Metabolism of arachidonic acid by the cytochrome P450 enzyme in patients with chronic Keshan disease and dilated cardiomyopathy

BING ZHOU[1,2], SHULAN HE[3], XI WANG[1,2], XIAOLONG ZHEN[4], XIAOHUI SU[1,2] and WUHONG TAN[1,2]

[1]School of Public Health, Xi'an Jiaotong University Health Science Center, Key Laboratory of Trace Elements and Endemic Diseases National Health and Family Planning Commission; [2]Key Laboratory of Environment and Genes Related to Diseases (Xi'an Jiaotong University), Ministry of Education, Xi'an, Shaanxi 710061; [3]Department of Epidemiology and Biostatistics, School of Public Health, Ningxia Medical University, Yinchuan, Ningxia 750004; [4]Wuqing People's Hospital of Tianjin, Tianjin 301700, P.R. China

Received September 10, 2015; Accepted December 18, 2015

DOI: 10.3892/br.2015.563

Abstract. Keshan disease (KD) is an endemic cardiomyopathy. The etiology of KD is selenium deficiency; however, it is not the only one and there is no effective approach to preventing and curing this disease. The aim of the present study was to explore the differences in the role of arachidonic acid (AA) by the cytochrome P450 enzyme between chronic KD (CKD), dilated cardiomyopathy (DCM) and control patients. Reverse transcription-quantitative polymerase chain reaction was used to detect the CYP1A1 and CYP2C19 gene expression levels in 6 CKD patients, 6 DCM and 6 healthy controls. An enzyme-linked immunosorbent assay kit was applied to detect serum protein expression of CYP1A1 and CYP2C19, AA and epoxyeicosatrienoic acids (EETs), and 20-hydroxyeicosatetraenoic acids (20-HETE) in 67 CKD patients, 28 DCM, and 58 controls. The present results showed that the expression levels of CYP1A1 and CYP2C19 genes were significantly upregulated compared with the control group (P<0.01). The expression level of the CYP1A1 protein in the CKD (49.55±35.11 pg/ml) and DCM (46.68 ±13.01 pg/ml) groups were enhanced compared with the control group (44.33±16.76 pg/ml) (P<0.01). The production of the CYP2C19 protein in the CKD (57.52±28.22 pg/ml) and DCM (56.36±11.26 pg/ml) groups was enhanced compared with the control group (51.43±10.76 pg/ml). The concentrations of AA in the CKD (126.27±47.91 ng/ml) and DCM (133.24±58.67 ng/ml) groups were also significantly increased compared to the control (78.16±23.90 ng/ml) (P<0.001). The concentration of 20-HETE in the CKD (198.34±17.22 ng/ml) and DCM (194.46±20.35 ng/ml) groups were also significantly increased compared to the control (130.10±16.10 ng/ml) (P<0.001). The only difference between CKD and DCM was for the expression of the CYP1A1 gene and protein. The maximum concentration of EETs was in the control group (44.37±6.14 pg/ml), and the other two groups were lower than the control group (P<0.001). These findings indicated that AA-derived CYP450 metabolites may have a critical role in the pathogenesis of KD and DCM. Upregulation of the CYP2C19 gene and frequent protein expression may be a protective compensation reaction, while CYP1A1 may aggravate myocardial injury.

Correspondence to: Professor Wuhong Tan, Key Laboratory of Environment and Genes Related to Diseases (Xi'an Jiaotong University), Ministry of Education, 76 Yanta West Road, Xi'an, Shaanxi 710061, P.R. China
E-mail: tanwh@mail.xjtu.edu.cn

Key words: dilated cardiomyopathy, Keshan disease, arachidonic acid, epoxyeicosatrienoic acids, 20-hydroxyeicosatetraenoic acids

Introduction

Keshan disease (KD) is an endemic cardiomyopathy occurring only in China, involving 327 counties of 16 provinces. Chronic KD (CKD) has similar clinical characteristics to dilated cardiomyopathy (DCM), such as acute or chronic episodes of heart disorder characterized by cardiogenic shock, arrhythmia and congestive heart failure, with cardiomegaly, as well as multifocal myocardial necrosis and fibrosis under the electron microscope (1). A differential diagnosis for the two diseases in clinical practice is extremely difficult. To further distinguish, the main characteristics of DCM are increased heart weight and cardiac hypertrophy, while KD is characterized by severe myocardial degeneration, evident necrosis and fibrosis, and even clear geographic characteristics. Previous studies have identified that the two types of disease were significantly different in ventricular cavity expansion and ventricular volume by summarizing the image and ultrasound information of DCM and KD patients (2). In recent years, certain studies have proposed that *ATM* and *NFATC2* may be considered significant differential genetic diagnoses in the two similar diseases through comparing the KD and DCM patient peripheral blood mononuclear cells using genome-wide expression microarray technology (3).

