The expression of UDP-glucose pyrophosphorylase 2 following acute myocardial infarction in rat

LuShun Zhang [1], LinBo Gao [2], WeiBo Liang [1], YunLiu [1], TianYi Chen [1] and Lin Zhang [1]

1 Department of Forensic Biology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, Sichuan 610041, China.
2 Laboratory of Molecular and Translational Medicine, West China Institute of Women and Children's Health, West China Second University Hospital, Sichuan University, Chengdu, Sichuan 610041, China
zhanglin@scu.edu.cn

Abstract: Acute myocardial infarction (AMI) is a major cause of sudden cardiac death (SCD) in patients with coronary and ischemic heart diseases. However, the age of AMI estimation in SCD is not fully known. The aim of this study was to investigate the expression of UDP-glucose pyrophosphorylase 2 (UGP2) in AMI. A total of 71 Sprague–Dawley male rats were divided into AMI group, sham-operated group and normal control group. After surgery, the myocardium was harvest following 0 min, 15 min, 30 min, 60 min, 120 min, 240 min in AMI group and sham-operated group. The expression of UGP2 in myocardium of rats was detected using quantitative real-time PCR and Western Blot. *UGP2* mRNA and corresponding protein expression were decreased after ligation 15 min, and significant differences were found in *UGP2* mRNA (after ligation from 30 min to 120 min) and UGP2 protein (after ligation from 30 min to 240 min) in the AMI zone compared with the control, respectively. These findings suggest that UGP2 may be used as a potential biomarker for the time estimation in AMI.
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Key words: UGP2; AMI; Age of AMI; SCD.

1. Introduction

Acute myocardial infarction (AMI) is a major cause of sudden cardiac death (SCD) in patients with coronary and ischemic heart diseases (Xu et al., 2001; Thygesen and Uretsky, 2004). Early diagnostic assessment of patients suspected of having acute coronary syndrome (i.e., ST-elevation myocardial infarction, non ST-elevation myocardial infarction or unstable angina) remains a challenge, especially when the electrocardiogram (ECG) is inconclusive. In patients presenting with ischemic-type chest pain, the ECG has only 50% sensitivity for the diagnosis of acute myocardial infarction (MI) (Menown et al., 2000). Currently, assessment of the myocardial infarction (MI) presence is based on the elevation of biochemical markers from myocardial necrosis in the context of clinical and ECG findings (Anderson et al., 2007; Bassandet al., 2007; MEMBERS et al., 2007; Thygesen et al., 2007). Recent studies have focused on the biomarkers that were used for diagnosis of AMI within initial 24 h in patients with chest pain. These biomarkers, are from myocyte injury (myeloperoxidase, metalloproteinase-9, soluble CD40 ligand, pregnancy-associated plasma protein A, choline, ischemia-modified albumin, unbound free fatty acids, glycogen phosphorylase isoenzyme BB, and placental growth factor) and myocardial necrosis (Cardiac Troponin I and T). Nowadays, CK-MB was used as the standard marker for diagnosing AMI (Apple et al., 2005).

Forensic autopsy data shows that SCD victims usually die less than 6 h, even within 1 h after AMI occurs (Pratt et al., 1996; de la Grandmaison, 2006). The current diagnostic approaches are not sensitive enough to make such an early diagnosis (Xu et al., 2001; Virmani et al., 2001). SCD remains one of the major challenges of contemporary cardiology and forensic pathology. In our previous work, we explored the different transcription gene between AMI groups and normal groups in rat using suppression subtractive hybridization (SSH) assay (Zhang et al., 2009). After hybridization, the UDP-glucose pyrophosphorylase 2 (UGP2) was detected. Considering that the UGPase act as a key enzyme in carbohydrate metabolism, we examined the expression levels of UGP2 mRNA and protein in myocardium by real-time PCR and Western-Blot to determine whether it can be utilized as an earlier biomarker for AMI age estimation.

2. Materials and methods

2.1. Animals, surgery and tissue preparation

A total of 71 adult healthy S-D male rats (300-350g) were provided by the West China Medical Animal Center of Sichuan University. The animal research was approved by Sichuan University Committee on Animal Research. The models were made following the methods described previously (Zhang et al., 2009). In brief, the animals were divided by the interval time 0 min, 15 min, 30 min, 60 min,120 min, 240 min in acute myocardial ischemia group (AMI, n=6 for each subgroup) and sham-operated group (Sham,

n=5 for each subgroup). We also set up a normal control group (normal, n=5). At the precise setting time, the heart of each animal was harvested. Tissue samples were taken out from ischemic in left ventricles of MI groups, and the tissue samples of sham groups and normal group were taken out from the left ventricles.

