Biochimica et Biophysica Acta 1842 (2014) 2120–2125

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Increased serum 2-oxoglutarate associated with high myocardial energy expenditure and poor prognosis in chronic heart failure patients

Ping-An Chen [a,b,c], Zhi-Hao Xu [b], Yu-Li Huang [b], Yi Luo [d], Ding-Ji Zhu [b], Peng Wang [b], Zhi-Yong Du [e], Yang Yang [d], Dai-Hong Wu [f], Wen-Yan Lai [a,b,c], Hao Ren [c,g,*], Ding-Li Xu [a,b,c,**]

[a] State Key Laboratory of Organ Failure Research, Department of Cardiology, Nanfang Hospital, Southern Medical University, Guangzhou, China
[b] Department of Cardiology, Nanfang Hospital, Southern Medical University, Guangzhou, China
[c] Key Laboratory For Organ Failure Research, Ministry of Education of the People's Republic of China, Guangzhou, China
[d] Department of Cardiology, Guangzhou First People's Hospital, Guangzhou, China
[e] Department of Cardiology, General Hospital of Guangzhou Military Command, Guangzhou, China
[f] Ultrasonic Department, Guangzhou First People's Hospital, Guangzhou, China
[g] Department of Rheumatology, Nanfang Hospital, Southern Medical University, Guangzhou, China

ARTICLE INFO

Article history:
Received 8 May 2014
Received in revised form 26 June 2014
Accepted 22 July 2014
Available online 28 July 2014

Keywords:
2-Oxoglutarate
Myocardial energy expenditure
Biomarker
Heart failure

ABSTRACT

Myocardial energy expenditure (MEE) and 2-oxoglutarate are elevated in chronic heart failure (CHF) patients compared with healthy controls. To explore whether 2-oxoglutarate could reflect the levels of MEE and predict the prognosis of CHF, 219 CHF patients and 66 healthy controls were enrolled. 2-Oxoglutarate was assayed with Liquid Chromatography–Mass Spectrometry/Mass Spectrometry (LC/MS/MS). CHF patients were divided into 4 groups according to interquartile range of MEE and followed for death or recurrent hospital admission due to CHF for the mean follow-up time 6.64 ± 0.16 months. 2-Oxoglutarate was increased in CHF patients compared with controls ($P < 0.01$) and correlated with estimated glomerular filtration rate ($r = 0.142$, $P = 0.036$), age ($r = -0.269$, $P < 0.01$) and MEE levels ($r = 0.307$, $P < 0.01$) in a multiple linear correlation analysis in CHF patients. Furthermore, 2-oxoglutarate (OR = 3.470, 95% CI = 1.557 to 7.730, $P = 0.002$), N-terminal pro-B-type natriuretic peptide (OR = 4.013, 95% CI = 1.553 to 10.365, $P = 0.004$), age (OR = 1.611, 95% CI = 1.136 to 2.283, $P = 0.007$) and left ventricular ejection fraction (OR = 7.272, 95% CI = 3.110 to 17.000, $P < 0.001$) were independently associated with MEE on multiple logistic regression analysis. Kaplan–Meier event curves showed that high 2-oxoglutarate levels were associated with adverse outcomes (Log Rank, $Chi^2 = 4.026$, $P = 0.045$). This study showed that serum 2-oxoglutarate is associated with MEE levels, which can be used as potential biomarkers for MEE, and it can reflect the clinical severity and short-term outcome of CHF.

1. Introduction

Heart failure (HF) is a complex syndrome characterized by mechanical dysfunction of the myocardium, abnormal metabolism and excessive, continuous neurohormonal activation [1]. Several myocardial metabolic abnormalities occur in chronic heart failure (CHF), including altered substrate utilization and decreased high energy phosphate content [2]. Despite recent great progress, the knowledge of metabolic abnormalities in CHF is still limited. Whether and how they alter according to etiology and the severity of CHF remain poorly understood.

Recently, studies showed that there were significant metabolic differences in serum [3], urine [4] and exhaled breath [5–7] samples between CHF patients and the healthy subjects. These findings suggested that some metabolites may associate with CHF and can reflect the state of cardiac energy metabolism. Of these, 2-oxoglutarate [3], a major intermediate metabolite of the tricarboxylic acid cycle, is a promising one due to its important roles in regulating myocardial energy metabolism.

