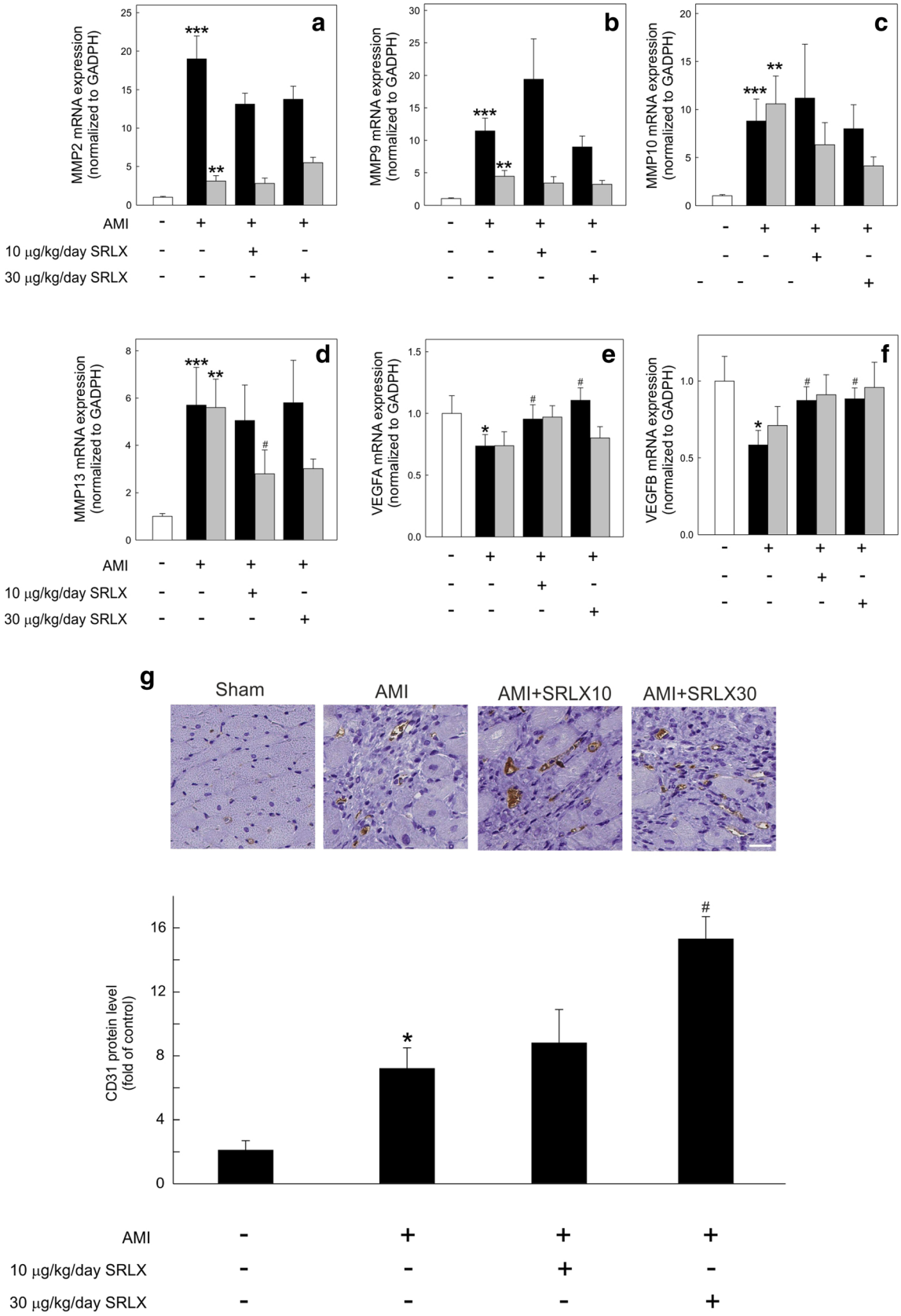


Fig. 1 Effect of serelaxin on inflammation. **a**–**c** Cardiac mRNA expression of pro-inflammatory molecules assessed by quantitative RT-PCR analysis in the infarcted myocardium (*black bars*) and border zone (*gray bars*). Data were normalized to GAPDH expression and are expressed as fold of the sham group. **d** Representative photomicrographs (×40) illustrating the immunohistochemical staining of CD-68 in the absence of AMI (sham) or in the infarcted myocardium from the infarcted groups. Bar graph shows the quantitative analysis of CD-68 expression in the infarcted myocardium. Data are expressed as fold of the sham group. *SRLX10*, 10 μg/kg/day serelaxin. *SRLX30*, 30 μg/kg/day serelaxin. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, AMI compared to the sham group; $^{\#}p < 0.05$, SRLX10 and SRLX30 compared to the AMI group

elevation and thereby confirm the success of surgery. The rats were randomly assigned to four treatment groups: (i) rats operated without LAD coronary artery ligation and treated with placebo (20 mM sodium acetate solution, pH 5.0) (sham group, $n = 10$), (ii) rats with AMI and treated with placebo (AMI group; $n = 12$), (iii) rats with AMI and treated with SRLX10 (10 μg/kg/day) (AMI + SRLX10 group, $n = 12$), and (iv) rats with AMI and treated with SRLX30 (30 μg/kg/day) (AMI + SRLX30 group, $n = 12$). The dose of serelaxin was selected according to clinical studies in acute HF, and it was adjusted to body weight [3, 4]. The treatment with SRLX or placebo began immediately after surgery at a continuous rate of 1 μl/h for 72 h, using ALZET® mini-osmotic pumps (model 1003D, from ALZA Corporation, Palo Alto, CA, USA). The pumps were placed in the subcutaneous space, and the catheter was inserted into the jugular vein (Supplementary material). LV function variables were assessed before sacrifice using a transthoracic echocardiographic examination (4–12-MHz phased array sectorial transducer, HD7 XE, Philips) as described in the Supplementary


a
MMP2 mRNA expression (normalized to GADPH)
b
MMP9 mRNA expression (normalized to GADPH)
c
MMP10 mRNA expression (normalized to GADPH)
