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Research on the relations between the variation of miRNA-184 before and after treatment of acute myocardial infarction and prognosis

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Abstract. – OBJECTIVE: Several miRNAs have been shown to be released into the circulation and play roles during acute myocardial infarction (AMI). This study aimed at detecting the variation of miRNA-184 before and after treatment of acute myocardial infarction and assessing its prognostic value.

PATIENTS AND METHODS: 72 AMI patients participated in the study, alongside 10 patients with stable coronary disease and 10 healthy volunteers for comparison. The expression levels of miRNA-184 were measured in AMI patients at 6 h, 12 h, 48 h, 7 d, and 14 d after the onset of symptoms, using blood samples and an RT-PCR method. The levels were compared to single-time levels in the other two groups of individuals. The correlations between the N-terminal pro-brain natriuretic peptide (NT-proBNP) and parameters of the ventricular function (LVEDd and LVEF) and miRNA-184 levels were analyzed taking samples during a one-month follow-up visit. Finally, the correlation between the occurrence rate of major adverse cardiac effects (MACE) and miRNA-184 levels was analyzed evaluating the occurrence of MACE at a one-year follow-up visit.

RESULTS: The expression levels of miRNA-184 (6h) were significantly higher than those of the other two groups ($p < 0.05$). The levels reached a peak 24 h after the onset of symptoms and fell back to normal after 7 to 14 days (at which point the levels were no different than the levels in the other two groups). NT-proBNP and left ventricular end-diastolic diameter (LVEDd) were significantly lower after treatment, whereas the left ventricular ejection fraction (LVEF) increased significantly ($p < 0.05$). After a relevant Pearson analysis the expression level of miRNA-184 mRNA was positively correlated with ΔNT-proBNP (before and after treatment) ($p < 0.05$), with ΔLVEDd (before and after treatment) and with ΔLVEF (before and after treatment) ($p < 0.05$). Finally, 22 cases (36%) of major adverse cardiac events, MACE, were found in AMI patients, and the expression levels of miRNA-184 of the MACE group were significantly higher than those of the non-MACE group at each time point ($p < 0.05$).

CONCLUSIONS: miRNA-184 shows a dynamic evolution before and after percutaneous coronary intervention (PCI) treatment of AMI, and it is closely correlated with recent ventricular remodeling indexes and a future occurrence rate of MACE.

Key Words:
miRNA-184, AMI, PCI, NT-proBNP, LVEDd, LVEF, MACE.

Introduction

Acute myocardial infarction (AMI) have the highest death rate of any type of coronary disease and are also the main cause of acute and chronic heart failure[1]. Timely revascularization with percutaneous coronary intervention (PCI) and coronary artery bypass graft (CABG) can significantly increase the survival rate of AMI, but there is no proof to showing that the ventricular remodeling process can be reversed[2]. A study[3] found that miRNA-184 is relatively stable in the circulation and could play a role in the reconstruction process during myocardial apoptosis by inhibiting the expression of target genes. Multiple miRNAs, including miRNA-210, mi-1, mi-320, mi-21, mi-29, mi-126, and mi-146A, are closely related to the occurrence and development of coronary heart disease[4]. However, more studies are needed to establish the value of measuring miRNA levels for prognostic purposes. This study analyzed the dynamic evolution of miRNA-184 levels before and after PCI treatment for AMI, and found that it is closely correlated with the ventricular remodeling indexes and the occurrence rate of major

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adverse cardiac effects (MACE) provides a reference frame for clinical diagnosis, treatment, and prognosis.

Patients and Methods

Patients

72 patients presenting with initial AMI, hospitalized in our hospital between July 2014 and January 2015, and treated with PCI (duration of the disease ≤ 48 h for NSTEMI, ≤ 12 h for STEMI) were enrolled in the study. AMI was diagnosed according to common standards, based on clinical symptoms, evolution of dynamic electrocardiogram and myocardial damage positive markers. Patients excluded included those with evident surgical contraindications (such as blood coagulation disorders, severe hypotension, disturbance of consciousness, etc.); those unsuited to undergo CPI treatment (left main coronary artery involved by coronary angiography, triple vessel disease, bifurcation lesions, calcified lesions and small vessel lesions); and others with contrast agent sensitivity, autoimmune disease, imperfect clinical data, poor compliance, serious condition of disease or expected lifetime shorter than 12 months.

