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Obestatin attenuated doxorubicin-induced cardiomyopathy via enhancing long noncoding Mhrt RNA expression

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ABSTRACT

Objective: The emergence of side-effect of doxorubicin in cardiomyopathy and heart failure has led to the search for diverse strategies to prevent its cytotoxic effects. This study was to determine the role of obestatin on doxorubicin-induced cardiomyocytes apoptosis and possible underlying mechanism.
Methods: Sprague Dawley rats were divided into 3 groups and received treatment for a total of 6 weeks: group1, untreated normal rats; group2, Doxorubicin-induced heart cardiomyopathy (DC) rats; and group3, obestatin treated HC rats. Doxorubicin (2.5 mg/kg) or obestatin (100 μg/kg/d) were discontinuously administered via intraperitoneal injection. Primary cardiomyocytes and H9C2 cell line were used for in vitro experiments. Mhrt and Nrf2 (nuclear factor erythroid 2 −related factor 2) mRNA expressions were determined using qRT-PCR. Expression of Nrf2 protein was determined using western blotting. TUNEL assay was performed to evaluate cell apoptosis.
Results: Administration of obestatin significantly improved doxorubicin-induced dysfunction of left ventricular contractility function, moreover, resulted in upregulation of Mhrt and Nrf2 in failing myocardial tissue. Co-incubation of obestatin and doxorubicin in primary cardiomyocytes also enhanced Mhrt and Nrf2 expression as well as prevented cell apoptosis in comparison with doxorubicin only. Manipulation of cellular Mhrt by pcDNA-Mhrt or si-Mhrt transfection positively regulated Nrf2 expression in doxorubicin-incubated cardiomyocytes. Silencing Mhrt reversed cardioprotective effects of obestatin both in vivo and in vitro.
Conclusion: Administration of obestatin attenuates doxorubicin-induced cardiac dysfunction via preservation of cardiomyocytes apoptosis in a Mhrt-Nrf2 dependent pathway.

1. Introduction

Doxorubicin is a one of clinical anthracycline drugs and is a widely used as cytotoxic agent for the treatment of human neoplasms such as acute leukemia, ovarian carcinoma and metastatic breast cancer [1,2]. Nevertheless, administration of doxorubicin also causes side-effect of cardiotoxicity that induce development of cardiomyopathy, eventually invariably resulting in congestive heart failure [3]. This consequence makes a huge challenge for survival quality in cancer patient. Therefore, development of medical therapies in the management of cardiac dysfunction is thus top priority for the long term survival of these individuals.

The peptide hormone obestatin, first discovered in 2005 as the ghrelin homologous gene, has a diverse range of anti-ghrelin physiological actions such as suppressed food intake, reduced intestinal peristalsis, slowed the absorption of food, and thereby caused decrease in body-weight [4]. Although there is no evidence focusing on the relevant metabolic or viability modifier for cardiomyocytes as ghrelin [5], Alloatti's group recently found that obestatin could activate some anti-apoptotic signaling pathways and protect cardiac cell against myocardial injury and apoptosis induced by ischemia-reperfusion [6]. Considering the same pathophysiological base of cardiomyocytes apoptosis underlying cardiomyopathy, we considered the benefit role of obestatin in doxorubicin-induced cardiomyopathy.

Although the biosynthesis and biological activities of small non-coding RNAs are well explored, the understanding of long non-coding RNAs (lncRNAs) is limited [7]. lncRNAs are defined as transcripts that are longer than 200 nt and do not code for proteins to separate them from protein-coding genes. Evolving research has identified diverse epigenetic regulatory roles for lncRNAs in

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development, homeostasis, and disease. lncRNAs are likely to play important regulatory roles in human heart failure as well [8]. Because the number of lncRNAs is more than twice the number of protein-coding genes in human, researchers are still in the infancy of naming, categorizing, and validating lncRNAs [9]. In the cardiovascular system, studies have detected and characterized the expression of lncRNAs under normal physiological condition and in disease states [10]. A cluster of antisense transcripts from the Myh7 locus (named myosin heavy chain-associated RNA transcripts [MyHEART or *Mhrt*]) was recently shown to interfere with cardiac hypertrophy and subsequent heart failure [11]. Mhrt is highly enriched in the nuclear fraction of cardiomyocytes and is down-regulated by pressure overload. Overexpression of Mhrt protects the heart from myocardial hypertrophy and cardiomyopathy [12]. We proposed the involvement of Mhrt in cardioprotective role of obestatin.

The physiological function of Mhrt is its post-transcriptional regulation on key protein. Nrf2 (nuclear factor erythroid 2-related factor 2) serves as an important transcription factor for genotoxicity of cells in cardiovascular system [13]. Herein, we used in vivo model of doxorubicin rats and in vitro model of doxorubicin-stimulated cardiomyocytes to determine the aberrant expression of Mhrt and examined its interaction role with Nrf2 in obestatin-treated doxorubicin-induced cardiomyopathy.