Arachidonic acid (AA) is oxidized through three major metabolic pathways: Cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYPs) (4). Previously, studies have focused on the CYP450 system of AA; epoxidation and

hydroxylation of AA are catalyzed by cytochrome P450s (CYPs) in heart disease (5). According to the CYP metabolic pathway followed by AA, the major metabolites are either epoxyeicosatrienoic acids (EETs) or 20-hydroxyeicosatetraenoic acids (20-HETE), which are primarily vasodilators or vasoconstrictors, respectively (6). In addition, it has been increasingly recognized that EETs and 20-HETE have crucial and opposing roles in the development of cardiac disease. Certain studies have reported the possible changes in expression levels of the main human CYP450 monooxygenase (2E1 isoform) at DCM progression at the end stage of heart failure using western blot analysis (7). While for KD, a number of previous studies have explored the mechanisms of the former two pathways (COX and LOX pathways of AA), which were abnormal in KD, little evidence is available regarding the CYP450 pathway in KD. Therefore, a comparison in the metabolism of AA by the CYP450 enzyme between CKD and DCM is noteworthy, and may provide more functional information for the pathogenesis of KD.

In the past several decades, a number of studies have explored the cause of KD, and its main etiology and pathogenesis is known to be selenium deficiency. At present, KD is widely considered as multifactorial environment-gene interaction complex diseases. KD has been suggested as a 'mitochondrial cardiomyopathy' endemic in China (8). Our previous study compared the mitochondrial-related gene expression profiles of peripheral blood mononuclear cells derived from KD patients and normal controls by mitochondria-focused cDNA microarray technology. The results indicated that the enzyme-related genes CYP1A1 and CYP2C19 were upregulated (ratios ≥2.0) (9), and these genes belong to the cytochrome P450 isoforms, whose metabolites are biologically active and have critical roles in the maintenance of essential body functions. Various CYP isoenzymes have been confirmed to have a role in the metabolism of xenobiotics and endogenous compounds. The model endogenous substrate of CYP is AA (10).

In the present study, the metabolism of AA by the CYP450 enzyme was investigated in CKD patients, DCM patients and health controls by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and enzyme-linked immunosorbent assay (ELISA) to examine the gene expression changes and biochemical parameters associated with the CYP-AA metabolism. The study may aid in the understanding of the pathogenesis of KD and distinguish a possible difference between KD and DCM.

Materials and methods

Sample collection and experiment group. The present study was approved by the Human Ethics Committee, Xi'an Jiaotong University (Xi'an, Shaanxi, China) and all participants provided informed consent. A total of 67 CKD patients were selected according to 'The Diagnostic Standard of the KD in China' for KD (WS/T 210-2011), who were from areas of Zhengning and Heshui (Gansu, China). A total of 28 DCM patients who did not live in endemic areas were diagnosed in three tertiary-level facilities hospitals in Xi'an in terms of the World Health Organization/International Society of Federation of Cardiology proposed standards (11). Three group subjects were matched by age and gender. These patients were selected as samples as they were not exhibiting coronary artery disease, myocarditis, congenital heart disease, pulmonary heart disease or other cardiovascular diseases. A total of 58 healthy controls without any chronic diseases, such as diabetes, hypertension and any other heart disease, were collected from the KD-affected area of Huangling (Shaanxi, China).

Peripheral venous blood (3 ml) from each subject was collected into vacuum blood tube, and serum was obtained through centrifugation at 2,683 x g for 8 min and stored at -80˚C for ELISA. Anticoagulant whole blood (2 ml) was acquired into a tube with 7.5 ml RNA stabilizing solution and mixed prior to storing at -80˚C using for RNA extraction.

