Expression of microRNA-26b-5p is related to the changes of random blood glucose after primary percutaneous coronary interventions

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Research article

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Abstract

Background: Stress hyperglycemia (SH) is prevalent in non-diabetic patients with ST-segment elevation myocardial infarction (STEMI), and it can significantly increase the risk of adverse cardiovascular events. The study aims to determine the changes of and correlation between circulating microRNA-26b-5p levels and random blood glucose levels after primary percutaneous coronary interventions (p-PCI).

Methods: INS-1 cells were used to establish model of insulin regulation. Overexpression of miR-26b was performed with lentiviral transfection, while downregulation of miR-26b was accomplished by using shRNA sequences. Insulin was quantitatively detected by an enzyme-linked immunosorbent assay kit (ELISA). we enrolled 48 patients with acute anterior wall myocardial infarction who successfully underwent p-PCI from June 2017 to November 2017 in Beijing Anzhen Hospital and Beijing Friendship Hospital, Capital Medical University. Plasma samples were collected before p-PCI, 24h and 72h after operation. The level of circulating microRNA-26b-5p were measured by Real Time PCR. The association between circulating microRNA-26b-5p and random blood glucose was calculated by Spearman correlation coefficient.

Results: The results of in vitro test showed inhibition of miR-26b leads to a decrease in insulin secretion while overexpression of miR-26b increases insulin secretion in INS-1 cells. 34 male patients accounting for 71% were included in this study. The circulating microRNA-26b-5p levels were 14.41 ± 8.52 and 19.63 ± 9.42 at 24h and 72h after operation respectively, as compared with 10.32 ± 6.62 before p-PCI ($P < 0.001$). Significantly decreased random blood glucose level were found at 24h and 72h after operation compared with that before p-PCI (7.35 ± 1.17 VS. 7.12 ± 1.17 VS. 8.28 ± 1.35 , $P < 0.001$). Spearman correlation coefficient revealed that there was an inverse correlation between circulating microRNA-26b-5p levels and random blood glucose levels ($r = -0.425$, $P < 0.001$).

Conclusion: MicroRNA-26b-5p may be involved in the regulation of blood glucose in non-diabetic patients with STEMI after p-PCI.

Background

Acute myocardial infarction (AMI) is often associated with a disorder of blood glucose metabolism. There was a prevalence of stress hyperglycemia (SH) in non-diabetic patients with ST-segment elevation myocardial infarction (STEMI), and the incidence rate in different studies ranged from 31–71% [1]. Previous studies have shown that SH increases the risk of in-hospital mortality and adverse cardiovascular events in patients with AMI [2, 3].

MicroRNA (miRNA) participates in the pathophysiology of many cardiovascular diseases, which can affect stem cell transplantation in the treatment of MI [4]. MicroRNA-26b (miR-26b) has been previously identified as a regulator of cardiac hypertrophy, miR-26b could inhibit PTGS2 to activate the MAPK pathway and reduce inflammatory response and improve myocardial remodeling in mice with MI [4]. MiR-

26b-5p could reverse the hypertrophic growth in Ang-II-induced neonatal mouse ventricular cardiomyocytes (NMVCs), as well as eliminate the pro-hypertrophic effect of circRNA_000203 in NMVCs^[5]. Some studies indicated that the expression of miR-26b was affected by a variety of factors that are correlated with obesity, glucose and insulin sensitivity^[6]. Recent studies show that mir-26b-5p may be closely related with adverse outcomes in patients with STEMI^[7]. However, to date, the mechanisms are not clear and it probably related with glucose and lipid metabolism. This study aims to examine the changes of and correlation between circulating microRNA-26b-5p and random blood glucose after primary percutaneous coronary intervention (p-PCI), and provide clinical evidence for further study.

Methods

In vitro test

Cell culture and cell transfection INS-1 cells were cultured in RPMI-1640 with 10% fetal bovine serum, 50μM β mercaptoethanol, 11.1mM glucose, 2mM glutamine, 100 U/ml penicillin, 100 U/ml streptomycin in a humidified incubator with an atmosphere of 5% CO₂ at 37 °C. The medium was changed every 2 days, and the cells passaged when reached 70% confluence. The cells between 7th and 10th passage were used in this study. Overexpression of miR-26b was performed with lentiviral transfection while interference of miR-26 was accomplished using shRNA sequences(miR-26bminics) were designed and synthesized. Thus the INS-1 cells can be divided into 3 groups: wild type INS-1 cells, miR-26b overexpression INS-1 cells as well as miR-26b suppression INS-1 cells.

