CLINICAL SIGNIFICANCE OF DOWNREGULATED PROTEIN KINASE C IOTA (PRKCI) GENE IN PERIPHERAL LEUKOCYTES OF PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

LIN FAN¹, HEYU MENG¹, YUSHUANG YANG³, KAIYAO SHI, DONGNA LIU¹, FANBO MENG¹*
¹Postgraduated student of Department of Cardiovascular Medicine, China-Japan Union Hospital of Jilin University, now work in Echocardiography Department, First Affiliated Hospital of Soochow University, Suzhou 215006, China - ²Medical College of Yanbian University, Yanji 133000, China - ³Department of Cardiovascular Medicine, China-Japan Union Hospital of Jilin University, Jilin 130033, China

ABSTRACT

Introduction: It has been accepted that gene play a role in the evolution of acute myocardial infarction (AMI). We found protein kinase C iota (PRKCI) gene had different expression in the peripheral leukocytes of patients with AMI through gene chip.

Materials and methods: 20 AMI patients were randomly selected and set as the AMI group, and 20 healthy people were selected as the control group. The clinical data of all study subjects were recorded. 6 ml of venous blood was sampled from both two groups for ribonucleic acid (RNA) and protein extraction; reverse transcriptionpolymerase chain reaction (RT-PCR) and Western blot were then performed to detect the expression of PRKCI gene using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin as the internal reference, respectively.

Results: There existed significant differences in low-density lipoprotein (LDL-ch) and high-density lipoprotein (HDL-ch) between the two groups. At the RNA level, the relative expression of PRKCI gene in the AMI group to the control group was 0.62 ± 0.11 (P=0.032); at the protein level, the PRKCI protein expression in the AMI group was also significantly reduced than the control group.

Conclusions: The low expression of PRKCI gene takes part in the development of AMI. PRKCI gene may be used as a molecular marker for the diagnosis of AMI, even treated as a therapeutic target for the clinical diagnosis and treatment of AMI patients.

Keywords: Acute myocardial infarction, PRKCI gene, RNA, PRKCI protein, molecular labeling.

DOI: 10.19193/0393-6384_2018_2_50

Received August 30, 2017; Accepted January 20, 2018

Introduction

Acute myocardial infarction (AMI) refers to the coronary artery stenosis or occlusion-based drastic reduction or interruption of coronary blood supply, thus causing severe ischemia-resulted myocardial necrosis in the corresponding myocardium. Studies in recent years have shown that AMI is a disease co-caused by a variety of factors such as heredity, environment, personal habits, or society, and it also has the characteristic of familial aggregation; however, its pathogenesis could not be fully explained by the Rules of Mendelian inheritance. Therefore, it is now considered as a polygenic inheritance disease⁹.

Present treatment methods such as anti-platelet aggregation, coronary artery expansion, blood fat reduction, or coronary stent implantation reduce the morbidity and mortality of AMI to some extent, and they also play important roles in the prognosis and recovery of these patients.

However, partial patients would still occur secondary AMI, and the treatment effects would thus be poor. Studies found that some patients still occur thrombosis when orally administrated clopidogrel, and it was related with the mutation of certain gene locus²⁴. Therefore, selecting one optimal drug treatment through clinically testing these patients’ gene sequences could greatly increase the treatment effects.
In recent years, microarray technologies have been successfully applied to investigate the genetic correlations of various diseases, including hypertension, type 1 diabetes, or pancreatic cancer. The whole genome association studies have shown that AMI is related with heredity, and the locus mutations of some genes are closely related with the occurrence of AMI. Related researches also made great progresses, and PCSK9 gene inhibitors that are closely related with lipid metabolism have been used in clinical treatments.

At the RNA level, certain research also found that when compared with the sham group, the myocardial cells in the AMI mice exhibited some gene expression differences, and this might have an impact on AMI. Meanwhile, some genes were found upregulated in the AMI patients with ST-segment elevation, and this could be used as an adjunct means for the STMI assessment. More and more evidence has proven that the gene expression levels in AMI patients were abnormal, and these abnormally expressed genes might be used as the molecular markers for the AMI diagnosis or the therapeutic targets. However, different ethnic groups have different gene expression profiles, so it would be vital to obtain the specific genes from the diseased race(s).

In our previous study, we found the expressions of some genes in the peripheral leukocytes of AMI patients in Han nationality in northeast China changed significantly than those non-AMI patients, among which the protein kinase C iota (PRKCI) gene was significantly downregulated (P=0.003517699, LogFC = -1.563554087).

