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Expression of ghrelin and its receptor in rats after coronary artery ligation

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ABSTRACT

Ghrelin is a novel growth hormone-releasing peptide, which has been shown to exert beneficial effects on cardiac function and ventricular remodeling. The present study aimed to investigate the expression of ghrelin and the growth hormone (GH) secretagogue receptor 1a (GHSR-1a), and the association with cardiac remodeling in rats with myocardial infarction (MI). Twenty-four hours after ligation of the anterior descending artery (LAD), adult male Sprague–Dawley rats were randomized to 3 d, 7 d and 28 d group. Sham animals underwent thoracotomy and pericardiotomy, but not LAD ligation. Expression of both ghrelin and GHSR-1a was assessed by means of immunohistochemistry and real-time PCR. Plasma ghrelin levels were measured by ELISA kit. In addition, cardiac remodeling was assessed by echocardiographic and hemodynamic measurements. Plasma and cardiac expression of ghrelin decreased on days 3, 7 and 28 compared with the sham group ($P < 0.05$). In contrast the GHSR-1a mRNA levels increased during the same days ($P < 0.05$). Decreased positive immunoreaction for ghrelin and increased positive GHSR-1a were also observed in the infarcted heart. Interestingly, plasma ghrelin correlated negatively with left ventricular end-diastolic pressure ($r = -0.59$, $P = 0.002$) and left ventricular end-diastolic dimension ($r = -0.73$, $P < 0.01$). The ghrelin system may play an important role regulating cardiac remodeling after MI and present as a potential significant target for pharmacological modulation and treating cardiac remodeling.

1. Introduction

Ghrelin, a peptide hormone from the stomach, has been identified as an endogenous ligand for the growth hormone secretagogue receptor (GHSR-1a) [1]. The ghrelin system is present in both vascular and cardiac tissues [2–4], and is synthesized and secreted by cardiomyocytes [5]. Apart from the ability to stimulate growth hormone secretion and to exert regulatory effects on appetite and metabolism, there is an increasing evidence that ghrelin has cardiovascular effects [6].

Previous studies have demonstrated that ghrelin inhibited cardiomyocyte apoptosis [7], improved left ventricular function and attenuates the development of cardiac cachexia in rats and humans with chronic heart failure (CHF) [8,9]. We also reported that ghrelin modulated sympathetic innervation and improved cardiac function after myocardial infarction through anti-inflammation and anti-apoptosis [10,11]. A recent study has indicated that CHF hearts exhibit an impaired ghrelin production and increased GHSR-1a expression, which indicates the potential of ghrelin system as a target for pharmacological modulation [12]. In contrast, earlier studies indicated that ghrelin plays a minor role in the control of cardiac function in the rats [13].

In the work described here, we aimed to investigate the dynamic expression of ghrelin and GHSR-1a in infarcted hears. In addition, we aimed to analyze whether the plasma level of ghrelin is related to the clinical parameters in the chronic heart failure model.

2. Materials and methods

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health, and the protocol was approved by the Institutional Animal Care Committee form Wuhan University.

2.1. Animals and experimental designs

The adult male Wister rats (250–300 g) were supplied by the animal experiment center of Wuhan University, China. Myocardial infarction was induced by ligation of the left anterior descending (LAD) coronary artery. Briefly, rats were anesthetized with an intraperitoneal injection of 3% pentobarbital sodium (30 mg/kg) and underwent left coronary artery ligation through a left thoracotomy to induce MI ($n = 60$) or sham coronary ligation ($n = 12$). The operation was performed with electrocardiogram monitoring. Successful ligation of the LCA was verified

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visually by the color change in the ischemic area and ECG leads I and aVL S–T segment elevations after the occlusion. In sham-operated rats, a suture was tied loosely around the left coronary artery without ligating it. Eighteen rats died within 24 h of coronary ligation.

2.2. Echocardiographic and hemodynamic measurements

Echocardiographic studies were performed at 3 d, 7 d and 28 d after operation. Each rat was lightly anesthetized with an intraperitoneal injection of pentobarbital sodium, and transthoracic echocardiography was performed using a Sequoia 512 (Acuson, Mountain View, CA, USA) equipped with a 3–7 MHz linear transducer. The anterior chest was shaven and a layer of acoustic coupling gel was applied to the thorax. Two-dimensional short-axis views of the left ventricle and M-mode tracings were recorded to measure LV end-systolic diameter (LVESD) and LV end-diastolic diameter (LVEDD). For hemodynamic measurements, a catheter was inserted into the right carotid artery and then advanced into the LV for recording of LV end-systolic pressure (LVESP) and LV end-diastolic pressure (LVEDP), and the data were measured by a multi-conductive physiological recorder (MP 150, Biopac, USA).

2.3. Laboratory investigation and immunoassay

The blood samples were collected following the above measurement and centrifuged for 10 min at 1000 ×g, and the serum was collected. The levels of ghrelin in serum were evaluated by using a commercial specific ELISA kit (B28007, Sigma). Samples from each rat were tested in duplicate, according to the detailed protocol provided by the manufacturers.