2.2. RNA Extraction and quantitative real-time PCR

Total RNA was extracted from cardiac muscle specimens with TRIzol reagent according to the manufacturer's instructions (Takara Biotechnology, Dalian, China). The purity and quantity of RNA were assessed using the Nano Drop spectrophotometer (Thermo Scientific, DE, USA). Integrity of RNA was determined by electrophoresis through 1.0% formaldehyde agarose gel. Samples RNA bands, staining with ethidium bromide, were visualized under UV illumination.

Synthesis of cDNA was performed with Prime Script TM RT reagent Kit (Takara Biotechnology) according to the manufacturer's instructions. Real-time PCRs were carried out on the Applied Biosystems 7500 Real-Time PCR System using SYBR® Premix Ex TaqTM II (Takara Biotechnology). Primers of UGP2 were designed based on sequences obtained from GenBank (Gene ID: 289827), and the primer sequences were: forward primer 5′-GAAGGTTCCCAGATACAA -3, and reverse primer 5′- CACGGTTTCAGGAGATG -3. The18S rRNA was chosen as an internal control, and the primer information was obtained from previously published (Teixeira-Clerc F et al., 2006). PCR DNA standards were from a pure conventional PCR product of UGP2 and 18S, respectively. PCR DNA standards were diluted over a 10-fold range (107-101) to prepare the standard curves (Simpson et al., 2000; Liss, 2002; Wozney and Wilson, 2012). All Q-PCRs were performed under conditions recommended by the manufacturer (95 ℃for 10 min, followed by 40 cycles at 95 ℃ for 15 s, 60 ℃ for 1min). Fluorescence signal was detected at the end of each cycle.

Melting curves performed with Dissociation Curves software (Applied Biosystems) to ensure only a single product was amplified, and sample products were run on a 3% agarose gel to confirm specificity. Samples were tested in triplicate and the average values were used for quantification. Sterile purified water was used as a negative control.

2.3. Western Blot

Equal amounts of protein extracts were resolved by SDS-PAGE and the interest proteins were probed on a nitrocellulose membrane, the primary antibody concentration for UGP2 (Novus) and Actin (Abcam) were 1:1000 and 1:2000, respectively. The Western blotting ECL Advance Detection Kit (Millipore, Billerica, MA, USA) was used for sensitive detection of enhanced chemiluminescence (ECL) of the antigen-antibody complexes. Each experiment was repeated at least three times.

2.4. Statistical analysis

All statistical analyses were performed with SPSS version 11.5 (SPSS, Chicago, IL). Group distributions were presented as mean ±SD. χ^2 test or Fisher's exact test was used to compare proportions. Analysis of variance (ANOVA) was used for comparisons within AMI groups. $P<0.05$ was taken as the minimum level of significance.

3. Results

3.1. The Quantitative PCR for AMI

We analyzed the melting curve of each PCR product in each PCR session and confirmed that no non-specific products were produced. There was rarely significant primer dimer formation during the numbers of cycles. It provided the quantification of the PCR products from a range of experimental samples. The amplification efficiency of the two genes was 95% and 92% from the standard cure, respectively. The quantitative values were defined as the PCR cycle at which the fluorescent signal of the reporter dye crosses an arbitrarily placed threshold in the exponential phase. In our assays, the constant threshold value was 0.04. There was no statistical significance of UGP2 mRNA level between the control group and sham group, suggesting that the animal model was constructed successfully. The expression of UGP2 mRNA in AMI group was decreased by 15 min after ligation, and the significant decreasing level was observed from 30 min to 240 min ($P < 0.05$) compared with the AMI 0 min group or control group. When compared subgroup with different myocardial ischemia time after ligation in operation groups, the expression of UGP2 mRNA in 30 min, 60 min 240 min was obviously changed with the former group ($P < 0.05$) (Figure 1).

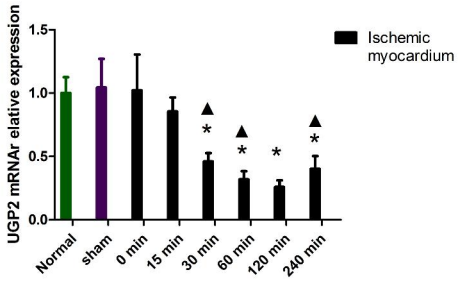

Figure 1 Relative expression of UGP2 mRNA in Normal control, Sham operation group, and Operation group during different myocardial ischemia time

Note: * P<0.05, compare with the expression of UGP2 mRNA in Operation groups and Control. ▲P<0.05, compared the former group with the expression of UGP2 mRNA in Operation groups.