Furthermore, according to the pathway analysis, this gene was involved in multiple metabolic pathways such as insulin metabolism, platelet activation, intercellular connections, and wnt signaling pathway, and these factors were related with the occurrence of multiple AMI risk factors. In this study, the expressions of PRKCI in AMI patients were studied at the RNA and protein levels, respectively, and the functions of this gene was also analyzed, aiming to find the molecular marker(s) of AMI and apply it (them) for targeting clinical treatment.

**Materials and methods**

**General data**

20 AMI patients hospitalized and treated in the Department of Cardiology, China-Japan Union Hospital of Jilin University, from November 2014 to January 2015 were randomly selected and set as the AMI group. In the meantime, 20 healthy volunteers were set as the control group. The clinical data of these two groups were recorded and analyzed in details. The diagnostic criteria of AMI referred to the diagnostic guidelines issued by the American College of Cardiology/American Heart Association in 2012, and the vascular lesions were further confirmed by coronary angiography.

The study subjects that met the diagnostic guidelines and existed severe vascular stenosis or occlusion were set as the AMI group, while those mismatching the diagnostic guidelines and with vascular stenosis <50% were divided into the control group. All the study subjects were excluded other diseases such as cancer, liver dysfunction, renal dysfunction, peripheral arterial diseases, or thyroid diseases. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Jilin University. Written informed consent was obtained from all participants.

**Peripheral lymphocytes sampling**

6 ml of fasting peripheral blood was sampled from each study subject in the morning, and then added into EDTA-anticoagulated tube and stored at 4°C. The lymphocytes were also sampled with 4h of blood sampling. The peripheral lymphocytes were then obtained referring to the instructions of human peripheral blood lymphocyte separation solution (Tianjin Haoyang Biological Manufacture CO., LTD, Tianjin). The peripheral lymphocytes of each subject were divided into two parts, with one part (4 ml) for the total RNA extraction and the other part (2 ml) for the total lymphocytic protein extraction.

**Detection by reverse transcriptionpolymerase chain reaction (RT-PCR)**

The total RNA was extracted from the acquired peripheral lymphocytes according to the kit instructions of total RNA extraction kit (Tiangen BioTechnology Co., Ltd. Beijing), and then detected the quality and concentration using agarose gel electrophoresis and spectrophotometer. The total RNA reverse-transcription was then performed according to the kit instructions of reverse transcription kit (TOYOBO, Shanghai); the obtained cDNA was then stored at -80°C for the next fluorescence quantitative PCR.
The obtained cDNA sample was firstly diluted by 10 folds, and then performed PCR amplification according to the specific instructions of SYBR fluorescence quantification kit (TaKaRa, Dalian). The reaction condition was at 95°C for 10 min, and the following three steps were repeated 40 cycles: 95°C for 15 sec, 60°C for 20 sec, 72°C for 20 sec; 95°C for 1 min, 55°C for 30 sec, and 95°C for 30 sec. The GAPDH gene was used as the reference gene, and the PRKCI gene was set as the candidate gene; the specific amplification conditions were determined according to the solubility curves of the software in Mx3005P. The PCR primer sequences were shown in Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>Upstream primer 5’-GACATCAA-GAAGGGTGAGGC-3’</td>
</tr>
<tr>
<td>PRKCI</td>
<td>Upstream primer 5’-CTTGCAGTGAGGGTCC-3’</td>
</tr>
<tr>
<td></td>
<td>downstream primer 5’-TGTCATTGAGAG-CAATGCCAGC-3’</td>
</tr>
<tr>
<td></td>
<td>downstream primer 5’-AGGCTTCTTCAACTC-GAAGTGG-3’</td>
</tr>
</tbody>
</table>

Table 1: RT-PCR primer sequences which were used in RT-PCR. RT-PCR: reverse transcription polymerase chain reaction

Western blot

The total protein was extracted from the acquired peripheral lymphocytes using RIPA lysate, and its concentration was then detected using a spectrophotometer; the obtained sample was then stored at -80°C for the further Western Blot. The total protein obtained was detected using classic Western Blot assay, with β-actin as the reference gene and PRKCI as the candidate gene. The primary antibodies were rabbit anti-mouse β-actin antibody and rabbit anti-mouse PRKCI antibody, and the secondary antibody was goat anti-rabbit IgG antibody. All the antibodies were purchased from Abcam Co.

Statistical analysis

All the data were analyzed using SPSS20.0 (Statistical Product and Service Solutions20.0, IBM, USA) statistical software; all the measurement data were expressed as mean±standard deviation, and the intergroup differences were performed independent T test; the counting data were expressed as frequency, and the intergroup comparison used the χ² analysis; with P<0.05 considered as statistically significant.