Total RNA extraction and cDNA synthesis. The total RNA from blood was abstracted with E.Z.N.A. Total RNA kit I (Omega Bio-Tek, Norcross, RA, USA) according to the manufacturer's protocol. The concentration and purity of total RNA was evaluated by measuring the absorbance 260/280 ratio with a Thermo Nanodrop 2000 (Thermo Fischer Scientific, Inc., Waltham, MA, USA). Following this, RNA was transcribed into cDNA using the PrimeScript RT Reagent kit (Takara Biotechnology, Dalian, China). The reverse transcription system was as follows: 0.5 μg of total RNA was added to a mix of 2.0 μl 5X PrimeScript buffer, 0.5 μl PrimeScript RT Enzyme mix I, 0.5 μl Oligo dT Primer, 0.5 μl Random 6-mers, finally adding Rnase-Free dH_2O to create a volume of 10 μl. The reaction mixture was maintained at 37˚C for 15 min, and heated for 85˚C for 5 sec.

RT-qPCR. RT-qPCR analysis was performed in the Bio-Rad IQ5 PCR Detection system (Bio-Rad, Hercules, CA, USA) using SYBR Premix Ex Taq™ II (Takara Biotechnology). A 25-μl reaction mixture was placed into 0.2 ml clear strips with 8-well tubes (Crystalgen, Commack, NY, USA), which contained 1 μl of 10 μM forward primer and 1 μl of 10 μM reverse primer (0.4 μM final concentration of each primer), 12.5 μl of SYBR Premix Taq II, 8.5 μl of nuclease-free water, and 2 μl of the cDNA sample. The specific primer sequences were CYP1A1 (forward, 5'-CCT CCT CAA CCT CCT GCT AC-3' and reverse, 5'-AAG CAA ATG GCA CAG AYG AC-3'); CYP2C19 (forward, 5'-GGT CCT TGT GCT CTG TCT CT-3' and reverse, 5'-CAT ATC CAT GCA GCA CCA CC-3'); glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward, 5'-TGC ACC ACC AAC TGC TTA GC-3' and reverse, 5'-GGC ATG GAC TGT GGT CAT GAG-3'), which were designed and synthesized by AuGCT DNA-SYN Biotechnology Corporation, Beijing, China. The thermocycling conditions were set at 95˚C for 30 sec, followed by 40 PCR cycles of denaturation at 95˚C for 5 sec, 60˚C for 30 sec, and annealing/extension at 65˚C for 15 sec. GAPDH was used as an internal standard for mRNA levels.

ELISAs. The ELISA kit is an accurate, precise sensitivity assay, particularly suitable for large sample detection. The levels of protein expression of CYP1A1 and CYP2C19, AA, EETs and 20-HETE in all the serum samples were tested using commercially available ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA) in accordance with the manufacturer's protocols.

Table I. Gender and age characteristics of research subject.

Analysis	Male, n	Female, n	Mean age ± SD, years
RT-qPCR			
CKD (n=6)	3	3	52.00±6.00
DCM (n=6)	3	3	49.33±2.08
Controls (n=6)	3	3	48.67±2.52
Statistical analysis	χ^2=1.706, P=1		F=0.6, P=0.579
ELISA			
CKD (n=67)	27	40	54.06±15.72
DCM (n=28)	11	17	58.00±16.64
Controls (n=58)	23	35	50.33±21.36
Statistical analysis	χ^2=0.825, P=0.662		F=0.135, P=0.876

CKD, chronic Keshan disease; DCM, dilated cardiomyopathy; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; SD, standard deviation.

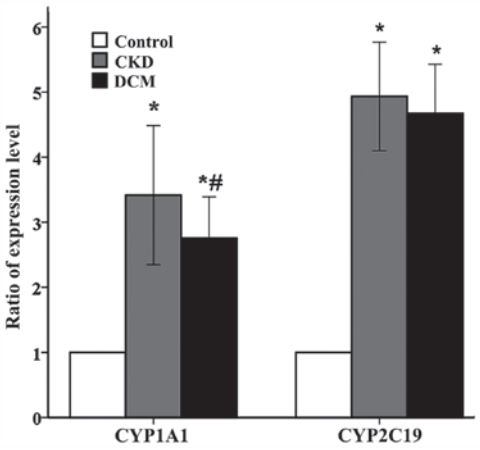

Figure 1. Reverse transcription-quantitative polymerase chain reaction was performed to analyze the gene expression profiles in the whole blood of the three groups. The expression of CYP1A1 and CYP2C19 in the CKD and DCM groups was increased significantly compared to the healthy control. Data are expressed as relative ratio. *P<0.01, CKD and DCM compared with control respectively. #P<0.05, for DCM versus CKD. CKD, chronic Keshan disease; DCM, dilated cardiomyopathy.

