Pharmacogenomics and Personalized Medicine

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ORIGINAL RESEARCH

Carvedilol Alters Circulating MiR-1 and MiR-214 in Heart Failure

This article was published in the following Dove Press journal:
Pharmacogenomics and Personalized Medicine

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Introduction: MicroRNAs (miRNAs) are recognized as major contributors in various cardiovascular diseases, such as heart failure (HF). These small noncoding RNAs that posttranscriptionally control target genes are involved in regulating different pathophysiological processes including cardiac proliferation, ifferentiation, hypertrophy, and fibrosis. Although carvedilol, a β-adrenergic blocker, and a drug of choice in HF produce cytoprotective actions against cardiomyocyte hypertrophy, the mechanisms are poorly understood. Here we proposed that the expression of hypertrophic-specific miRNAs (miR-1, miR-133, miR-208, and miR-214) might be linked to beneficial effects of carvedilol.

Methods: The levels of four hypertrophic-specific miRNAs were measured in the sera of 35 patients with systolic HF receiving carvedilol (treated) and 20 HF patients not receiving any β-blockers (untreated) as well as 17 nonHF individuals (healthy) using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Systolic HF was defined as left ventricular ejection fraction <50% by transthoracic echocardiography.

Results: We demonstrated that miR-1 and miR-214 were significantly upregulated in the treated group compared to the untreated group (P=0.014 and 5.3-fold, 0.033 and 4.2-fold, respectively). However, miR-133 and miR-208 did not show significant difference in expression between these two study groups. MiR-1 was significantly downregulated in the untreated group compared with healthy individuals (P=0.019 and 0.14-fold).

Conclusion: In conclusion, it might be postulated that one of the mechanisms by which carvedilol may exert its cardioprotective effects can be through increasing miR-1 and miR-214 expressions which may also serve as a potential therapeutic target in patients with systolic HF in future.

Keywords: microRNA, β-blocker, carvedilol, systolic heart failure, cardiac hypertrophy

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide.[1] Systolic heart failure (HF) is becoming the most prevalent form of chronic CVD and is defined as a state of inadequate cardiac output to meet the tissues' metabolic needs.[2] Despite efforts made towards preventing the disease, the prevalence of HF remains over 26 million worldwide[3]. Not enough evidence regarding the prevalence of HF is provided in the Middle East region; however, it is assumed that it will soon be equal to that of western countries.[4] Pathologic remodeling, which is the hallmark of chronic HF is triggered and aggravated by a variety of stimuli including pressure and volume overload in cardiac chambers. Valvular heart diseases, hypertension, dilated and hypertrophic cardiomyopathies induce acute or chronic stress to the heart and culminate in a hypertrophic and/or apoptotic response which is frequently seen in HF.[5,6]

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http://doi.org/10.2147/PGPM.S263740

Among the pharmacologic remedies for systolic HF, β-blockers have a promising role in reducing mortality. Since proposing the beneficial effects of β-blockers in HF in 1975,[7] much evidence is in favor of the role of these agents in improving cardiac function and survival rates in patients with systolic HF.[8–10] Among the β-blockers, carvedilol is currently the first-line therapy for symptomatic HF in adults.[11,12]

The discovery of micro ribonucleic acid (miRNA), small noncoding RNAs (~22 nucleotides), in regulating key protein-coding genes, has opened up new paths in understanding the underlying pathogenic conditions of CVDs, which may have implications in diagnosis, treatment and prognosis of the disease.[13,14] MiRNAs as detectable intracellular RNAs, are present in serum or plasma in a remarkably stable form[15] and can alter cardiac proliferation, differentiation and other pathological remodeling responses. The proposed mechanisms of posttranscriptional regulation of protein-coding genes, explain how these small molecules affect the cardiovascular system and select them as biomarkers of cardiac pathologic remodeling.[16] Several studies corroborate the role of miRNAs in processes of myocyte hypertrophy and apoptosis.[17–22] MiR-1 and miR-133, two miRs belonging to one transcriptional unit, are expressed at low levels in both animal and human models of cardiac hypertrophy[23] and are among the key regulators in these processes.[24,25] Deletion of these miRNAs which are highly abundant in the heart may be linked to cardiac defects.[26] MiR-208 as well as miR-1 and miR-133 are defined as key regulators of the gene network which is involved in cardiomyocyte differentiation.[27] There are implications that some miRNAs like miR-208b, which play a functional role in cardiomyocyte remodeling, may be useful targets for therapeutic purposes.[28] Another miRNA, miR-214, is proposed to play a regulatory role in cardiac hypertrophy and negative remodeling involved in HF.[29] Downregulation of miR-214 has been reported in the myocardium of patients diagnosed with cardiac hypertrophy.[30]

