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Chronic lower-dose relaxin administration protects from arrhythmia in experimental myocardial infarction due to anti-inflammatory and anti-fibrotic properties

Thomas Beiert [a,*,1,2], Vincent Knappe [a,1,2], Vedat Tiyerili [a,2], Florian Stöckigt [a,2], Verena Effelsberg [a,2], Markus Linhart [a,2], Martin Steinmetz [a,2], Sabine Klein [b,2], Robert Schierwagen [b,2], Jonel Trebicka [b,c,d,e,2], Wilhelm Roell [f,2], Georg Nickenig [a,2], Jan W. Schrickel [a,2], René P. Andrié [a,2]

[a] Department of Internal Medicine II, University Hospital Bonn, Rheinische Friedrich-Wilhelms University, Bonn, Germany
[b] Department of Internal Medicine I, University Hospital Bonn, Rheinische Friedrich-Wilhelms University, Bonn, Germany
[c] Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark
[d] European Foundation for the Study of Chronic Liver Failure – EF Clif, Barcelona, Spain
[e] Institute for Bioengineering of Catalonia, Barcelona, Spain
[f] Department of Cardiac Surgery, University Hospital Bonn, Rheinische Friedrich-Wilhelms University, Bonn, Germany

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ABSTRACT

Background: The peptide hormone relaxin-2 (RLX) exerts beneficial effects during myocardial ischemia, but functional data on lower-dose RLX in myocardial infarction (MI) is lacking. Therefore, we investigated the impact of 75 μg/kg/d RLX treatment on electrical vulnerability and left ventricular function in a mouse model of MI.

Methods and results: Standardized cryoinfarction of the left anterior ventricular wall was performed in mice. A two week treatment period with vehicle or RLX via subcutaneously implanted osmotic minipumps was started immediately after MI. The relaxin receptor RXFP1 was expressed on ventricular/atrial cardiomyocytes, myofibroblasts, macrophages and endothelial but not vascular smooth muscle cells of small coronary vessels. RLX treatment resulted in a significant reduction of ventricular tachycardia inducibility (vehicle: 91%, RLX: 18%, $p < 0.0001$) and increased epicardial conduction velocity in the left ventricle and borderzone. Furthermore, left ventricular function following MI was improved in RLX treated mice (left ventricular ejection fraction; vehicle: 41.1 ± 1.9%, RLX: 50.5 ± 3.5%, $p = 0.04$). Interestingly, scar formation was attenuated by RLX with decreased transcript expression of connective tissue growth factor. Transcript levels of the pro-inflammatory cytokines interleukin-6 and interleukin-1β were upregulated in hearts of vehicle treated animals compared to mice without MI. Application of RLX attenuated this inflammatory response. In addition, macrophage infiltration was reduced in the borderzone of RLX treated mice.

Conclusion: Treatment with lower-dose RLX in mice prevents post-infarction ventricular tachycardia due to attenuation of scar formation and cardiac inflammation. Therefore, RLX could be evaluated as new therapeutic option in the treatment of MI.

1. Introduction

Despite a reduction of age-standardized death rates for cardiovascular diseases in developed countries since 1990, there has been a worldwide increase in the total number of cardiovascular deaths [1]. Ischemic heart disease was the major cause of global years of life lost in 2013 [1] and therefore remains a pivotal target for the development of new pharmacological therapies.

Relaxin-2 (RLX) is a protein hormone mediating maternal hemodynamic and reno-vascular adaption during pregnancy [2,3]. In addition, RLX also acts as an auto- and paracrine factor in many different tissues outside pregnancy, including the heart [2]. Diverse animal models of cardiovascular disease could demonstrate an improved outcome following application of RLX [4–8]. Unfortunately, these promising experimental results could not yet be translated into clinical applications. The reduction in cardiovascular and all-cause mortality at 180 days following a 48-hour continuous intravenous infusion of RLX observed in the

* Corresponding author at: Department of Internal Medicine II, University Hospital Bonn, Sigmund-Freud-Str. 25, 53127 Bonn, Germany.
E-mail address: Thomas.Beiert@ukbonn.de (T. Beiert).
[1] T.B. and V.K. contributed equally to this work.
[2] These authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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clinical trial RELAXin in Acute Heart Failure (RELAX-AHF) [9] was not confirmed in the recently completed RELAX-AHF 2 study (Novartis press release on March 22, 2017).

Infarct size limiting effects of RLX have been described after temporary/reperfused [10,11] and permanent [6,7] ligation of the left anterior descending artery (LAD). Whereas anti-arrhythmic effects of RLX are well documented for atrial fibrillation [12–14], evidence whether infarct attenuation translates into reduced susceptibility to ventricular tachycardia (VT) is limited. Previous reports showed a RLX-induced reduction of spontaneous VT during the ischemic [10] and reperfusion phase [10,15]. Only Wang et al. described reduced VT inducibility following two weeks of RLX treatment after LAD ligation using electrical burst stimulation [7]. But burst stimulation alone should be used with caution due to potential inaccuracies in selectivity and low specificity [16].