A calibration standard curve on a semi-log scale was subsequently drawn by collecting and analyzing the corresponding absorbencies at different dilution extents of standards in duplicate. The Infinite M2000 multimode microplate reader (Tecan, Männedorf, Switzerland) was used to test the absorbance of the samples and standards. In carrying out these assays, the serum of each patient was detected in two replicates, which prevented from repeated freezing and thawing of the samples.

Statistical analysis. All the experimental data were analyzed with SPSS version 18.0 software (IBM, Corp., Armonk, NY, USA). The gender-matched RT-qPCR sample set and ELISA sample set was determined using χ^2 test and age was compared by one-way analysis of variance (ANOVA). The $2^{-\Delta\Delta CT}$ method was used to calculate the relative fold-changes in gene expression determined from the RT-qPCR result (12). ELISA data were analyzed using ANOVA, and were tested by pairwise comparisons [least significant difference (LSD)-t-test]. P<0.05 was considered to indicate a statistically significant difference.

Results

Baseline data. The characteristics of the CKD and DCM patients and the controls are shown in Table I. No significant difference was apparent between the gender constituent of the RT-qPCR sample set and ELISA sample among the three groups by χ^2 test (P<0.05). Age distribution in the three groups was balanced, with no significant difference through one-way ANOVA (P<0.05). The LSD-t-test analysis of age distribution indicated no significant difference in any two groups (P<0.05). These results suggest there were no significant gender and age differences; and these characteristics were balanced and comparable among the three groups.

Results of RT-qPCR. The concentration of total RNA was examined with the Thermo Nanodrop 2000. The absorbance value 260/280 was 1.94-2.09, maintained at ~2.0. Therefore, the RNA samples were of a good purity and quality. RT-qPCR was performed to assess the mRNA levels of CYP1A1 and CYP2C19. As shown in Fig. 1, multiple bar diagrams show the expression ratio of the CYP1A1 and CYP2C19 genes. The expression levels of the two target genes in the CKD and DCM groups were markedly enhanced compared with the control group, which were consistent with the former microarray experiment (5).

ELISA results. To further investigate the CYP1A1 and CYP2C19 protein expression levels and the relevant metabolites, ELISA kits were used. Fig. 2 presents the various expression levels of CYP1A1 and CYP2C19 protein production. The expression level of the target proteins in the CKD and DCM patients was significantly increased compared with the healthy controls (P<0.001), consistent with the former immunohistochemical results. In addition, between the CKD and DCM groups, the CYP1A1 protein exhibited a significant difference (P<0.05), whereas there was no difference for the CYP2C19 protein (P>0.05).

In order to investigate the associated product expression level of the AA-CYP metabolic pathway, the concentrations of AA, EETs and 20-HETE in the blood serum were measured by the ELISA kits. As described in Fig. 3, the content of AA and 20-HETE had a similar trend, as the content was significantly increased in the CKD and DCM groups compared to the controls (P<0.001). However, the expression level of EETs exhibited the opposite tendency, with a maximum concentration in the healthy control, and a reduction of the concentration in the CKD and DCM groups (P<0.001).

Discussion

The present study evaluated the possible differences between CKD and DCM based on the upregulation of CYP1A1 and CYP2C19 genes. RT-qPCR and ELISA were applied to compare the differences in the CYP1A1 and CYP2C19 gene expression levels and biochemical parameters associated with CYP-AA metabolism in the CKD patients, DCM patients and