Although studies have shown that carvedilol inhibits cardiomyocyte hypertrophy, the exact cardioprotective mechanism is still unknown.[31] An in vivo study on rat neonatal cardiomyocytes showed that carvedilol protects cardiomyocytes against oxidative stress-induced apoptosis by upregulating miR-133.[32] Therefore, the protective role of carvedilol in HF might be linked to alterations in levels of different miRNA expression. In this study we investigated, for the first time, the effects of carvedilol on miRNA expressions in a clinical setting using sera of HF patients registered in a regional hospital-based systolic HF disease registry system, Fasa Registry of Systolic HF (FaRSH).

Methods

Sample Collection

This study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and Uniform Requirements for manuscripts submitted to biomedical journals and was approved by the local committee for ethics of medical experiments on human subjects of Shiraz University of Medical Sciences. We obtained written consent from all participants prior to the interview and all participants were informed about the purpose of the study.

We used the infrastructure of a local hospital-based disease registry system FaRSH which has enrolled patients admitted with a diagnosis of systolic HF since July 2015. The main inclusion criterion was left ventricular ejection fraction (LVEF) of <50%. Fifty-five patients with diagnosis of systolic HF secondary to ischemic heart disease were recruited in the study. Patients were matched according to age, sex, and body mass index (BMI). Those with severe valvular heart diseases, GFR <30 cc/min and significant comorbidities were excluded in the first place.

Thirty-five patients (treated) received carvedilol for at least three months, the dose of which ranged from 6.25 mg to 12.5 mg in two divided doses. β-blockers were contraindicated in the other 20 patients (untreated) due to severe sinus bradycardia and partial atrioventricular (AV) block.

Seventeen nonHF individuals (healthy) were also enrolled in the study. The exclusion criteria for healthy individuals was the absence of cardiovascular diseases, hyperlipidemia, malignancies, and autoimmune, neurologic and psychiatric diseases.

Serum Isolation and Storage

Five mL of blood was collected from each participant. Whole blood was allowed to stand at room temperature for five minutes before being centrifuged at 4°C at 12000 g for 20 min. The resultant serum was aliquoted into endonuclease-free Eppendorf tubes and stored at – 80°C.

RNA Extraction and cDNA Synthesis

Total RNA was extracted from serum samples using TRIzol LS Reagent (Invitrogen, Carlsbad, CA, USA). Half a mL of serum was incubated at 37°C overnight with 10 µL proteinase K (Cinaclone, Iran). For RNA

isolation 0.25 mL of serum was homogenized in 0.75 mL of TRIzol LS. Two hundred µL of chloroform was added to the sample for phase separation, mixed and centrifuged. RNA was left exclusively in the aqueous phase. After the removal of chloroform and precipitation with isopropanol, the pellet was washed by centrifugation with 75% ethanol. The RNA pellet was dried at room temperature for 5– 10 min and dissolved in 20 µL of diethyl pyrocarbonate (DEPC)-treated water. RNA concentrations and quality was calculated using Nanodrop® Hellma, Denmark. Total RNA samples were kept at −80°C for further analysis.

For reverse transcription (RT) reaction of the miRNAs and synthesis of cDNA strands, specific RT primers (Exiqon, Vedbaek, Denmark) and cDNA synthesis kit (Exiqon, Vedbaek, Denmark) were used according to the manufacturer's protocol. Briefly, in order to fabricate the poly A strand, we mixed 2 µL buffer (10×), ATP 2 µL, polyA polymerase 0.5 µL, 1.5–2 µL of total RNA was incubated at 37°C for 10 min. cDNA synthesis was performed as follows: two µL 5× reaction buffer, 1 µL dNTP (10 mM), 0.5 µL RT enzyme, 0.5 µL microRNA cDNA synthesis specific primers, 2 µL polyadenylated RNA. The mixture was incubated for 60 min at 44°C, followed by heat-inactivation enzyme of reverse transcriptase (RT-enzyme for one minute at 85°C in a thermal cycler).