d
MMP13 mRNA expression (normalized to GADPH)
e
VEGFA mRNA expression (normalized to GADPH)
f
VEGFB mRNA expression (normalized to GADPH)
AMI
10 μg/kg/day SRLX
30 μg/kg/day SRLX
g
Sham
AMI
AMI+SRLX10
AMI+SRLX30
CD31 protein level (fold of control)

◀ **Fig. 2** Effect of serelaxin on angiogenesis. **a**–**f** Cardiac mRNA expression of metalloproteinases (*MMPs*) and pro-angiogenic molecules assessed by quantitative RT-PCR analysis in the infarcted myocardium (*black bars*) and border zone (*gray bars*). Data were normalized to GAPDH expression and are expressed as fold of the sham group. **g** Representative photomicrographs (×40) illustrating the immunohistochemical staining of CD-31 in the absence of AMI (sham) or in the infarcted myocardium from the infarcted groups. Bar graph shows the quantitative analysis of vessel density in the infarcted myocardium. Data are expressed as the number of stained blood vessels per field. *SRLX10*, 10 μg/kg/day serelaxin. *SRLX30*, 30 μg/kg/day serelaxin. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, AMI compared to the sham group. $^{\#}p < 0.05$, SRLX10 and SRLX30 compared to the AMI group

material. Animals were sacrificed 7 days after surgery, and their hearts were carefully removed and processed for analysis. The right ventricle (RV) and the LV, including the septum, were separated in ice-cold saline. A transverse section was cut from the middle LV (papillary muscles) and formalin-fixed and paraffin wax-embedded for immunohistochemical examination. Infarcted myocardium and border zone were separated from the rest of the LV and immediately frozen in liquid nitrogen and stored at −80 °C for gene expression studies.

Serelaxin Detection by Enzyme-Linked Immunosorbent Assay

Blood samples (0.5 ml) were collected by tail vein puncture and centrifuged (1500 rpm, 15 min) to separate plasma, before storing at −80 °C until analysis. Circulating serelaxin levels were measured 24 and 72 h after osmotic pump implantation by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (R&D Systems; Minneapolis, MN, USA). Each sample was measured in duplicate following the manufacturer's instructions.