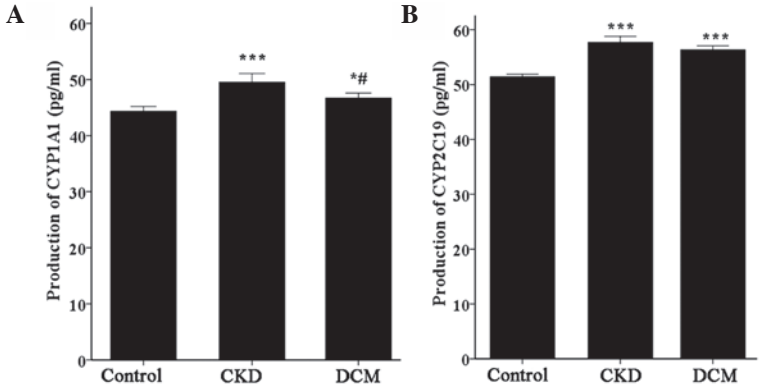

Figure 2. Production of (A) CYP1A1 and (B) CYP2C19 protein in blood serum. Data were analyzed with one-way analysis of variance, and multiple comparison by least significant difference t-test. Each bar is the mean ± standard deviation. ***P<0.001, *P<0.05, compared with the healthy control. #P<0.05, for DCM versus CKD. DCM, dilated cardiomyopathy; CKD, chronic Keshan disease.

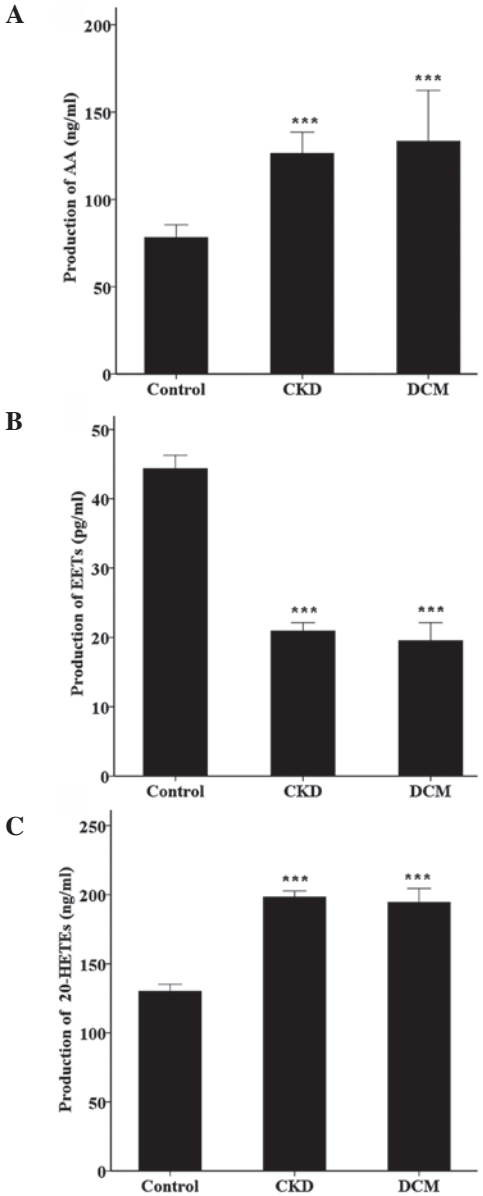

Figure 3. Expression level of (A) AA, (B) EETs and (C) HETEs in blood serum. Data were analyzed with one-way analysis of variance, and multiple comparison by least significant difference t-test. Each bar is the mean ± standard deviation. ***P<0.001, compared with healthy control. AA, arachidonic acid; EETs, epoxyeicosatrienoic acids; 20-HETE, 20-hydroxyeicosatetraenoic acids; CKD, chronic Keshan disease; DCM, dilated cardiomyopathy.

normal controls. The RT-qPCR results were presented in Fig. 1, and the expression levels of the two target genes in the CKD and DCM groups were markedly enhanced compared with the control group, which were completely consistent with previous research results. In the healthy human heart, CYP1A1 mRNA is expressed in an extremely low amount (5), which has a vital physiological activity on endogenous substrates that are involved in the metabolism of AA and tryptamine (13). CYP2C19 mainly exists in the hepatic microsome, which is a type of important liver microsomal enzyme, mainly involved in a wide variety of drugs' metabolism and arachidonic acid epoxidation (13,14). Previous studies have reported that the expression levels of CYP genes 1A1 and 2C19 in the heart were predominant in the right ventricle (15). In CKD and DCM patient blood, mRNA levels for the CYP1A1 and CYP2C19 were higher in comparison to the healthy control, indicating that certain changes are caused by genetics in the metabolic pathways mainly associated with the cardiac structure and function injury, rather than starting factors, as an approach of original cause leading to myocardial pathological changes. There is a possible interrelationship of cause and effect between the pathological changes of the heart and AA-dependent CYP metabolic pathways.