Results

Baseline analysis

The baseline data of the study subjects were shown in Table 2. There was no significant difference in the age, sex, and comorbidities between the two groups; however, there were significant differences in low-density lipoprotein (LDL-ch) and high-density lipoprotein (HDL-ch) between the two groups, and the AMI group exhibited higher LDL-ch level and lower HDL-ch level.

<table>
<thead>
<tr>
<th>Clinical info</th>
<th>AMI group(n=20)</th>
<th>Control(n=20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years)</td>
<td>51±13</td>
<td>58±11</td>
<td>0.13</td>
</tr>
<tr>
<td>Gender(F/M)</td>
<td>6/4</td>
<td>13-Jul</td>
<td>1</td>
</tr>
<tr>
<td>Hypertension=yes/no</td>
<td>8/12</td>
<td>14-Jun</td>
<td>0.74</td>
</tr>
<tr>
<td>Diabetes=yes/no</td>
<td>5/15</td>
<td>18-Feb</td>
<td>0.405</td>
</tr>
<tr>
<td>Fasting blood sugar(mmol/L)</td>
<td>7.16±2.63</td>
<td>6.04±3.51</td>
<td>0.306</td>
</tr>
<tr>
<td>Total cholesterol(mmol/L)</td>
<td>4.94±1.18</td>
<td>4.54±0.79</td>
<td>0.311</td>
</tr>
<tr>
<td>Triglyceride(mmol/L)</td>
<td>2.11±0.92</td>
<td>1.66±1.22</td>
<td>0.303</td>
</tr>
<tr>
<td>LDL-ch(mmol/L)</td>
<td>3.49±0.96</td>
<td>2.84±0.57</td>
<td>0.044</td>
</tr>
<tr>
<td>HDL-ch(mmol/L)</td>
<td>0.85±0.15</td>
<td>1.14±0.36</td>
<td>0.004</td>
</tr>
<tr>
<td>Blood platelet count(10^9/L)</td>
<td>242.35±63.92</td>
<td>234.92±44.75</td>
<td>0.724</td>
</tr>
</tbody>
</table>

Table 2: Baseline information of the study subjects: there were no significant differences between the two group except LDL-ch and HDL-ch. LDL-ch: low-density lipoprotein; HDL-ch: high-density lipoprotein; AMI: acute myocardial infarction; Note: *, P<0.05.

RT-PCR results

In this study, the solubility curves of the candidate gene and the internal reference were both single-peak form, indicating the amplification products had higher specificity (Figure 1), and no non-specific amplification occurred. The Δcycle threshold (ΔCT) value of each RT-PCR sample was the average of three-time repetitions, and the standard deviations were all in the reasonable ranges. The relative expression of PRKCI used the 2-ΔΔCT method, and the results showed that at the mRNA level, the expression of PRKCI in the AMI group was lower than the control group, and the relative expression of was 0.62 ± 0.11, P=0.032 (Table 3), consistent with the results of the microarray assay[19].
Western blot

The results of Western blot showed no significant difference in the expression of β-actin between the two groups, but the protein expression of PRKCI in the AMI group was significantly lower than the control group (Figure 2).

Discussion

Our previous microarray experiments revealed that the PRKCI gene in the peripheral leukocytes of AMI patients was significantly downregulated, and this study confirmed that PRKCI was downregulated in Han AMI patients in northeast China through more samples, no matter at the RNA level nor at the protein level, this gene was detected significantly downregulated.

The PRKCI gene is one member of the protein kinase C family, which participates in a variety of cellular responses, and whose activation plays important roles in the early stage of various biological processes as muscle contraction, gene expression, or cell proliferation\(^{21,22}\). In myocardial tissues, the protein kinase C is considered to be able to activate the K-ATP channel, thus impacting the adaptive regulation in the early ischemic stage; meanwhile, it also participates in the occurrence of a variety of post-myocardial ischemia complications, such as cardiac arrhythmia or cardiac dysfunction\(^{23-25}\). The PRKCI gene was found to be involved in the insulin-mediated glucose transportation\(^{26,27}\). It was found in the extensive ischemic myocardial tissues of rats that the PRKCI protein was decomposed into fine granular fragments and then transported, and showed no significant change after reperfused with 1μM phorbol ester\(^{28}\). Recent studies showed that PRKCI was related with the occurrence of a variety of cancers, so it was considered as an oncogene\(^{29}\).

In the insulin pathway, the pathway analysis on the website of the Japanese genes and Genomes Encyclopedia (http://www.kegg.jp/pathway/hsa04910) showed that the combination of insulin and insulin receptor could then activate PIP3 and proline-directed protein kinase 1 (PDPK1), whose activation would further activate the PRKCI protein. The activated PRKCI protein then indirectly activated the sterol regulatory element binding protein 1 (SREBP-1C) and fatty acid synthase (FAS), thus promoting adipogenesis indirectly; meanwhile, it could promote the absorption of acetylcoenzyme a carboxylase α and glucose.