Quantitative Reverse Transcription Polymerase Chain Reaction

Quantitative reverse transcription polymerase chain reaction (RT-PCR) was performed according to the manufacturer's protocol with minor modifications (Supplementary material). The primer sequences are described in the Supplementary material (Table S1).

Immunohistochemistry

Macrophage infiltration and blood vessel density were assessed by immunohistochemical examination using antibodies against CD-68 (Abcam, Cambridge, MA, USA) and CD-31 (Dako, Denmark), respectively. Expanded protocols are provided in the Supplementary material. CD-68 or CD-31 staining was quantified by averaging results for seven to ten representative high-powered fields (×40) in the infarcted myocardium using the Tissue Image Analysis package (SlidePath, Leica Microsystems, Gateshead, UK). CD-68 expression was expressed as the CD-68-stained area/total myocardium area ratio and referred to the sham group. Blood vessel density was calculated as the average number of CD-31-stained blood vessels per field.

Interstitial Fibrosis

Formalin-fixed sections (5 μm) from the LV transverse section were stained with Masson's trichrome using standard techniques. High-resolution images were obtained through a slide scanner Leica SN400F (Leica Microsystems). The collagen area fraction was assessed in images acquired at ×40 magnification by blinded digital color separation and thresholding to calculate the ratio of green (collagen) to non-white (total tissue) pixels in five fields per animal. Collagen volume was calculated as the average of all slices and expressed as the ratio of Masson's trichrome-stained collagen area to total myocardium area. Measurements independently performed by two blinded observers were averaged, and the agreement between observers was assessed (intraclass correlation coefficient = 0.948 (95% CI = 0.918–0.969); supplementary material).