2.4. Tissue preparation

Rats were killed at 3 d, 7 d, and 28 d after the operation and the heart was dissected. Left ventricular myocardium was cut in half through the center of the infarct along the baso-apical axis; one half was immediately stored in −80 °C for further processing, and the other half for immunofluorescence staining.

2.5. Real-time PCR

Total RNA was prepared from the infarcted border with Trizol reagent (Invitrogen, USA), and reversely transcribed to cDNA using TaqMan Reverse Transcription Reagents (A1500, Biosystems). The expression levels of candidate genes were measured by real-time quantitative RT-PCR using a SYBR Green PCR Master mix (B 25004, Biosystems) as described. The following primers were used: ghrelin: 5′-GAAGCCACCAGCTAAACTGC-3′ (forward primer) and 5′-TG CTGGTA CTGAGCTCCTGA-3′ (reverse primer); GHSR-1a: 5′-CTCGGATCTGCTC ATCTTCC-3′ (forward primer) and 5′-GGAAGCAGATGGCGAAGTAG-3′; and GAPDH: 5′-CACGATGGAGGGGCCGGACTCATC-3′ (forward primer) and 5′-TAAAGACCTC TATGCCAACACAGT-3′ (reverse primer). PCR parameters were as follows: 1 cycle at 94 °C for 10 min followed by 40 cycles of 94 °C for 10 s, 58 °C for 15 s, and 72 °C for 30 s. The expected size amplicons were confirmed by gel electrophoreses. The mRNA levels of each gene were calculated using the $2^{-\Delta\Delta CT}$ method [14].

2.6. Immunofluorescence staining

Three sections of each heart were used for immunofluorescence study. Paraffin-embedded sections were performed at a thickness of 5 μm. Tissues were incubated with either anti-ghrelin or anti-GHSR-1a antibodies (1: 100, Abcam) overnight at 4 °C. Slides were then washed and incubated with TITC-labeled goat anti-rabbit Ab (1/500; Molecular Probes) for 1 h at 37 °C. The samples were then rinsed with PBS and observed under a fluorescence microscope (Leica, Germany).

2.7. Statistical analysis

ANOVA with Newman–Keuls test was used for multiple comparisons. $P < 0.05$ was considered statistically significant. Correlation coefficients between ghrelin and clinical parameters were calculated by linear regression analysis.

3. Results

3.1. Experimental model

The study was completed in the 44 surviving animals (10 in the sham group, 12 in the MI-3 d group, 12 in the MI-7 d group, and 10 in the MI-28 d group).

3.2. Alterations in plasma ghrelin levels after MI

Plasma levels of ghrelin in sham group were 0.99 ± 0.03 ng/ml. At 3 d, 7 d and 28 d after MI, plasma levels of ghrelin decreased by 22%, 32% and 57% respectively compared with sham group ($P < 0.05$). As showed in Fig. 1, at 28 days after MI, the plasma ghrelin levels were lower than that on days 3 and 7 ($P < 0.05$). There is no significant difference of plasma ghrelin levels at 3 days and 7 days after MI.

3.3. Ghrelin expression after MI

Ghrelin mRNA levels were analyzed on days 3, 7 and 28 after MI by using real-time q RT-PCR. As shown in Fig. 2A, the ghrelin mRNA levels are decreased on days 3, 7 and 28 compared with the sham group ($P < 0.05$). At 28 days after MI, ghrelin mRNA expression was lower than that on days 3 and 7 ($P < 0.05$). The ghrelin mRNA expression at 3 days after MI was not significantly different from at 7 days. The dynamic change of mRNA expression is similar with the plasma ghrelin levels. As shown in Fig. 3, the healthy rat myocardium showed a strong immunoreactivity for ghrelin. There was a significant decrease of immunoreaction for ghrelin in MI rats compared with that in sham group ($P < 0.05$).

3.4. GHSR-1a expression after MI

The GHSR-1a mRNA levels increased on days 3, 7 and 28 compared with the sham animals ($P < 0.05$). After MI, significantly altered expression patterns of GHSR-1a were found. Only a weak positive diffuse immunostaining of GHSR-1a in the cardiomyocytes was detected in sham rats. In contrast, an increased positive immunoreaction for GHSR-1a in rats with MI was found. Immunostain results were similar with the qPCR results (Fig. 4).

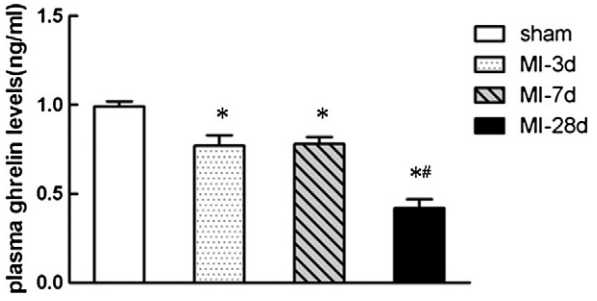

Fig. 1. Plasma ghrelin level in rats with myocardial infarction in different time points. *P < 0.05 vs sham-operated group; #P < 0.05 vs MI group.