In addition, PDPK1 could cause the threonine protein kinase 3 (Akt3) phosphorylated, and the latter could inhibit the fat metabolism by inhibiting the synthesis of cAMP. On the other hand, the activated SREBP-1C could indirectly activate the pyruvate kinase (PYK) and glucokinase (GK), and these two enzymes could reduce the blood sugar via the glycolytic pathway.

In the platelet activation pathway, the combination of ADP and G protein-coupled purine P2Y receptor 1 (P2Y1) on the platelet membrane would activate the G protein polypeptide (Gq), and the lat-
ter could activate the phospholipase C-β1 (PLCβ) and thus further activate diacylglycerol (DAG), thereby activating the PRKCI protein. The activated PRKCI protein then could activate the Ras related protein RAP-1a, and then continuously induce the activation of RIAM protein and talin 1 protein, thereby causing the activation of the platelet membrane integrin α-2b, which was also a component of the platelet protein IIb/IIIa complex, so the activated integrin α-2b could activate the fibrinogen α chain, thus further promoting the platelet aggregation indirectly.

The pathway analysis showed that the normal expression of the PRKCI protein could be prone to promoting the adipogenesis, blood glucose degradation, and platelet aggregation, so the crowd with downregulated PRKCI protein should have lower blood lipid levels and higher blood sugar levels. However, it is generally believed AMI patients have higher LDL-ch lever, which is also one of the initiating links of coronary atherosclerosis, contradicting this pathway analysis. In our previous cell experiments, we used RNA interference vectors to specifically interfere the in vitro rat cardiomyocytes, and the non-interfered cells were used as a control so as to avoid the reduce the RNA gene and protein expressions of PRKCI in the experiment group while the expressions of other genes were not affected. The comparative observation with the control group revealed that the LDL concentration in the media of the experiment group was gradually increased over time\(^{[30]}\), indicating that the downregulation of the PRKCI gene could promote the synthesis of blood lipids.

In the present study, the LDL-ch level in AMI patients was higher than the control group, consistent with the results of our previous cell interference experiments and the large clinical big data. The result that the HDL-ch level in AMI patients was lower was also consistent with the general belief that this factor was one protective media, and it could do favor to the formation of anti-atherosclerosis. According to the pathway analysis, the patients with downregulated PRKCI gene should have higher blood sugar levels, and in this study, the AMI patients had higher average blood sugar level than the control group, but no significant difference was found, so it still needed to be further clarified with cell experiments. The platelet count between the two groups had no significant difference, but the PRKCI gene affected the platelet aggregation; however, this study did not test the platelet functions, so this phenomenon still needed further study.

Through analyzing the results in our study, we considered that: such biological processes as the lipid metabolism, lipid synthesis, carbohydrate metabolism, carbohydrate synthesis, and platelet aggregation in human were subject to the regulation of a variety of factors, so it would not be accurate to analyze the results from only one single gene or one metabolic pathway, so there would exist differences with the actual situations. AMI is a polygenic disease caused by the combination of multiple factors, and lots of pathways are involved in, so the interactions among these pathways would be complex, and more clinical data and gene function data would help to improve the accuracy of further detailed analysis.

In recent years, individualized treatment of certain disease has been gradually paid high attention in the medical field, and the targeting individualized treatment would greatly improve the effectiveness of the treatment. Based on the results of previous microarray experiments, this study used blood samples, which could be easily obtained clinically, to analyze the PRKCI gene expression in the AMI patients, and the results revealed that the expression of this gene was reduced in the AMI patients, which thus promoted the synthesis of LDL-ch and thus induced the coronary atherosclerosis, so it could be considered as one of the causes of AMI. Therefore, the PRKCI gene could be used as a molecular marker for the diagnosis of AMI, or a therapeutic target for the clinical diagnosis and treatment of AMI patients.

Conclusions

The PRKCI gene was downregulated in the peripheral leukocytes in AMI patients, and its impacts on the lipid metabolism was one of the causes of AMI.

References


Acknowledgments

This study was funded by the Excellent Talent Projects in New Century of Ministry of Education (2008), Projects of Jilin Provincial Science and Technology Department in 2009 (20090734), and Projects of Jilin Provincial Department of Finance in 2012 (2012009), and special acknowledgements should be given to Professor Zhihui Zhao and his research team for their technical guidance.

Corresponding Author:
FANBO MENG
Department of Cardiovasular Medicine,
China-Japan Union Hospital of Jilin University,
No. 126 Xiantai Street
Changchun 130033
E-mail: docfanbomeng@126.com
(China)