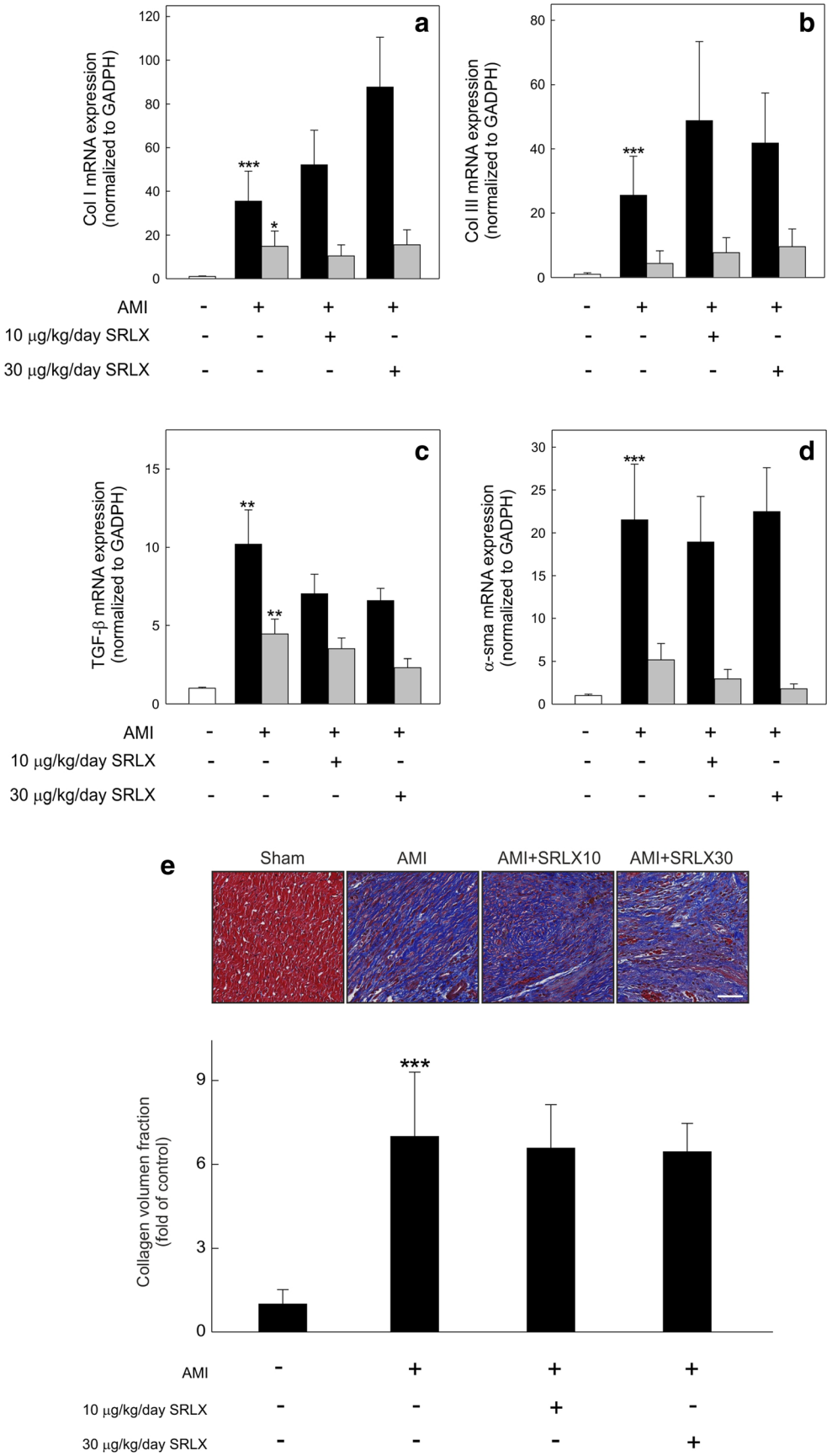
Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM), except for echocardiographic data expressed as mean ± standard deviation (SD). Data were analyzed using SPSS Statistics 22 (IBM Corp., USA). Normality was tested using the Kolmogorov-Smirnov test. An unpaired *t* test or Mann-Whitney *U* test was used for comparisons between the AMI group and the sham group, as appropriate. For comparisons between infarcted groups (AMI, AMI + SRLX10, AMI + SLRX30), a one-way analysis of variance or Kruskal-Wallis test was used as appropriate. As post hoc analysis, Dunnett's test was applied to test significances between the AMI group and AMI + SRLX10 and AMI + SLRX30, respectively. Spearman's correlation was used to assess associations with plasma levels of serelaxin. Graphing was performed using SigmaPlot 11.0 (Systat Software, Inc., USA). Statistical significance was assumed at $p < 0.05$.

Results

Anatomical and Echocardiographic Parameters

Mortality rates within 48 h after surgery did not differ between groups (Table 1, $p = 0.601$). There was no mortality after day 2 in any of the groups. After 7 days, immediately before the sacrifice, the AMI group exhibited body weight loss and


a
Col I mRNA expression (normalized to GADPH)
120
100
80
60
40
20
0
***
*
b
Col III mRNA expression (normalized to GADPH)
80
60
40
20
0
***
AMI - + + +
10 μg/kg/day SRLX - - + -
30 μg/kg/day SRLX - - - +
c
TGF-β mRNA expression (normalized to GADPH)
15
10
5
0
**
**
d
α-sma mRNA expression (normalized to GADPH)
30
25
20
15
10
5
0
***
AMI - + + +
10 μg/kg/day SRLX - - + -
30 μg/kg/day SRLX - - - +
e
Sham
AMI
AMI+SRLX10
AMI+SRLX30
Collagen volumen fraction (fold of control)
9
6
3
0
***
AMI - + + +
10 μg/kg/day SRLX - - + -
30 μg/kg/day SRLX - - - +