Insulin secretion and measurement. The three groups of INS-1 cells described above were cultured separately in 6-well plates until reached their 80% confluent. The culture supernatant was collected and the insulin level was quantitatively detected by an enzyme-linked immunosorbent assay (ELISA).

In vivo test

We did this prospective observational study at two major hospitals in Beijing, China (Beijing Anzhen Hospital, and Beijing Friendship Hospital, Capital Medical University). We recruited patients without diabetes mellitus with acute anterior wall myocardial infarction who successfully underwent p-PCI in the CCU from June 2017 to November 2017. Exclusion criteria included: 1) diabetes mellitus; 2) failed p-PCI therapy; 3) Thrombolysis In Myocardial Infarction (TIMI) 0-2 flow; 4) heart failure with Killip class 2-4; and, 5) cardiogenic shock, stent thrombosis, recurrent myocardial infarction, or cardiac death.

Blood sampling and Real Time qPCR analysis

Total RNA were Extracted from Plasma samples collected before p-PCI, 24h and 72h after operation using an RNA extraction kit (cwbio.co.ltd,cat#cw0581). Complementary DNA(cDNA) was then synthesized by using the cDNA synthesis kit (cwbio.co. ltd, cat# CW2141) and miRNA specific reverse transcription primers. cDNA fragments containing microRNA-26b-5p were amplified using the following **primers**: 5'-TTCAAGTAATTCAGGATAGGT -3'; 5'- GGCCAACCGCGAGAAGATG -3'. relative microRNA-26b-5p expression

were analysed by quantitative real-time PCR(qRT-PCR) in an Applied Biosystems 7500 Sequence Detection System (ABI 7500 SDS; Foster City, CA, USA) and calculated by $2^{-\Delta\Delta CT}$ method.

Statistic analysis

The SPSS statistical software (Version 21.0, SPSS, Inc., Chicago, IL, USA) had been utilized for statistical analysis. Continuous data were expressed as mean $\pm$ standard deviation(SD) or median range. Groups of continuous data were compared by one-way analysis of variance (ANOVA) or kruskal-wallis *H* test. The association between circulating microRNA-26b-5p and random blood glucose was calculated by the Spearman correlation analysis.

Results

The results of in vitro test showed inhibition of miR-26b leads to a decrease in insulin secretion while overexpression of miR-26b increases insulin secretion in INS-1 cells .Inhibition of miR-26b leads to a markedly decrease by 45.5% in insulin secretion while overexpression of miR-26b can increase insulin release by 68.9% (Table 1).

A total of 54 STEMI patients without diabetes mellitus undergoing p-PCI were enrolled in this study. 6 patients were ineligible because there was either no qualified blood sampling (n=4), or new onset diabetes(n=2). Thus, 48 patients (34 men and 14 women) were included in the final statistical analyses. Table 2 provides the basic characteristics of the study patients by gender. Men had a higher smoking rate compared with women ($P=0.005$). There were no significant differences between male and female with respect to other characteristics.

The circulating microRNA-26b-5p levels were 14.41 ± 8.52 and 19.63 ± 9.42 at 24h and 72h after operation respectively, as compared with 10.32 ± 6.62 before p-PCI ($P<0.001$). Significantly decreased random blood glucose level were found at 24h and 72h after operation compared with that before p-PCI 7.35 ± 1.17 VS. 7.12 ± 1.17 VS. 8.28 ± 1.35 , $P<0.001$. And the detail information was shown in table3.

Correlation between the circulating microRNA-26b-5p levels and random blood glucose values was displayed in Figure 1, there was an inverse correlation between circulating microRNA-26b-5p levels and random blood glucose values $y = -0.062x + 8.499$ $r = -0.425$ $P < 0.001$.

Discussion

In order to evaluate the correlation between circulating microRNA-26b-5p and random blood glucose, in vitro test was made to test the hypothesis that miR-26b could regulate the insulin secretion in INS-1 cells. Our experiments found inhibition of miR-26b leads to a decrease in insulin secretion while overexpression of miR-26b increases insulin secretion in INS-1 cells. Previous study also showed that knockdown of miR-26 in cultured β -cells or in isolated primary islets resulted in reducing insulin promoter activity and insulin mRNA, which is associated with upregulation of transcriptional repressors, including Bhlhe22 and Sox6^[8].