Myocardial energy expenditure (MEE) is an important indicator reflecting myocardial energy metabolism. Different ways to estimate MEE in failing heart were provided in recent years, including positron emission tomography [8,9], nuclear magnetic resonance [10,11] and Doppler echocardiography [12,13]. It has been reported that elevated MEE is associated with decreased left ventricular ejection fraction (LVEF) and can be used as an independent predictor of cardiovascular mortality [12]. Recently, our preliminary results showed that in patients with CHF, elevation of MEE was associated with significant changes in serum metabolomic profiles by ^{1}H-NMR-based metabolic analysis and

* Correspondence to: H. Ren, Department of Rheumatology, Nanfang Hospital, Southern Medical University, 1838 Northern Guangzhou Ave, Guangzhou, Guangdong 510515, China. Tel.: +86 20 61641515; fax: +86 20 61360416.
** Correspondence to: D. Xu, Department of Cardiology, Nanfang Hospital, Southern Medical University, 1838 Northern Guangzhou Ave, Guangzhou, Guangdong 510515, China. Tel.: +86 20 61641493; fax: +86 20 61360416.
E-mail addresses: renhao67@aliyun.com (H. Ren), dinglixu@fimmu.com (D.-L. Xu).

http://dx.doi.org/10.1016/j.bbadis.2014.07.018
0925-4439/

suggested that these compounds could be used as potential serum biomarkers to explore myocardial energy mechanism in CHF patients [14].

The purpose of this study was to evaluate the relationship between 2-oxoglutarate and MEE in CHF patients. Our goals were: 1) to test the hypotheses that whether the serum concentration of 2-oxoglutarate is associated with MEE levels and can reflect the severity of CHF; and 2) to assess the predictive value of 2-oxoglutarate for the prognosis of CHF.

2. Materials and methods

2.1. Study population

219 patients with CHF were consecutively enrolled after obtaining informed consent in 2 participating centers (Nanfang Hospital and Guangzhou First People's Hospital, China). Patients with acute coronary syndrome, diabetes mellitus and other metabolic diseases, estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m^2, sepsis, malignancy, autoimmune disease or severe hepatic disease were excluded. The underlying causes of CHF were classified as hypertension, ischemic heart disease, valvular heart disease and dilated cardiomyopathy on the basis of the patients' history, cardiac morphology and coronary angiography. Consensus of 2 experienced clinical cardiologists was required for the classification of New York Heart Association (NYHA) functional classes. The severity of CHF was evaluated by NYHA classification and MEE. Follow-up events, including all-cause mortality and recurrent hospital admission due to CHF, were ascertained via hospital database, medical records and contact with patients and their family members. Sixty-six age-matched control subjects with normal cardiac function were recruited from the health management center and outpatient department in Guangzhou First People's Hospital. The study complied with the Declaration of Helsinki and was approved by the institutional ethics committee of Nanfang Hospital and Guangzhou First People's Hospital, China. All subjects were provided with a hard copy of informed consent before recruitment.

2.2. Biochemistry detection

Antecubital venous blood was drawn into pyrogen-free tubes with or without EDTA as anticoagulant respectively on the same day of MEE measurement. After centrifugation at 3000 *g* at 4 °C for 10 min, all serum or platelet-poor plasma samples were stored at −80 °C. Serum 2-oxoglutarate was assayed with Agilent 6460 LC/MS/MS (USA). Chromatographic separations of prepared samples were achieved using an Eclipse Plus C 18 column (3.5 μm, 2.1 mm × 100 mm). The mass spectrometer was operated in the positive ion ESI mode with MRM for the analytes. The following optimized ESI parameters were applied: drying gas flow rate, 10 L/min; drying gas temperature, 350 °C; nebulizing gas pressure 30 psi; capillary voltage 4000 V; and fragmentor voltage 50 V. Free thyroxine, free triiodothyronine and thyroid stimulating hormone (TSH) were measured with a direct chemiluminescence immunoassay (Siemens Healthcare Diagnostics Inc., USA). N-terminal pro-B-type natriuretic peptide (NT-proBNP) was analyzed with the Elecsys NT-proBNP immunoassay (Roche Diagnostics). Estimated glomerular filtration rate (eGFR) was calculated based on MDRD formula. All subjects underwent oral glucose tolerance test (OGTT) with 75 g of oral anhydrous glucose as described previously [15].