Real-time PCR

Aliquots of cDNA were used for quantitative PCR using Real-time PCR Master Mix (Exiqon, Vedbaek, Denmark), specific primers (Exiqon, Vedbaek, Denmark) and ABI 7500 (Applied Bio systems, Foster City, USA). Four µL of synthesized cDNA was mixed with 10 µL of SYBER green, 0.4 µL ROX dye, and mixed with specific miR primers 1 µL (10 mM) as shown in Table 1.

As an internal reference gene, 5S rRNA was used in order to normalize miRNA expressions.

In order to check the accuracy of amplifications we included a negative control (NTC) in each run by eliminating the cDNA sample in the tube. RT-PCR was run in duplicate using ABI 7500 REA real-time quantitative PCR system (Applied Bio System, USA) with the following cycling conditions: preliminary denaturation at 95°C for five minutes, followed by 45 cycles of denaturation at 95°C for 5 seconds, annealing at 63°C for 20 seconds, and elongation at 72°C for 30 seconds.

Table 1 MiRNA Specific Primers for cDNA Synthesis Reaction

MiRNAs	Sequence
MiR-1	5′AACAUACUUCUUUAUAUGCC3′
MiR-133	5′AGCUGGUAAAAUGGAACCAAAUC3′
MiR-208	5′GAGCUUUUGGCCCGGGUUA3′
MiR-214	5′CUGCCUGUCUACACUUGCUGUGC3′

Statistical Analysis

Statistical analysis was performed using SPSS® 21.0 for Windows® (IBM Corporation, Armonk, NY, USA). Graphs were plotted using GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA, USA). Distribution of all variables was tested for normal distribution using Kolmogorov–Smirnov test. Comparisons between two groups were performed with an independent sample *t*-test.

A widely used method for presenting relative expression of miRNA is called the 2-ΔΔct method which presents the data of the miRNA of interest ($Ct_{\text{miRNA of interest}}$) relative to internal control gene ($Ct_{\text{internal control miRNA}}$), termed Δct. Results are calculated as Δct± standard error of the mean (SEM). All values of miRNAs are expressed as ±SEM. *P*-value<0.05 was considered statistically significant.

Results

A total of 72 Individuals comprised of 17 healthy subjects (male/female: 10/7; age: 67.25±11.06; and 35 HF patients treated with carvedilol (male/female: 19/16; age: 64.83 ±11.03) and 20 HF patients receiving no β-blockers (male/female: 11/9; age: 67.25±11.06) were enrolled in our study. Demographical characteristics of nonHF (healthy) subjects are presented in Table 2. Clinical characteristics and demographics of HF patients receiving carvedilol (treated) and HF patients not treated with

Table 2 Characteristics of HF Patients Receiving Carvedilol (Treated) and NonHF Patients (Healthy)

Characteristics	Treated (n=35)	Healthy (n=17)	*P*-value
Age (years)	64.83±11.03	67.25±11.06	0.631
Male/female (n/n)	19/16	10/7	0.736
BMI (kg/m^2)	26.0±0.65	24.9±3.5	0.076
Diabetes mellitus, n (%)	37.1%	35.3%	0.900
Smoking, n (%)	31.4%	40.0%	0.137
HTN, n (%)	40.0%	29.4%	0.461

Note: Mean ±SD.
Abbreviation: HTN, hypertension.

Table 3 Characteristics of HF Patients Receiving Carvedilol (Treated) and HF Patients Not Receiving Carvedilol (Untreated)

Characteristics	Treated (n=35)	Untreated (n=20)	*P*-value
Age (years)	64.83±11.03	67.25±11.06	0.44
Male/female (n/n)	19/16	11/9	0.60
BMI (kg/m^2)	26.0±0.647	24.8±2.45	0.60
Diabetes mellitus, n (%)	37.1%	40.0%	0.528
Smoking, n (%)	31.4%	40.0%	0.999
HTN, n (%)	40.0%	65.0%	0.096
LVEF	33.6±1.68	30.8±2.72	0.35
AF, n (%)	14.2%	5%	0.399
Heart valve disease (%)	17.1%	15%	0.999
DCM, n (%)	8.5%	15%	0.657
Hyperlipidemia, n (%)	57.1%	50%	0.779
Stroke (%)	2.8%	5%	0.999
Previous MI	54.2%	55%	0.999
History of malignancy	5.7%	10.0%	0.61

Note: Mean +SD.
Abbreviations: HTN, hypertension; LVEF, left ventricular ejection fraction; AF, atrial fibrillation; DCM, dilated cardiomyopathy.