Additionally, the only two studies with chronic RLX administration after MI [6,7] (excluding those with cell transplantation) administered, like other studies in cardiovascular disease [4,5], a high dose of 500 μg/kg/d RLX and presented conflicting results regarding cardiac function. There is no data on RLX-mediated cardioprotection during MI for lower-dose RLX.

Therefore, the aim of this study was to determine the influence of chronic lower-dose RLX administration (75 μg/kg/d) on VT vulnerability and hemodynamic parameters in cryoinfarcted mice.

2. Material and methods

An expanded Material and methods section is available in the *Supplementary material online*.

2.1. Animals

Male (16%) and female (84%), 12-week-old mice (genetic background: CD1, Charles River, Sulzfeld, Germany) were used for this study (Permit Number: 84-02.04.2014.A159).

2.2. Cryoinfarction

MI by cryolesion was performed under general anesthesia as previously reported [17,18].

2.3. Osmotic minipump implantation

Osmotic minipumps (Alzet Model 1002; Durect Corporation, Alzet Osmotic Pumps, Cupertino, California, USA) were implanted subcutaneously and released either RLX (75 μg/kg/d; Novartis, Nürnberg, Germany) or vehicle (sodiumacetate) for two weeks [12].

2.4. Electrophysiological investigation (EPI) and left ventricular catheterization

EPI [12,18–20] and left heart catheterization [17,18,21] were both performed consecutively under conditions of inhalation anesthesia as previously reported.

2.5. Epicardial mapping

Myocardial conduction velocities were analyzed by epicardial activation mapping in Langendorff-perfused hearts [19,20].

2.6. Sirius red staining and fibrosis quantification

Ventricular cryosections were stained with sirius red according to standard protocols [20]. Quantifications of infarct size and ventricular septal fibrosis were performed as previously described [12,22].

2.7. Hematoxylin eosin staining and myocyte cross sectional area

Hematoxylin eosin staining was performed according to standardized protocols [17]. The average cross sectional size of cardiomyocytes in the interventricular septum was calculated and expressed as myocyte cross sectional area.

2.8. Immunofluorescence

Immunfluorescence staining of paraffin- or cryosections was performed as reported before [8,12,20].

2.9. RNA preparation and quantitative PCR

Hearts were dissected into left ventricle/borderzone/infarct. Expression levels were analyzed using Taqman probes as described previously [8,12,23].

2.10. Statistical analysis

All data are shown as mean ± SEM. Statistical analyses were performed using Chi square, Fisher's exact test, unpaired two-tailed student's *t*-test, one-way ANOVA with Tukey post-test, Mann-Whitney *U* test or Kruskal-Wallis test with Dunn's multiple comparison test as appropriate. The extreme studentized deviate method (Grubb's test) was applied to detect significant outliers, which were excluded from further analysis. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Effect of RLX on baseline parameters

After induction of MI by cryolesion, CD1 wild-type mice were treated with either RLX or vehicle for two weeks via subcutaneously implanted osmotic minipumps (Fig. 1A). Plasma levels of human relaxin-2 were elevated to 6.6 ± 1.1 ng/ml in RLX treated mice (Fig. 1B). Age and body weight did not differ significantly between control animals without MI, vehicle or RLX treatment. Cryoinfarction led to a significant increase in heart weight and heart weight/body weight ratio. The latter was significantly attenuated by RLX (Table S1). Mortality was comparable between the groups (vehicle: 8.6%, RLX: 5.6%, $p = 1.00$).

3.2. RXFP1 expression in cryoinfarcted mouse hearts

The relaxin/insulin like family peptide receptor 1 (RXFP1) was expressed in ventricular and atrial cardiomyocytes (Fig. S1A and S1B), α-smooth muscle actin positive myofibroblasts (Fig. S1E), CD68 positive macrophages (Fig. S1F) and endothelial (Fig. S1D) but not vascular smooth muscle cells (Fig. S1C) of small coronary vessels of vehicle treated mice.

3.3. Reduction of VT inducibility by RLX

Invasive EPIs were performed and mice tested for inducibility of VT by programmed and burst endocardial electrical stimulation (Fig. 1C). After MI, VT inducibility increased from 30% (control) to 91% in vehicle treated mice ($p = 0.001$). Treatment with RLX reduced inducibility to 18% ($p < 0.0001$ compared to vehicle; Fig. 1D) with concomitant reduction of the amount of VT episodes per animal (Fig. 1E). In animals with induction of at least one VT the total amount of VT episodes was similar (Fig. 1F) and average as well as cumulative VT duration were comparable between the groups (Fig. 1G and H). VRP was 27.0 ± 3.0 ms in control mice, 23.9 ± 1.4 ms in vehicle and 28.2 ± 1.5 in RLX treated mice ($p = 0.1658$ for overall comparison).