The Ethics Committee of our hospital approved the study. The informed consents were obtained from the patients or their family members. The patients included 40 men and 32 women; in total 43 cases of STEMI (ST elevation myocardial infarction), and 29 cases of NSTEMI (non-STEMI). The elapsed time from onset of symptoms to operation varied from 8 to 60 hours, with a mean of 30.5 h. Also, for comparison 10 patients with stable coronary disease and 10 healthy volunteers were also enrolled. All AMI patients were treated with standard medical processes which were done by the same surgical and nursing teams in accordance with guidelines.

RT-PCR Methods

The RT-PCR method was chosen to get the expression level of serum miRNA-184 mRNA at different time points (6 h, 12 h, 48 h, 7 days and 14 days after the onset of symptoms). 5 ml of venous blood were collected from each patient. After a centrifugation step at 3000 rpm/min for 15 min, the supernatant was saved at -80°C for subsequent examination. RNA was extracted by TRIzol reagent (MRCGENE), and the RNA concentration and purity were determined by the NanoDrop ND-1000 (Qiagen, Hilden, Germany) ultraviolet absorption method and denatured agarose gel electrophoresis. Then, cDNA was synthesized. Primers were designed using the Primer 5.0 ABI PRISM7900 system (Applied Biosystems, Foster City, CA, USA) and ordered from Shanghai Yingjun Biotechnology Company. All the primers were resuspended to a final l0 pmol/μl concentration. Primer sequences are the following: miRNA-184-F: 5`-TGCTGCCAGTCACGTCCCCTTATCACTTTTCCA-3`, miRNA-184-R: 5'-CCTGTCAATCACCTACCCTTATCAGTTCTCCTG-3', β-actin-F: 5`-GGGTCAGGGCAGTATCTCTC-3`, β-actin-R: 5`-AGGACAGCACCAGAGTAACC-3'. The reaction system was a 2 × Master Mix 2 μl cDNA + 0.5 μl of 10 μM PCR specific primer + ddH_2O = 8 μl. The thermocycler was programmed to run an initial denaturating step of 95°C for 10 min. Then, a 40X cycle of a denaturing step at 95°C for 10 s, and then an annealing step at 60°C for 60 s. The products were analyzed by the $2^{-\Delta\Delta CT}$ method and shown as target gene/reference gene.

The correlation between N-terminal pro-brain natriuretic peptide NT-proBNP, left ventricular end diastolic diameter (LVEDd), left ventricular ejection fraction (LVEF) and miRNA-184 levels were analyzed at a one-month follow-up visit. The correlation between the occurrence rate of MACE and the miRNA-184 levels were analyzed during a one-year follow-up visit. NT-proBNP was examined by chemiluminescence immunoassay.

Statistical Analysis

The SPSS19.0 software (SPSS Inc., Chicago, IL, USA) was used for recording data and performing statistical analyses. The quantitative data was shown in the form of average ± standard deviation; comparisons between multiple groups were analyzed by One-way ANOVA; while intra-group comparisons were examined by repeated measurement data. Qualitative data was shown in the form of case number and percentages (%) and qualitative data comparisons among groups were examined by χ^2. The Pearson's correlation was used to analyze relevant correlations between variables. Differences with a *p*-value lower than 0.05 were considered statistically significant.