β-blockers (untreated) are shown in Table 3. The two groups were matched according to age, sex, and BMI and were similar regarding smoking habits, HNT and diabetes (P>0.05). Two groups were matched according to age, sex, and BMI and were similar regarding smoking habits, history of malignancies and diabetes (P>0.05).

Figure 1 and Supplementary Figure 1 represent fold-change comparison of different miRNA expressions in the treated group compared with healthy individuals. As shown, regarding expression levels of the four miRs, no significant difference was observed between the two groups (P>0.05).

As demonstrated in Figure 2 and Supplementary Figure 2, miR-1 was significantly (P=0.019) and by 0.14-fold downregulated in untreated group compared with healthy subjects.

Fold changes in median expression of the study miRNAs of enrolled patients (treated vs untreated) are demonstrated in Figure 3 and Supplementary Figure 3. Real-time polymerase chain reaction (RT-PCR) analysis of miRNA, using precise significance criteria of a two-fold or greater difference in expression level and P value <0.05, revealed that two miRNAs, miR-1 and miR-214 were significantly upregulated in patients with HF who were treated with carvedilol compared to the control group (P=0.014 and 0.033 respectively). Regarding miR-214, its expression was significantly and by 4.2-fold higher in carvedilol receiving patients compared to patients in the

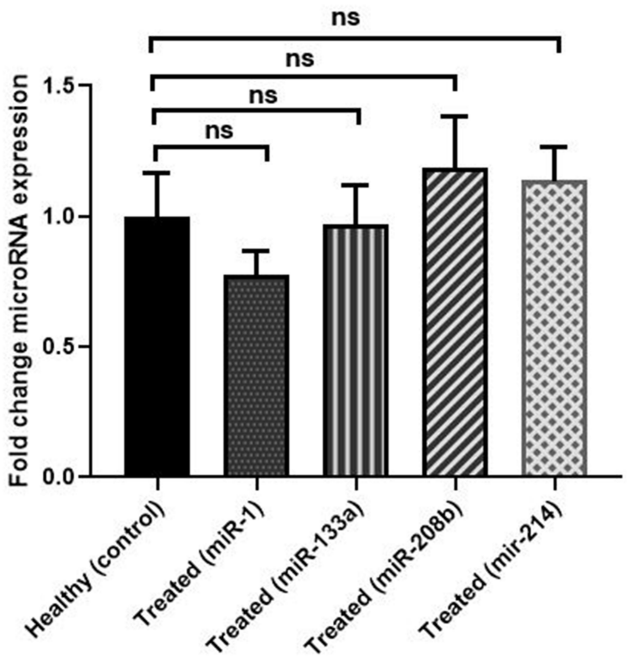

Figure 1 Fold-change comparison of different miRNA expressions in HF patients receiving carvedilol (treated) compared with non-HF (healthy) individuals. Fold-change comparison of miRNA expressions (miR-208b, miR-133a, miR-214, and miR-1) between HF patients receiving carvedilol (treated) compared with nonHF (healthy) individuals. (P>0.05) between miRNA expressions in HF patients receiving carvedilol (treated) compared with nonHF (healthy) group.
Abbreviation: ns, no significant difference.

control group who did not receive carvedilol (P=0.033). Considering miR-1 expression, a noticeable increase by 5.3-fold was observed in cases compared to the control group (P=0.014). Similarly, the expression level of miR-133 in individuals taking carvedilol showed an increased trend by 3.1-fold compared to the control group; however, this observed difference was not statistically significant (P=0.052). Regarding miR-208, no significant difference was observed with respect to the two study groups (P=0.171).

Discussion

MiRNAs are prominent regulators of cell growth, proliferation, differentiation and apoptosis.[33,34] Alteration in miRNAs expressions, apart from their role in the pathophysiology of CVDs, would nominate them as emerging sensitive and noninvasive biomarkers in diagnosis, treatment surveillance and prognosis in many disease states such as systolic HF. In this study, we speculated whether the expression of hypertrophy-related miRNAs is involved in the protective mechanism of carvedilol in systolic HF.