Several years ago, a number of studies manifested that low selenium and vitamin E could increase the AA produced by enhancing the activity of PLA2 in the initial stage of AA metabolism, thereby affecting the final products level of AA metabolites. As expected, the present study also identified that the content of AA in the two patient groups was notably higher compared to the normal control group. KD is mainly distributed in the selenium deficient areas of China from southeast to northwest, which may result in a higher AA concentration in KD. In addition, lipid peroxide levels were enhanced in dietary vitamin E deficiency in the heart (16). AA is one of the diverse free fatty acids, that not only participates in myocardium energy supply, but AA and its CYP-derived metabolites may also act as secondary messenger molecules for protein kinases, including protein kinase C, mitogen-activated protein kinase, protein kinase A, Erk1/2, and Akt (13,17,18). AA is synthesized from linoleic acid, and previous studies have reported that dietary linoleic acid supplementation was beneficial to DCM patients. This may explain the reason for a higher AA content in DCM patients. However, more linoleic acid content

may aggravate myocardial ischemia and debase the cardiac function. Thus, excessive AA may lead to severe cardiac function reduction of DCM. There was no difference between the content of AA in the two patient groups. Therefore, a higher AA content may have a certain protective effect on the heart, but may cause side effects. This effect has no association with the geographical environment factors.

There is evidence that CYP450 modulates contractility of cardiomyocytes through metabolism of AA (14). CYPs are known to metabolize AA to HETEs and EETs through ω-hydroxylases and epoxidation, respectively (19). Biological activities of EETs and the opposing actions of 20-HETE within the heart are now well established (6). EETs can be applied to a variety of ion channels of cardiovascular cells, and protect myocardial cells by regulating the expression of genes and proteins associated with apoptosis (20). 20-HETE has detrimental effects, particularly in the heart ischemia. A previous study reported that inhibition of 20-HETE can reduce the infarct size in canines (21). In the present results, the content of EETs in CKD and DCM was clearly lower compared to the healthy control. Therefore, EETs may have a key role during the development and/or progression of KD and DCM. CYP2C are the predominant epoxygenase isoforms involved in EETs formation, and they are abundantly expressed in the endothelium, myocardium and kidney in humans (20). As a consequence, the CYP2C19 gene was upregulated and the frequent protein expression may be a protective compensation reaction. According to certain studies, CYP1A1 metabolizes AA to different regioisomers of HETEs (22). Additionally, previous studies have shown that the induction of CYP1A1 in the hypertrophied hearts caused a significant increase in the formation of 20-HETE (23). In the present study, the experimental data also documented that the concentration of 20-HETE was significantly increased compared to the healthy control subjects. Therefore, an increase in the expression level of CYP1A1 may exacerbate myocardial injury. The findings summarized above demonstrate that AA metabolites are disordered in endemic DCM and idiopathic DCM. The purpose of these changes may adapt to the pathological and physiological of the heart through regulating content, structure and stability of cell membrane lipid, as well as AA metabolites. A wide cardioprotective role of fatty acids has been studied thus far, particularly CYP-mediated AA metabolites may have the more complex role in KD and DCM.

In conclusion, there is a strong correlation between P450-mediated AA metabolites and the pathogenesis of dilated cardiomyopathy. The overall balance of CYP genes changes have resulted in a higher production of 20-HETE and lower production of EETs in the KD and DCM hearts. Speculation regarding the therapeutic implications of automatically selective expression of CYP450 enzymes should be made with caution. Further studies are required to investigate the mechanisms by which KD modulates P450 gene expression.

Acknowledgements

The authors thank all the research subjects who submitted peripheral blood. The present study was supported by the National Natural Scientific Foundation of China (grant no. 81273008) and the National Natural Scientific Foundation of China (grant no. 30872192).