◀ **Fig. 3** Effect of serelaxin on fibrosis. **a**–**d** Cardiac mRNA expression of fibrosis markers assessed by quantitative RT-PCR analysis in the infarcted myocardium (*black bars*) and border zone (*gray bars*). Data were normalized to GAPDH expression and are expressed as fold of the sham group. **e** Representative photomicrographs (×40) illustrating Masson's trichrome staining in the absence of AMI (sham) or in the infarcted myocardium from the infarcted groups. Bar graph shows the quantitative analysis of interstitial fibrosis expressed as collagen volume fraction with respect to control. *SRLX10*, 10 μg/kg/day serelaxin. *SRLX30*, 30 μg/kg/day serelaxin. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, AMI compared to the sham group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, SRLX10 and SRLX30 compared to the AMI group

increased lung weight, as well as an increase in LV volumes and a decrease in LVEF, compared with the sham group. Serelaxin-treated groups did not differ from the AMI group. Table 1 shows the anatomical and echocardiography parameters of the respective groups of rats.

Inflammation

Gene expression of inflammatory markers was assessed by real-time RT-PCR, distinguishing between the infarcted myocardium and the border zone (Fig. 1). The AMI group exhibited signs of inflammation compared to the sham group. In the infarcted myocardium, there were increased levels of interleukin (IL)-6 (16.04 ± 3.86, $p < 0.001$), tumor necrosis factor alpha (TNF-α) (2.58 ± 0.28, $p < 0.001$), and monocyte chemoattractant protein 1 (MCP-1) (15.03 ± 2.47 vs. 1.00 ± 0.18, $p < 0.001$). In the border zone, there were also increased levels of IL-6 (4.56 ± 1.00, $p = 0.005$), TNF-α (1.93 ± 0.37, $p = 0.04$), and MCP-1 (12.00 ± 3.34, $p = 0.001$).

Compared to the AMI group, treatment with SRLX30 was associated with lower expression of IL-6 (7.43 ± 1.05, $p = 0.04$) and MCP-1 (7.74 ± 1.35, $p = 0.01$) in the infarcted myocardium, and lower expression of IL-6 (1.69 ± 0.46, $p = 0.04$), TNF-α (0.93 ± 0.22, $p = 0.04$), and MCP-1 (3.46 ± 1.19, $p = 0.03$) in the border area. This anti-inflammatory effect was not present in the group treated with SRLX10. In agreement with the RT-PCR analysis, the infarcted myocardium of the AMI group showed greater macrophage infiltration compared with the sham group (Fig. 1d). The treatment with SRLX30 attenuated the macrophage infiltration induced by infarction compared with the AMI group; however, macrophage infiltration did not differ in those animals treated with SRLX10.

Angiogenesis

Cardiac expression of extracellular matrix (ECM)-degrading proteases of the matrix metalloproteinase (MMP) family and pro-angiogenic molecules was assessed through real-time RT-PCR (Fig. 2a–d). In the infarcted myocardium, the AMI group exhibited increased levels of MMP2 (18.97 ± 3.05, $p < 0.001$), MMP9 (11.44 ± 1.99, $p < 0.001$), MMP10 (8.80 ± 2.29, $p = 0.001$), and MMP13 (5.760 ± 1.64, $p < 0.001$) compared with sham group of rats. In the border zone, there was a significant increase in MMPs, compared with sham group levels. However, no significant reduction in MMPs was associated with serelaxin infusion at any dose in the infarcted myocardium or the border zone, compared with the AMI group.

The pro-angiogenic molecules, vascular endothelial growth factor A (VEGFA) (0.73 ± 0.09, $p = 0.04$) and VEGFB (0.58 ± 0.09, $p = 0.03$), were downregulated in the infarcted myocardium of the AMI group, compared to the sham group (Fig. 2, panels E-F). The treatment with SRLX30 was associated with upregulation of VEGFA (1.10 ± 0.10, $p = 0.01$) and VEGFB (0.880 ± 0.07, $p = 0.02$), reaching expression levels similar to those of the sham group. The analysis of vascular density pointed to an increased number of blood vessels in the infarcted myocardium of the AMI group compared to the sham group (7.20 ± 1.30 vs. 2.10 ± 0.60, $p = 0.02$) (Fig. 2g). The treatment with SRLX30 significantly increased the number of vessels as compared with the AMI group (15.30 ± 1.40, $p = 0.01$). The results of treatment with SRLX10 did not show significant differences in this respect from those obtained for the AMI group (8.80 ± 2.10, $p = 0.5$). No significant changes were observed in the border zone in terms of pro-angiogenic molecules and vascular density for any treatment.