Results of miRNA expression in our enrolled patients with systolic HF who received carvedilol compared to the

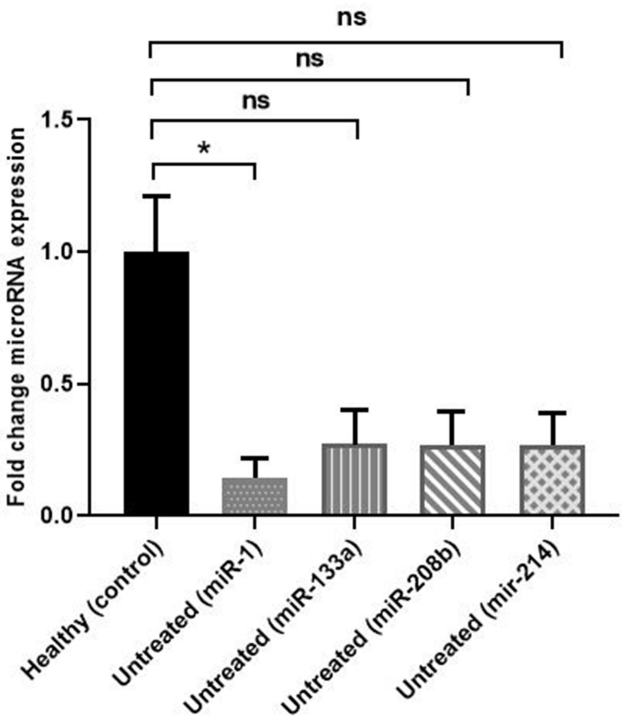

Figure 2 Fold-change comparison of different miRNA expressions in HF patients not receiving carvedilol (untreated) compared with nonHF (healthy) individuals. Fold-change comparison of miRNA expressions (miR-208b, miR-133a, miR-214, and miR-1) between HF patients not receiving carvedilol (untreated) compared with nonHF (healthy) individuals. *Significant difference (P<0.05) between miRNA expressions in HF patients not treated with carvedilol (untreated) and nonHF (healthy) group. (P>0.05) between miRNA expressions in HF patients receiving carvedilol (untreated) compared with nonHF (healthy) group.
Abbreviation: ns, no significant difference.

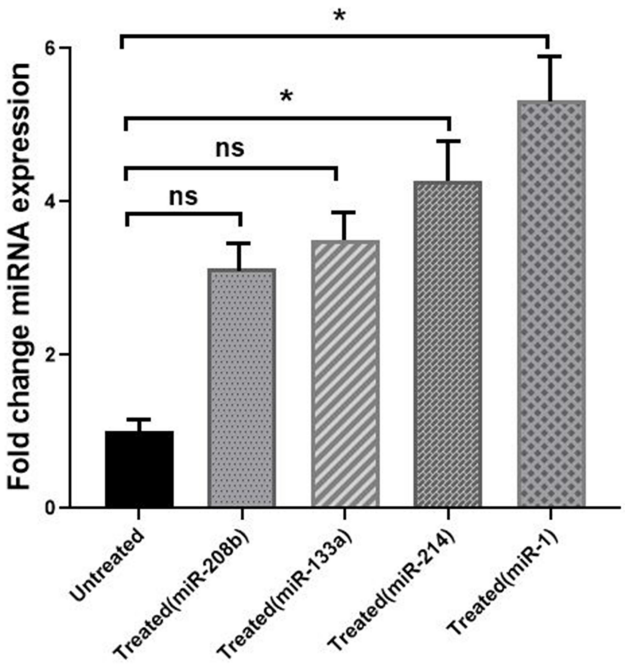

Figure 3 Fold-change comparison of different miRNA expressions in HF patients treated with carvedilol (treated) and untreated HF group (untreated). Fold-change comparison of miRNA expressions (miR-208b, miR-133a, miR-214, and miR-1) between HF patients treated with carvedilol and untreated HF patients. Significant upregulation of circulating miR-214 and miR-1 in patients treated with carvedilol compared to untreated group. *Significant difference (P<0.05) between miRNA expressions in HF patients treated with carvedilol and untreated HF group. (P>0.05) between miRNA expressions in HF patients treated with carvedilol and untreated HF group.
Abbreviation: ns, no significant difference.

group who were not treated with carvedilol revealed: (1) significant upregulated miR-214 expression; (2) markedly upregulated miR-1 expression; (3) no significant effect on miRNA-133; and (4) miR-208 expression. Furthermore, comparison of expression of the four studied miRs between untreated and healthy subjects showed that miR-1 was significantly downregulated in untreated patients. No significant difference was observed between expression levels of miRs in healthy individuals compared with treated patients.