Normal, Normal control group; Sham, Sham operation group; 0 min, 15 min, 30 min, 60 min,120 min, 240 min were different myocardial ischemia time after ligation the coronary artery in operation group during.

3.2. Western Blot

Immunoreactive proteins for UGP2 protein and actin protein with molecular masses of approximately 56kDa and 42kDa were detected respectively (Figure 2). Similar to *UGP2* mRNA expression, the level of UGP2 protein in AMI group after 15 min ligation was lower than the other two groups, and significant changes from 60 min to 240 min ($P < 0.05$) in AMI group compared with the AMI 0 min group or control group (Figure 3).

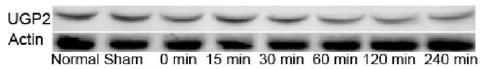

Figure 2 Expression of UGP2 and actin protein in rat myocardial tissue of Control group, Sham group and Operation group with different myocardial ischemia time.

Note: Normal: Normal group；Sham：Sham group；0 min, 15 min, 30 min, 60 min, 120 min, 240 min were the ischemia time, respectively.

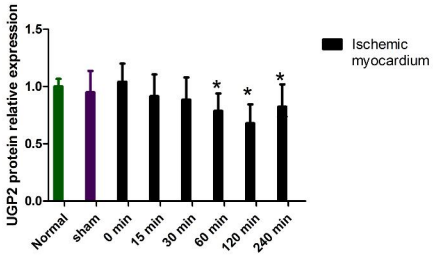

Figure 3 Relative expression of UGP2 protein comparing the Control in Sham operation group and Operation group during different myocardial ischemia time

Note: * $P<0.05$, compared with the expression of UGP2 protein in Operation groups and Normal Control.

4. Discussion

The *UDP-glucose pyrophosphorylase* (*UGPase*), encoded by *UGPase* mRNA, was widely observed in animals, microorganisms and plants (Peng and Chang, 1993). *UGPase* gene has two isoforms in Rat and human being, UGP1 and UGP2. And the UGP2 locates at Chr. 2 p13-p14 (Cheng et al., 1997). UGPase is a key enzyme for carbohydrate metabolism, catalyzing the reversible production of UDP-glucose (UDP-Glc) and pyrophosphate from glucose 1-phosphate (Glc 1-P) and UTP, depending on the cellular metabolic status. This reaction is necessary in several tissues. In liver and muscle, UDP-Glc, a substrate/product of UGPase, is the energetically activated form of glucose and is required for the synthesis of glycogen. While in lactating mammary gland it is converted to UDP galactose and thence lactose (Bishop et al., 2002). Previous work (Zhou et al., 2011) has shown that over-expressing *UGPase* genes can increase the product of UDP-Glc in Streptomyces. And *UGPase* gene was proved to be a rate limiting factor in vegetative and reproductive phases in arabidopsis thaliana (Park et al., 2010). Our study was undertaken to detect the change of UGP2 in AMI tissue after ligation of the left anterior descending coronary artery in rats. The results demonstrate that *UGP2* mRNA and corresponding protein expression were decreased after ligation 15 min, and significant changes *UGP2* mRNA (after ligation from 30 min to 120 min) and UGP2 protein (after ligation from 30 min to 240 min) in the AMI zone, respectively. It confirmed that UGP2 may be a potential biomarker of the time estimation in AMI.

Considering the injury associated stress response may affect the entire body and operator skills (Bai et al., 2008), the sham-operated group was setup. No significant changes in the expression levels of UGP2 mRNA and protein of myocardium between the sham-operated group and the normal control group were found, suggesting that the animal model was successfully constructed.

In conclusion, although the molecular mechanism of UGP2 changing is unclear, our AMI animal model data of UGP2 gave us a clue to estimate the time of acute myocardial infarction in forensic science practice, and additional studies should be carried out to better understand those changes in AMI.

Conflict of Interest

The authors have declared that no conflict of interest exists.

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Corresponding author:

Lin Zhang, Department of Forensic Biology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, P.R. China.

Email: zhanglin@scu.edu.cn.

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