Fibrosis

Cardiac expression of fibrosis markers was also assessed through real-time RT-PCR (Fig. 3a–d). In the infarcted myocardium of the AMI group, all fibrosis markers increased compared with the levels observed in sham rats: collagen I (35.44 ± 13.75, $p < 0.001$), collagen III (25.54 ± 12.16, $p < 0.001$), α-SMA (21.53 ± 6.49, $p < 0.001$), and transforming growth factor beta (TGF-β) (10.190 ± 2.18, $p < 0.001$). Treatment with 10 or 30 μg/kg/day of serelaxin did not attenuate this upregulation of any fibrosis marker post AMI. In the border area of the AMI group, the expression of collagen I (14.86 ± 6.99, $p = 0.01$) and TGF-β (4.45 ± 0.95, $p = 0.003$) was also higher compared with the sham group, and again, treatment with serelaxin did not result in any decrease. Myocardial fibrosis was also assessed by measuring interstitial fibrosis through histological staining, and compared with sham rats, the collagen volume fraction was increased in the AMI group (6.90 ± 2.10, $p < 0.001$, Fig. 3e). Again, serelaxin therapy was not either associated with a reduction in collagen volume fraction compared with the AMI group.

Galectin-3 and Soluble Isoform of Suppression of Tumorigenicity 2

The cardiac expression of other molecules involved in cardiac remodeling was also assessed by real-time RT-PCR (Fig. 4).

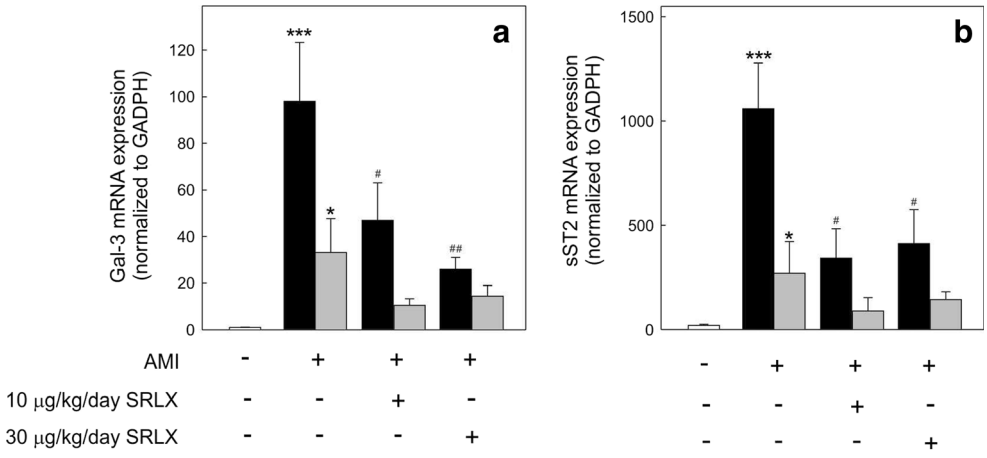


Fig. 4 Effect of serelaxin on cardiac expression of galectin-3 and sST2. Quantitative RT-PCR analysis of galectin-3 (**a**) and sST2 (**b**) in the infarcted myocardium (*black bars*) and border zone (*gray bars*). Data were normalized to GAPDH expression and are expressed as fold of the sham group. *SRLX* serelaxin. ***$p < 0.001$, *$p < 0.05$, AMI compared to the sham group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, SRLX10 and SRLX30 compared to the AMI group