Systolic HF is a complex disorder with many possible causes, the most prevalent of which is coronary artery disease leading to myocardial ischemia.[3] β-blockers were first advocated for treatment of HF in the late 1970s.[7] Results from clinical trials suggest that among β-blockers, carvedilol, metoprolol succinate and bisoprolol reduce mortality rates when added to conventional therapy.[35–37] Carvedilol consistently improves survival compared with metoprolol.[38] However, molecular mechanisms underlying this favorable effect remain elusive. HF is a pathological condition in which the regulation among specific genes is disrupted. As a consequence, the expression of oncogenes is increased, while the expression of normal and mature genes decreases leading to cardiac hypertrophy and/or remodeling, a pathological status that ultimately causes further damage to the failing cardiomyocytes, thus perpetuating the impaired cardiac function.[39] Compelling evidence points at the role of miRNAs in the regulation of cardiac remodeling and advocates the role of these small molecules as potential therapeutic biomarkers in HF.[40–42] In agreement with such findings, our results showed that miR-214 and miR-1 expressions were significantly upregulated in sera of patients with systolic HF who were treated with carvedilol. It should be mentioned that no significant difference was observed between expression levels of miRs in healthy individuals compared with treated patients, which advocates the beneficial role of carvedilol therapy by means of miRs. Moreover, miR-1 was significantly downregulated in untreated patients vs healthy subjects as observed in previous studies.[43,44] Therefore, overexpression of miR-1 and miR-214 in treated patients might propose a molecular mechanism by which carvedilol benefits HF patients.

Numerous studies have reported that overlap mechanisms exist in ventricular remodeling and HF, but little is known about the role of key regulators in this regard.[45] Biological role and clinical importance of miRNAs in different tissues as well as different cardiovascular diseases stand controversial[46,47] and these controversies may be secondary to tissue-specific miRNA features and their different functions in different cells and pathophysiologic conditions.

Apoptosis of cardiomyocytes has been identified as a fundamental process in HF and is strongly associated with this illness.[48] MiR-214 was first introduced for its role in apoptosis.[49] Overexpression of miR-214 has been shown to inhibit H_2O_2-mediated apoptosis in rat cardiomyocytes. Furthermore, H_2O_2-mediated apoptosis was exacerbated following downregulation of miR-214 expression.[50] Beside subsiding cellular apoptosis, overexpression of miRNA-214 significantly ameliorates left ventricular remodeling and hemodynamics of the heart in animal models.[22] According to many subsequent reports, miR-214 is involved in muscle cell differentiation, proliferation and hypertrophy.[51–53] MiR-214 knocked out mice have been shown to exhibit reduced cardiac function and thus be more susceptible to death. Mir-214 protects cardiomyocytes by preventing the release of excessive Ca^{2+} into the cytoplasm and induces its cardioprotective action by suppressing Ca^{2+} effector kinase, CaMKII, and cell death mediators. In the absence of miR-214 expression in the heart, higher levels Ca^{2+} effectors further perpetuate Ca^{2+} overload and cell death, resulting in greater impairment of cardiac function.[46] A report by Tang et al demonstrated that miR-214 exhibits antihypertrophic properties rather than prohypertrophic effect in both an animal model of cardiac hypertrophy and in patients diagnosed with HF. MiR-214 inhibits cardiomyocytes hypertrophy by downregulating *MEF2C* (myocyte enhancer factor-2C) expression which has been involved in cardiac transcriptional program and is upregulated during cardiac hypertrophy.[30] Mef2a, a transcription factor, is a critical factor in the growth process of cardiomyocytes. In line with these observations and considering that this miRNA does not further aggravate the process of cardiac remodeling,[25] we measured a significant upregulation of miR-214 in HF patients receiving carvedilol compared to the control group (fold= 4.28, P=0.033). Therefore, we can propose that carvedilol's cardioprotective effects may be mechanistically linked to regulating the expression of miR-214, which itself exerts favorable effects on cardiac remodeling and ultimately leads to cardiac function stabilization or improvement.