Results

Comparison of the Expression Level of miRNA-184 mRNA

The expression level of miRNA-184 mRNA in AMI patients (6 h after symptoms appearance)

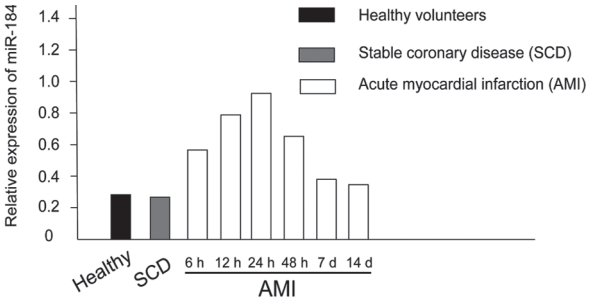

Figure 1. Comparison between the relative expression levels of miRNA at different time-points of AMI group and that of its control group.

was significantly higher than that in the other two groups ($p < 0.05$). There were 72 cases at the time of 6 h, 20 cases underwent PCI treatment by the 12 h time point, and all cases had had PCI treatment by 24 h. The expression level of miRNA-184 mRNA of AMI patients reached its summit after 24h, and fell back to normal after 7d and 14d (no difference with the other two groups) (Figure 1).

Correlation Between the Expression Quantity of miRNA-184 mRNA and Ventricular Remodeling Parameters

NT-proBNP and LVEDd were significantly lower after treatment, whereas LVEF increased, and the differences were of statistical significance ($p < 0.05$). NT-proBNP: (425.6 ± 55.3) to (874.9 ± 73.6) pg/ml, $t = 8.632$, $p < 0.001$. LVEDd: (52.3 ± 2.4) to (56.5 ± 2.7) mm, $t = 7.524$, $p < 0.001$. LVEF: (46.7 ± 3.3) to (40.5 ± 3.4) %, $t = 7.623$, $p < 0.001$ (Table I). The expression level of miRNA-184 was positively correlated with ΔNT-proBNP, ΔLVEDd and ΔLVEF (r = 0.324, $p = 0.027$; r = 0.362, $p = 0.022$; r = 0.411, $p = 0.016$) by making a relevant analysis of the expression level of miRNA-184 mRNA at the time of 14d, amount of NT-proBNP, LVEDd, and LVEF after treatment, ΔNT-proBNP (before and after treatment), ΔLVEDd (before and after treatment) and ΔLVEF (before and after treatment).

Correlation Between the Expression Level of miRNA-184 mRNA and the Occurrence Rate of MACE

22 AMI patients (30.6%) developed MACE, including 6 cases of target vessel revascularization, 5 cases of recurrent angina pectoris and myocardial infarction, 7 cases of acute and chronic heart failure and 4 cases of cardiac death. It was found that the expression levels of miRNA-184 in the MACE group were significantly higher than the levels of the non-MACE group at each time, and the differences were of statistical significance ($p < 0.05$) (Figure 2).

Discussion

A previous work[5] found that the miRNA level was correlated with a negative control of gene expression. Many translations of mRNAs get suppressed by the complementary pairing of miRNAs with the 3'end non-coding region of the target molecule mRNA. It is estimated that 1/3 of genes are regulated and controlled by miRNAs. Multiple studies[6] have found that some miRNAs are highly expressed in myocardial cells and influence the occurrence, development, and prognosis of coronary heart disease. Several miRNAs have been considered as biomarkers of acute coronary disease[7]. The miR-320 in a rat model of myocardi-

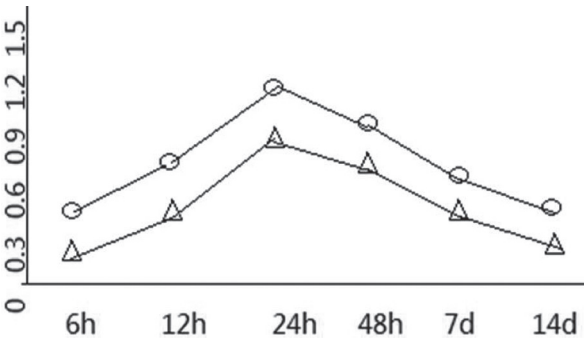

Figure 2. The expression level of miRNA at different time-points of MACE group and non-MACE group.

Table I. Levels of NT-proBNP, LVEDd, and LVEF before and after treatment.