Compared with sham rats, the messenger RNA (mRNA) expression of galectin-3 and soluble isoform of suppression of tumorigenicity 2 (sST2) increased in the infarcted area of the AMI group (galectin-3 = 98.04 ± 25.19, $p < 0.001$; sST2 = 1060 ± 219, $p < 0.001$). Compared with the AMI group, treatment with either 10 or 30 μg/kg/day of serelaxin significantly curbed the observed increase of galectin-3 (SRLX10 = 46.97 ± 16.12x, $p = 0.04$; SRLX30 = 25.97 ± 5.06, $p = 0.008$) and sST2 (SRLX10 = 342 ± 141, $p = 0.014$; SRLX30 = 412 ± 163, $p = 0.031$). In the border zone, the mRNA expression of galectin-3 and sST2 slightly increased in AMI rats compared with sham rats (galectin-3 = 33.15 ± 14.5, $p < 0.04$; ST2 = 269 ± 152, $p < 0.05$) and a non-significant trend to lower expression was observed with serelaxin therapy.

Plasma Concentrations of Serelaxin

The concentrations of serelaxin were measured in the plasma 24 and 72 h after starting infusion. At 24 h, the respective levels were 0.76 ± 0.81 and 2.54 ± 0.39 ng/ml in rats treated with SRLX10 and SRLX30, respectively. At 72 h, levels were 0.63 ± 0.15 and 1.92 ± 0.27 ng/ml, respectively. As expected, serelaxin was not detected in the plasma from untreated rats. Serelaxin plasma concentrations measured at 72 h correlated inversely with the expression of IL-6 ($r = -0.886$, $p = 0.019$), MCP-1 ($r = -0.786$, $p = 0.036$), VEGFA ($r = -0.857$, $p = 0.014$), MMP10 ($r = -0.821$, $p = 0.023$), galectin-3 ($r = -0.811$, $p = 0.027$), and collagen III ($r = -0.690$, $p = 0.037$) in rats treated with 30 μg/kg/day of serelaxin. No correlation was observed in rats treated with 10 μg/kg/day of serelaxin.

Discussion

The present study evaluated the short-term effects on myocardial remodeling of serelaxin administered intravenously during the first 72 h after AMI in a well-established rat model. Serelaxin resulted in early beneficial effects at 7 days, consisting of a reduction of inflammation, the promotion of angiogenesis, and lower cardiac levels of ST2 and galectin-3. In addition, a dose-dependent response was observed, given that the end-point assessment was more beneficial overall in the rats that received a dose of 30 μg/kg/day compared to the rats receiving the dose of 10 μg/kg/day, matched by circulating serelaxin concentrations. However, at this early time point (7 days), no clear improvement in LV function nor a reduction in interstitial fibrosis was observed.

Previous studies have described the anti-inflammatory effects of serelaxin in a variety of animal models of ischemic injury in the kidney [13], lung [14], or intestine [15]. In the heart, serelaxin administration reduced inflammatory cell infiltration and attenuated mast cell release in ischemia-reperfusion animal models [6, 7, 9]. Our results confirm these anti-inflammatory effects of serelaxin in the infarcted heart, as reflected by a significant reduction in the cardiac expression of inflammatory cytokines and chemokines (IL-6, TNF-α, and MCP-1), and the consequent inhibition of macrophage infiltration in the infarcted myocardium. In addition to the anti-inflammatory effect, serelaxin exerted pro-angiogenic actions by increasing both VEGF expression and vessel density, together with higher levels of MMPs. Our results agree with previous studies conducted in myocardial infarction models, where serelaxin administration for a longer period of time (7 and 30 days) also increased microvessel density and MMP activity [10, 12]. In the present study, 72 h of treatment was seen to be sufficient to produce benefits in terms of inflammation and angiogenesis in the infarcted myocardium, compared with the longer treatments conducted by others [10, 12].