Serum levels of miR-1 in patients receiving carvedilol were significantly elevated compared with the control group (fold=5.33, P=0.01). This alteration in expression is consistent with most of the previous studies regarding the role of miR-1 as one of the key regulating miRNAs in cardiac defects that attenuates cardiac hypertrophy.[2,54-56] Downregulation of miR-1 is one of the earliest changes, before any other miRNA expression change, after increase in cardiac overload, and precedes increase in cardiac mass and contractile dysfunction.[43] Data from animal models indicate that among the earliest changes due to increase in pressure overload observed in the heart, is the reduction of miR-1, prior to cardiac mass increase.[56] In vitro and in vivo data suggest that reduced expression of miR-1 is required for increased cardiac mass. Insulin growth factor (IGF)-1 as one of the main regulators of cardiac myocyte growth and differentiation, is repressed by miR-1[57] and its mRNA expression is inhibited by carvedilol.[58] Studies in neonatal myocyte culture are suggestive of downregulation of some miR-1-related hypertrophic target genes, such as Ras GTPase-activating protein (RasGAP), cyclin-dependent kinase 9 (*CDK9*), Ras homolog enriched in brain (Rheb) and fibronectin. Moreover, miR-1 inhibits the CaN/NFAT signaling pathway in cardiomyocytes by influencing the expression of Mef2a and GATA binding protein 4 (Gata4). In addition, administration of a miR-1 mimic to rats with left ventricular hypertrophy causes reversion of cardiac hypertrophy and alleviation of fibrosis and apoptosis which are recognized to be a major feature of cardiac remodeling and HF. Although a report by Hu et al is suggestive of downregulation of miR-1 by means of carvedilol in an animal model of myocardial infarction (MI),[59] it seems that miRNAs play different roles in different species as well as different physiological and pathological conditions.[46,47] Altogether, it can be postulated that miR-1 may reduce cardiac hypertrophy,[60] that mediates the protective effects of carvedilol or at least this inference deserves further studies to obtain supporting evidence.

MiR-133 has been identified as a regulatory biomarker associated with cardiac hypertrophy which is expressed at low levels in HF.[23] Recent studies show that miR-133 exhibits anti-apoptotic properties by means of suppressing the expression of caspase-3 and caspase-9.[61] MiR-133 protection against cardiomyocyte cell death can be viewed as a mechanism of action for β-blockers in HF. Upregulation of miR-133 expression by carvedilol and

the resultant anti-apoptotic effect, may contribute to the protective role of carvedilol in many pathological conditions such as MI and HF.[32] In our study, a trend toward the upregulation of MiR-133 was observed in carvedilol treated patients, although the difference between the two study groups was not significant (P=0.052) which might be due to the small sample size of patients recruited.

MiR-208 is essential for expression of the genes involved in cardiac fibrosis and hypertrophy and is expressed in the heart.[62,63] Overexpression of miR-208 has been shown to reduce the expression of antihypertrophy markers which ultimately leads to cardiomyocyte hypertrophy.[54] We observed lack of association between miR-208 expression and treatment with carvedilol in HF. MiR-208 is not advocated as a possible biomarker for favorable effects of carvedilol in HF.

The limitation of our study is the small sample size of enrolled patients. Moreover, the cohort of patients not receiving β-blockers were clinically more ill, which might have interfered with the expression of the studied miRNAs. We have taken into account these baseline differences to a high extent while choosing the controls for them, however, residual confounding factors might remain.

In summary, our study will serve as a base study for future investigations. Taken together, our findings indicated that the differential expressions of circulating miRNAs may relate to protective effects of carvedilol in cardiac remodeling and may be promising in HF treatment and prognosis. MiRNAs could be proposed as potential biomarkers in diagnosis, prognosis and treatment of HF and this study specifically provides evidence of altered miRNA expression in HF patients receiving carvedilol which can be viewed as explanations for mechanism of action of β-blockers in HF. However, to fully understand the mechanistic cardioprotective role of carvedilol in HF by means of these miRNAs investigating posttranscriptional changes are required to determine whether these miRNAs confer possible overlapping/compensatory effects on carvedilol-mediated cardioprotection.

Acknowledgments

This project was a part of MS thesis conducted by Elham Shirazi-Tehrani. The grant number of this project is 95-01-05-13912, Shiraz University of Medical Sciences, Shiraz, Iran.

Disclosure

The authors report no potential conflicts of interest in this work.

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