	NT-proBNP (pg/ml)	LVEDd (mm)	LVEF (%)
Before treatment	874.9 ± 73.6	56.5 ± 2.7	40.5 ± 3.4
After treatment	425.6 ± 55.3	52.3 ± 2.4	46.7 ± 3.3

al ischemia/reperfusion contributed to atherosclerosis by the expression of a myocardial protective heat shock protein 20[8]. Also, the expression of miR-21 in myocardial ischemic areas clearly decreased after 6h of ischemia in rats, while the levels increased in surrounding marginal areas[9]. It has also been found in an *in vivo* experiment that miR-1 and miR-133 are counterproductive to apoptosis[10]. At present, multiple miRNAs that increase or decrease their expression in patients with coronary heart disease can be detected from the peripheral veins of such patients[11]. The pathophysiological significance of such fluctuations is still unclear and further studies are needed. Based on the theory above, we expect that understanding the effects of miRNAs will open the door for new targets for intervention and prognosis of AMI.

From a genome-wide differential expression analysis, a study found that miRNA-184 probably plays an important role in myocardial apoptosis, ventricular remodeling, myocardial fibrosis and some other post-AMI myocardial cell disease compensatory mechanisms[12]. The above processes can be accomplished by miRNAs regulating and controlling cell growth and apoptosis, extracellular matrix reconstruction, neuroendocrine activation and other mechanisms activated by intercellular transport[13]. The hypoxia-inducible factor-lα (HIF-lα) plays a key role stimulating new vessels during anoxia by regulating endothelial cell factor and angiogenic protein factor-2[14,15]. miR-210 is now universally recognized as the most important miRNA in regulating anoxia[16]; besides, also being induced by HIF-lα, miRNA-184 can also be highly expressed, taking part in regulation of the cell cycle, differentiation, and apoptosis by regulating and controlling target genes (EFNA3, E2F3, NPTXI, CASP8AP2, etc.)[17]. The increasing expression levels of miR-1 and miRNA-184 have also been related to ischemia-perfusion injury and apoptosis after myocardial infarction, functioning by translational depression of Bcl-2 and IGF-1[18]. In the mouse's heart failure fibroblasts, the levels of miR-21 and miRNA-184 significantly increased, probably by inhibiting the Sprouty homologue 1 and PTEN gene and activating the MAPK pathway, thus increasing the survival rate of cardiac fibroblasts and promoting myocardial fibrosis and reconstruction[19].

In our study, the expression levels of miRNA-184 in AMI patients 6h after the onset of symptoms were significantly higher than the levels in the stable angina pectoris group or the healthy volunteers group. The AMI levels reached a peak 24 h after the onset of symptoms and then fell back to normal at 7 and 14 days after the onset of symptoms. miRNA-184 can be considered a valuable marker during the disease but it can also be an aid for the diagnosis of AMI together with troponins and creatine kinase-MB[20]. It is thought that the summit and fall back to normal levels of miRNA-184 during AMI may be related to the intervention of PCI, and the resulting reperfusion of ischemic areas[21].

It was found at a one-month follow-up visit measuring ventricular remodeling indexes that the expression level of miRNA-184 mRNA was positively correlated with ΔNT-proBNP, ΔLVEDd, and ΔLVEF; a reminder that miRNA-184 could play a role in the recovery and reconstruction process of injured myocardial cells[22]. Moreover, a one-year follow-up visit found that the occurrence of MACE was also correlated with the expression level of miRNA-184 mRNA, as the miRNA-184 levels of the patients who experienced MACE were significantly higher than the levels in the non-MACE group at each time point. Therefore, the expression level of miRNA-184 was increased during the ischemia, reperfusion, and reconstruction of myocardial cells. Whether miRNA-184 is an independent factor or it has a causative role in the development of MACE needs to be further studied.

Conclusions

miRNA-184 has a dynamic evolution before and after PCI treatment for AMI. It is closely correlated with recent ventricular remodeling indexes and the future occurrence rate of MACE.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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