After AMI, the inflammatory response switches to a fibrotic stage of remodeling, which begins between 3 and 7 days post AMI through the activation of myofibroblasts that synthesize fibrillar collagen to facilitate scar formation [16]. The replacement fibrosis per se may be also useful in the very first days of myocardial infarction, since it contributes to the

solidity of the ventricle. The ability of serelaxin to reduce cardiac fibroblast proliferation and differentiation, thereby reducing the left ventricular collagen content, has been observed in other models of cardiac fibrosis, such as those induced by hypertension [17], β-2 adrenergic receptor overexpression [18], angiotensin II infusion [19], or isoproterenol [20]. In the context of AMI, subcutaneous treatment with serelaxin (0.5 mg/kg/day until sacrifice) reduced the interstitial collagen content as measured 7 and 30 days post infarction, which was associated with a reduction in TGF-β expression and myofibroblast differentiation (more evident after 30 days) [12]. Serelaxin treatment started 1 month after AMI (1 μg/day, intraperitoneally for 28 days) has also been associated with a reduction in the amount of collagen fibers and activated myofibroblasts after 3 months [10]. The lack of an anti-fibrotic effect in the present study could be explained by the lower concentration of circulating serelaxin in our treated animals. While 2.5 ng/ml was the circulating serelaxin concentration reached in our study, the serelaxin concentration in other studies was 75 ± 29 ng/ml [10] and 20–40 ng/ml [12]. Another relevant difference between the studies was treatment duration; in contrast to the 3 days of therapy in our study, longer treatments of 28–30 days were used in the above studies [10, 12]. However, the treatment conducted in our study is closer to that used in humans [3, 4], in terms of dose, time of treatment, and route of administration.

Despite the lack of a direct anti-fibrotic effect, our results show that serelaxin was able to reduce the expression of galectin-3 and sST2. These two biomarkers have emerged as pro-fibrotic molecules with potential prognostic implications and have been associated with post-AMI cardiac fibrosis and suggested as targets for therapy [21, 22]. The modulation of galectin-3 and sST2 mediated by serelaxin in the early phase of infarction suggests a long-term effect on cardiac fibrosis. Indeed, the inhibition of sST2 has been proposed as a potential therapeutic strategy to reduce adverse cardiac remodeling [23, 24]. The ability of serelaxin to block the increase of sST2 in response to AMI could be translated into a long-term benefit, through its ability to attenuate sST2-mediated IL-33 sequestration and, consequently, by enhancing the cardioprotective IL-33 signaling [23, 24]. To date, this finding is the first scientifically acceptable evidence linking serelaxin with the IL-33/ST2 system.

Finally, the benefits as regards inflammation and angiogenesis associated with serelaxin therapy did not result in a significant improvement of cardiac function, as assessed by echocardiography. Our results agree with the study of Samuel CS and co-workers, which found that serelaxin treatment had no detectable effects on LV parameters of diastolic or systolic function at 7 or 30 days post AMI [12]. By contrast, treatment with serelaxin in a later phase (30 days post AMI) and for a longer period (28 days) was seen to preserve cardiac function and to attenuate adverse cardiac remodeling in infarcted rats [10, 11]. However, differences between doses, routes, and times make it difficult to give precise reasons for discrepancies between studies. However, based on our results and Samuel CS et al. [12], longer follow-up intervals are probably necessary to detect the effect on left ventricular function and dimensions. Recently, it has been communicated the disappointing results of the RELAX-AHF-2 trial, which represents the larger study designed to specifically evaluate the clinical benefits of serelaxin administration in patients with acute HF [5]. This study showed no benefits of serelaxin infusion in terms of mortality or re-hospitalization [25]. The results of our study suggest the setting of AMI as another potential scenario to get benefit from the anti-inflammatory, anti-remodeling, and pro-angiogenic actions of serelaxin; however, the lack of effect on cardiac function measures might represent a signal of no clinical benefit translation.

In conclusion, the 72-h administration of intravenous serelaxin resulted in early beneficial effects, consisting of a reduction in inflammation, the promotion of angiogenesis, and lower levels of ST2 and galectin-3 in the infarcted myocardium, following an experimental AMI with left ventricular systolic dysfunction. These results are of value from a translational point of view and support the need for further studies in order to assess this therapy in the setting of AMI.

Compliance with Ethical Standards

Clinical Relevance The intravenous infusion of serelaxin for 72 h after acute myocardial infarction might have a favorable effect on early processes of myocardial remodeling, in a dose-dependent manner.

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Conflict of Interest Dr. Pascual-Figal and Dr. de Boer received speaker fees from Novartis. All other authors report no conflicts of interest.

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