Some studies have found that miR-26b expression decreased in patients with AMI [9], which is consistent with the results of our study. In the present study, we found that random blood glucose decreased gradually after p-PCI, however circulating microRNA-26b-5p was gradually increased. And we also found that there was a negative correlation between circulating microRNA-26b-5p and random blood glucose. There are various mechanisms as to why hyperglycemia portends poor prognosis in non-diabetic patients with STEMI. First, Hyperglycemia promotes the binding of inflammatory cells with endothelium^[10], and increases the production of inflammatory cytokines. This may drive plaque instability^[11]. Second, acute hyperglycemia significantly attenuated endothelium-dependent vasodilation. As proved by the previous study, hyperglycemia impairs the forearm blood flow response to methacholine during hyperglycemia^[12]. In addition, hyperglycemia improve superoxide (O₂⁻) generation and impair platelet nitric oxide (NO) responsiveness, resulting in platelet activation and thrombotic events.^[13]

Insulin play a vital role in regulating glucose metabolism, and many evidence addressed miR-26b may participate in regulation of insulin resistance (IR). Previous study had pointed that MiR-26b expression is downregulated in visceral adipose tissue from obese rodent Models^[14]. Kirby TJ et al^[15] used mRNA-microRNA microarray approach to identify difference of microRNAs in subcutaneous adipose tissue between insulin-sensitive (IS) and IR patients, and found that 16 microRNAs are downregulated in IR patients including miR-26b, miR-30b, and miR-145. Additionally, Xu G et al^[14] showed that MiR-26b targeted the PTEN gene, activated PI3K/AKT signaling pathway so as to increase insulin-associated glucose uptake and promote insulin-associated glucose transporter 4(GLUT4) translocation to plasma membrane in human mature adipocytes.

miR-26b also have an important role in insulin synthesis. Tal Melkman-Zehavi et al^[16] shown that inactivating miRNA in β -cells of adult mice results in a dramatic decrease producing of insulin. When They knock down miR-24, miR-26, miR-182 or miR-148 of cultured β -cells, insulin promoter activity is repressed by upregulation of insulin transcriptional repressors including Bhlhe22 and Sox6. MiR-26b is a good discriminator of adverse cardiac events in patients with STEMI. In an ACS cohort which included 1002 STEMI patients, researchers selected 14 miRNAs for validating the relationship between miRNAs and MACE. Finally 3 miRNAs including miR-26b-5p, mir-320a and miR-660-5p are considered to involving in adverse cardiovascular events in patients with STEMI. As previous experimental studies shown, the 3 miRNAs are suggested to regulating adverse cardiac remodeling, cardiomyocyte death and apoptosis, and platelet activation, which may trigger heart failure, myocardial infarction, and cardiac death^[17-19]. Receiver-operating characteristic (ROC) curve showed that miR-26b-5p showed greater power to discriminate patients with MACE from patients without compared with high-sensitivity troponin T and pro B-type natriuretic peptide (pro-BNP). [Area under the curve (AUC) = 0.707 VS. 0.666 VS. 0.674]^[7].

In summary, we find that there is an inverse relationship between circulating microRNA-26b-5p and random blood glucose in non-diabetic patients with STEMI. In the present study, we can only draw a conclusion, which needed more experimental evidence, that MicroRNA-26b-5p may be involved in the regulation of blood glucose in non-diabetic patients with STEMI after p-PCI. Additionally, evidence have

shown that stress hyperglycemia and miR-26b-5p are associating with MACE in patients with STEMI. And miR-26b participates in regulation of insulin synthesis and IR. Therefore, we may hypothesize that microRNA-26b are involved in adverse outcomes in patients with STEMI by regulating hyperglycemia through pathway we don't know, which needed to further basic research to confirm.

Limitations

We acknowledge limitations of our study related with design and conduct. Firstly, the size of population was small. Secondly we did not enroll patients with diabetes because hypoglycemic agents have effect on blood glucose so that we couldn't access the impact of MicroRNA-26b-5p on the regulation of blood glucose. Thirdly we couldn't control every step of blood sampling. Therefore, the measurement of MicroRNA-26b-5p and blood glucose maybe not entirely accurate.