References

1. Lei C, Niu X, Wei J, Zhu J and Zhu Y: Interaction of glutathione peroxidase-1 and selenium in endemic dilated cardiomyopathy. Clin Chim Acta 399: 102-108, 2009.
2. Hwang JJ, Allen PD, Tseng GC, Lam CW, Fananapazir L, Dzau VJ and Liew CC: Microarray gene expression profiles in dilated and hypertrophic cardiomyopathic end-stage heart failure. Physiol Genomics 10: 31-44, 2002.
3. Xiaohui S, He S and Hong TW: Comparative analysis of gene expression profile between Keshan disease and dilated. Experimental and Clinical Cardiology 20: 1373-1384, 2014.
4. Proctor KG, Falck JR and Capdevila J: Intestinal vasodilation by epoxyeicosatrienoic acids: Arachidonic acid metabolites produced by a cytochrome P450 monooxygenase. Circ Res 60: 50-59, 1987.
5. Chaudhary KR, Batchu SN and Seubert JM: Cytochrome P450 enzymes and the heart. IUBMB Life 61: 954-960, 2009.
6. Fer M, Dréano Y, Lucas D, Corcos L, Salaün J, Berthou F and Amet Y: Metabolism of eicosapentaenoic and docosahexaenoic acids by recombinant human cytochromes P450. Arch Biochem Biophys 471: 116-125, 2008.
7. Sidorik L, Kyyamova R, Bobyk V, Kapustian L, Rozhko O, Vigontina O, Ryabenko D, Danko I, Maksymchuk O, Kovalenko VN, *et al*: Molecular chaperone, HSP60 and cytochrome P450 2E1 co-expression in dilated cardiomyopathy. Cell Biol Int 29: 51-55, 2005.
8. Fuyu Y: Keshan disease and mitochondrial cardiomyopathy. Sci China C Life Sci 49: 513-518, 2006.
9. He SL, Tan WH, Zhang ZT, Zhang F, Qu CJ, Lei YX, Zhu YH, Yu HJ, Xiang YZ and Guo X: Mitochondrial-related gene expression profiles suggest an important role of PGC-1alpha in the compensatory mechanism of endemic dilated cardiomyopathy. Exp Cell Res 319: 2604-2616, 2013.
10. Elbekai RH and El-Kadi AO: Cytochrome P450 enzymes: Central players in cardiovascular health and disease. Pharmacol Ther 112: 564-587, 2006.
11. Maisch B: Classification of cardiomyopathies according to the WHO/ISFC Task Force - more questions than answers. Med Klin (Munich) 93:199-209, 1998 (In German).
12. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
13. Gottlieb RA: Cytochrome P450: Major player in reperfusion injury. Arch Biochem Biophys 420: 262-267, 2003.
14. Raucy JL, Mueller L, Duan K, Allen SW, Storm S and Lasker JM: Expression and induction of CYP2C P450 enzymes in primary cultures of human hepatocytes. J Pharmacol Exp Ther 302: 475-482, 2002.
15. Thum T and Borlak J: Gene expression in distinct regions of the heart. Lancet 355: 979-983, 2000.
16. Melin AM, Carbonneau MA, Thomas MJ, *et al*: Dietary vitamins A and E affect differently lipid peroxidation in rat heart and testis. J Clin Biochem Nutr 18: 19-33, 1995.
17. Westphal C, Konkel A and Schunck WH: CYP-eicosanoids - a new link between omega-3 fatty acids and cardiac disease? Prostaglandins Other Lipid Mediat 96: 99-108, 2011.
18. Zeldin DC: Epoxygenase pathways of arachidonic acid metabolism. J Biol Chem 276: 36059-36062, 2001.
19. Seubert JM, Zeldin DC, Nithipatikom K and Gross GJ: Role of epoxyeicosatrienoic acids in protecting the myocardium following ischemia/reperfusion injury. Prostaglandins Other Lipid Mediat 82: 50-59, 2007.
20. Xu X, Zhang XA and Wang DW: The roles of CYP450 epoxygenases and metabolites, epoxyeicosatrienoic acids, in cardiovascular and malignant diseases. Adv Drug Deliv Rev 63: 597-609, 2011.
21. Gross ER, Nithipatikom K, Hsu AK, Peart JN, Falck JR, Campbell WB and Gross GJ: Cytochrome P450 omega-hydroxylase inhibition reduces infarct size during reperfusion via the sarcolemmal KATP channel. J Mol Cell Cardiol 37: 1245-1249, 2004.
22. Zordoky BN and El-Kadi AO: 2,3,7,8-Tetrachlorodibenzo-p-dioxin and beta-naphthoflavone induce cellular hypertrophy in H9c2 cells by an aryl hydrocarbon receptor-dependant mechanism. Toxicol In Vitro 24: 863-871, 2010.
23. Zordoky BN, Aboutabl ME and El-Kadi AO: Modulation of cytochrome P450 gene expression and arachidonic acid metabolism during isoproterenol-induced cardiac hypertrophy in rats. Drug Metab Dispos 36: 2277-2286, 2008.