2. Material and methods

2.1. Animals and experimental protocols

Male Sprague-Dawley rats (weighted at 250 ± 10 g; obtained from the Animal Experiment Center of Anhui Medical University) were applied to produce doxorubicin-induced cardiomyopathy model. For experimental protocols, animals were divided into three groups: Group1, normal rats with no treatment as control; Group2, DC rats with doxorubicin-induced heart cardiomyopathy; and Group3, obestatin treated DC rats. During in vivo process,

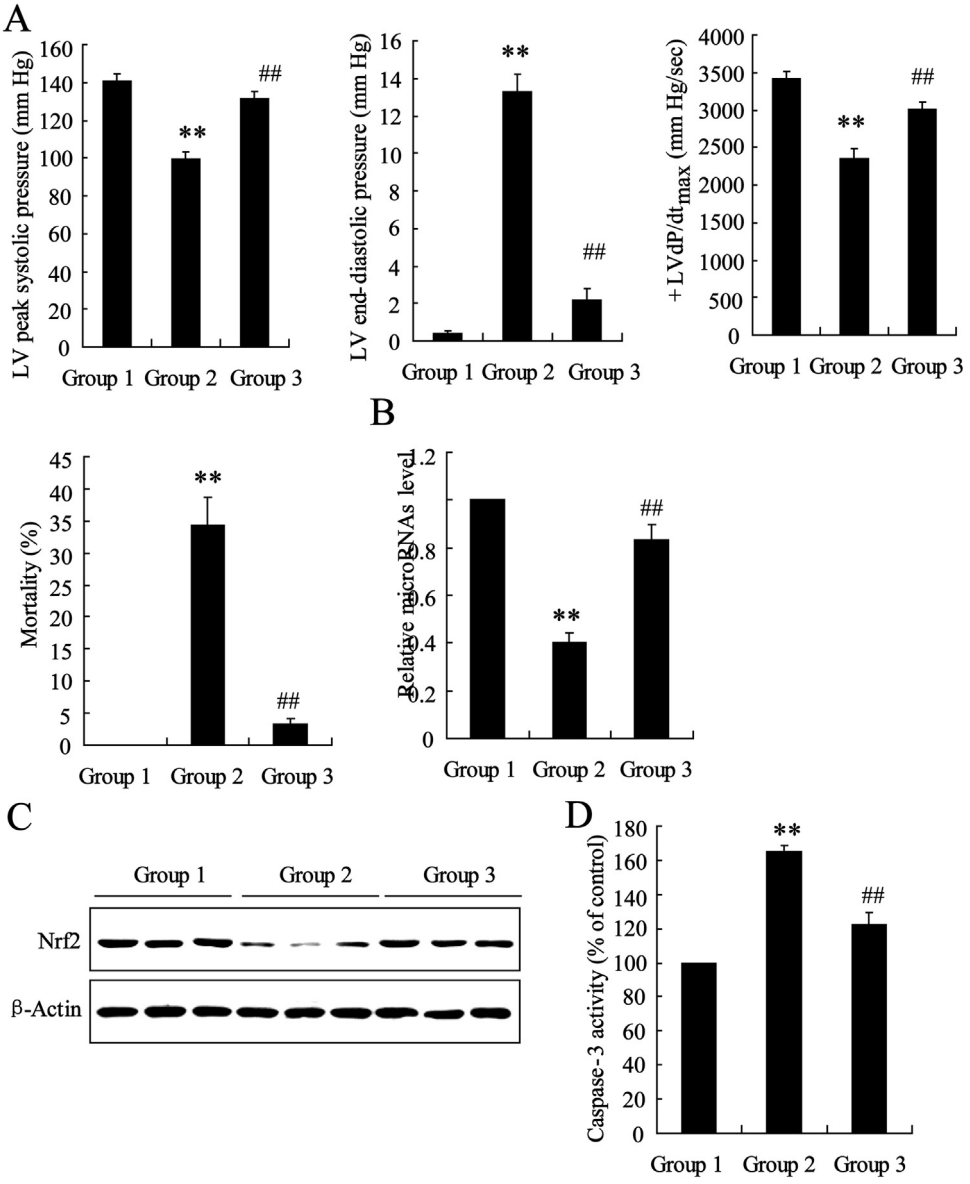

Fig. 1. Alteration of myocardial contractility and gene phenotypes in obestatin-treated DC rats. Sprague-Dawley rats were divided into three groups; Group1, 2 and 3 and were received 4 weeks obestatin treatment, (A) Left ventricle (LV) peak systolic pressure, LV end-diastolic pressure and +LV dP/dt_{max} were monitored, and mortality of rats were recorded. Myocardial tissue was isolated from all rats for gene expression analysis: (B) qRT-PCR was performed for analysis of Mhrt expression; (C) representative immunoblotting of Nrf2 expression. (D) Caspase-3 activity. Data are presented as mean ± SD. $^{**}p < 0.01$ vs. Group 1; $^{\#\#}p < 0.01$ vs. Group 2.