Conclusion

There is an inverse relationship between circulating microRNA-26b-5p and random blood glucose in non-diabetic patients with STEMI. MicroRNA-26b-5p may be involved in the regulation of blood glucose in non-diabetic patients with STEMI after p-PCI.

Abbreviations

AMI Acute myocardial infarction

SH stress hyperglycemia

STEMI ST-segment elevation myocardial infarction

miRNA MicroRNA

NMVCs neonatal mouse ventricular cardiomyocytes

p-PCI primary percutaneous coronary intervention

ELISA enzyme-linked immunosorbent assay

cDNA Complementary DNA

qRT-PCR quantitative real-time PCR

NO nitric oxide

IR insulin resistance

Declarations

Ethics approval and consent to participate

This study was approved by anzhen Hospital institutional medical ethical committee. All patients provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from Institutional Review Board of the Beijing Anzhen hospital and Beijing Friendship Hospital of Capital Medical University but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Institutional Review Board of the Beijing Anzhen hospital and Beijing Friendship Hospital of Capital Medical University.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Jianwei Zhang and Lingjie He designed the experiments; Jianwei Zhang and Lingjie He performed the experiments; Jianwei Zhang and Lingjie He analyzed the experimental results and wrote the manuscript. Both authors read and approved the final manuscript.

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Tables

Table 1 Effect of expression of miR-26b on insulin release (** P<0.01 ; * P<0.05)

Table 2. Basic characteristics of subjects by gender

	Men	Women	t/ χ^2	P-value
Subject number	34	14	NA	NA
Age (years)	55±16	53±18	0.379	0.706
BMI, kg/m ²	24.6±4.2	25.1±4.5	0.367	0.715
Left ventricular EF, %	54.1±7.2	53.4±7.8	0.299	0.766
WBC counts (10 ⁹ /L)	10.21 ±2.9	10.17 ±3.1	0.043	0.966
Serum creatinine, mg/dL	0.88±0.22	0.85±0.27	0.402	0.690
Total Cholesterol (mg/dl)	198±45	190±47	0.553	0.583
Triglycerides (mg/dl)	98±31	88±27	1.052	0.298
HDL-C (mg/dl)	54±13	60±15	1.390	0.171
LDL-C (mg/dl)	124±31.5	115±27.6	0.931	0.357
Preoperative random BG (mmol/l)	8.16±1.48	8.14±1.54	0.042	0.967
Coronary risk factor (n,%)				
Hyperlipidemia	17 (50)	6(43)	0.203	0.653
Hypertension	20(59)	5(36)	2.122	0.145
Current smoking	20(59)	2(15)	7.923	0.005
Family history	6 (18)	2(14)	0.020	0.887
Obesity	9(26)	4(29)	0.043	0.835
Previous medication [n(%)]				
Aspirin	27(84)	8(85)	1.490	0.222
ACEI or ARB	19(56)	7(50)	0.138	0.710
β-Blockers	16(47)	6(43)	0.071	0.791
Statin	15(44)	7(50)	0.138	0.710
Angiographic data[n(%)]				
Single vessel	16(47)	6(43)	0.071	0.791
Double vessels	12(35)	4(29)	0.013	0.911
Triple vessels	6(18)	4(29)	0.208	0.648
Main stem involved	4(12)	2 (14)	0.058	0.810

Data given as mean ± SD or n (%).

BMI, body mass index; EF ,ejection fraction; WBC, White blood cell; HDL-C, High density lipoprotein-cholesterol ; LDL-C ,Low density lipoprotein-cholesterol; BG, blood glucose

Table 3. Comparison of the circulating microRNA-26b-5p levels and random blood glucose values over 3 days

	before p-PCI	24 hours after p-PCI	72 hours after p-PCI	F-value	P-value
microRNA-26b-5p levels	10.32±6.62	14.41±8.52	19.63±9.42	15.28	0.001
RBG values[mmol/l]	8.28±1.35	7.35±1.17	7.12±1.17	11.94	0.001

RBG, Random blood glucose;p-PCIprimary percutaneous coronary interventions

Figures

Figure 1

Correlation between the circulating microRNA-26b-5p levels and random blood glucose values