2.3. MEE measurement

MEE was measured with a Siemens Sequoia 512 Encompass ultrasound system, using the method described previously [12,16]. Systolic blood pressure (SBP), left ventricular internal diameter at systole (LVIDs), left ventricular posterior wall end-systolic thickness (PWTs), left ventricular ejection time (LVET), LVEF and left ventricular stroke volume (LVSV) were measured. Finally, MEE was calculated as [12,16]: MEE (cal/min) = left ventricular circumferential end-systolic wall stress (cESS) × LVET × LVSV × heart rate × 4.2×10^{-4}.

$$\text{cESS} = \frac{\text{SBP} \times (\text{LVID}_s/2)^2 \times \left\{1 + \frac{(\text{LVID}_s/2 + \text{PWT}_s)^2}{(\text{LVID}_s/2 + \text{PWT}_s/2)^2}\right\}}{(\text{LVID}_s/2 + \text{PWT}_s)^2 - (\text{LVID}_s/2)^2}$$

2.4. Statistical analysis

The continuous normal variables were expressed as mean ± SD, and medians were presented with the 25th to 75th percentiles for skewed continuous variables. CHF patients were divided into quartiles on the basis of the levels of MEE. Categorical variables were compared with Pearson's χ^2 test. Differences between mean or median values for continuous variables were evaluated with Kruskall–Wallis test or 1-way ANOVA with S–N–K analysis, as appropriate. Pearson correlation for the normal and logarithmically transformed skewed variables was used to assess associations between study parameters. Multicollinearity (strong correlations among independent variables) was examined by collinearity diagnostic statistics. Variance inflation factor (VIF) values > 4.0 or tolerance < 0.25 may indicate a concern for multicollinearity in multivariate regression models [17]. The concentrations of 2-oxoglutarate were skewed and thus, were logarithmically transformed (Log 2-oxoglutarate) for calculation of associations with biochemical parameters (fasting blood glucose, postprandial blood glucose, hemoglobin A1c, alanine aminotransferase, aspartate aminotransferase) and other clinical parameters (NT-proBNP, eGFR, NYHA classification, LVEF, age, sex and MEE) in Pearson correlation and multiple linear regression analysis. Multivariable logistic regression analysis was used to investigate associations between MEE levels (dependent variable) and other parameters (independent variables) including fasting blood glucose, postprandial blood glucose, hemoglobin A1c, age, sex, creatinine, eGFR, 2-oxoglutarate, NYHA classification, free triiodothyronine, free thyroxine, thyroid-stimulating hormone, body mass index, LVEF and NT-proBNP. Events of recurrent hospital admission due to CHF or death in 8 months were investigated with Kaplan–Meier analysis by Log rank test. *P* values were two-sided and considered significant when <0.05. Statistical analyses were carried out using the software package SPSS version 17.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Baseline characteristics

Serum 2-oxoglutarate was higher in CHF patients compared with controls (median, 13.02 μg/mL [IQR 6.14 to 26.89] versus 10.58 μg/mL [IQR 7.69 to 13.42], $P < 0.01$). Patients with CHF were divided into 4 groups according to the interquartile range of MEE. Those with a MEE < 59.51 cal/min were included in the MEE 1 group; 59.51 cal/min ≤ MEE < 99.94 cal/min in the MEE 2 group; 99.94 cal/min ≤ MEE < 184.18 cal/min in the MEE 3 group; and MEE ≥ 184.18 cal/min in the MEE 4 group. Basic characteristics of 4 groups were presented in Table 1. Clinical parameters in 4 groups were similar except for higher NT-proBNP, left ventricular mass index (LVMI), Tei index, NYHA classes, as well as lower free triiodothyronine and high-density lipoprotein in the MEE 4 group compared with the MEE 1 group. Patients belonging to NYHA classes III and IV were with significantly higher MEE values than class I and II patients. NYHA classes were higher with greater MEE (Fig. 1).

Serum 2-oxoglutarate levels were lower in the MEE 1 group than those in the MEE 3 and 4 groups (both $P < 0.01$). Compared with the MEE 3 and 4 groups, the similar results were found in the MEE 2 group (both $P < 0.05$). However, there were no significant differences