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Clinical Research

Ceramide Is Upregulated and Associated With Mortality in Patients With Chronic Heart Failure

Jingjia Yu, MD,[a] Wei Pan, MD,[b] Ruizheng Shi, MD,[b] Tianlun Yang, MD,[b] Yuanjian Li, PhD,[c] Guolong Yu, MD,[b] Yongping Bai, MD,[b] Edward H. Schuchman, PhD,[d] Xingxuan He, MD,[d] and Guogang Zhang, MD[b]

[a] *The State Key Laboratory of Medical Genetics, Xiangya Medical School, Central South University, Changsha, Hunan, China*

[b] *Department of Cardiovascular Medicine, Xiangya Hospital, Central South University, Changsha, China*

[c] *Department of Pharmacology, School of Pharmaceutical Sciences, Central South University, Changsha, China*

[d] *Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, New York, USA*

ABSTRACT

Background: Ceramide is involved in apoptosis, inflammation, and stress responses, which are among the pathogenic components of chronic heart failure (CHF). However, no one has documented the levels of ceramide itself in CHF or determined its potential prognostic value.

Methods: In this study we recruited patients with heart failure consecutively from the hospital, of whom 423 stable patients were eventually selected to participate in this study after an observation period of at least 3 months after hospital discharge. All patents were followed up for all-cause death to December 31, 2013.

Results: Plasma ceramide levels were increased stepwise with New York Heart Association functional class (I, 5.32 ± 1.98; II, 5.81 ± 1.63; III, 6.14 ± 2.14; IV, 6.66 ± 2.61 ng/mL). During a mean follow-up of 4.4 years (interquartile range: 3.5-5.3 years), a total of 200 CHF

RÉSUMÉ

Introduction : Le céramide est impliqué dans l'apoptose, l'inflammation et la réponse au stress, qui sont parmi les composantes pathogènes de l'insuffisance cardiaque chronique (ICC). Cependant, personne n'a documenté les niveaux de céramide en soi dans l'ICC, ni déterminé valeur pronostique potentielle.

Méthodes : Dans cette étude nous avons recruté des patients consécutivement à une hospitalisation pour insuffisance cardiaque, dont 423 patients stables ont finalement été sélectionnés pour participer à cette étude, après une période d'observation d'au moins 3 mois après la sortie de l'hôpital. Chaque patient a été examiné pour toutes causes de décès confondues au 31 décembre 2013.

Résultats : Les niveaux de céramide plasmatique ont été augmentés progressivement selon la classification fonctionnelle de la New York Heart Association (I, 5,32 ± 1,98; II, 5,81 ± 1,63; III, 6,14 ± 2,14; IV,

Ceramide is one of the major sphingolipid second messengers participating in signal transduction.[1] Because of this bioactivity, the levels of ceramide are carefully regulated in cells, including cells within the cardiovascular system, and increased levels of ceramide are critically involved in apoptosis, inflammation, stress responses, and contraction of muscle cells.[2]

Recent research reveals that inflammatory mediators increase ceramide production as part of a conserved cellular response.[3] The resultant ceramide accumulation is thought to mediate stress-induced apoptosis in a variety of cell types.[4] As far as we know, inflammation and the progressive loss of cardiac myocytes are among the most important pathogenic components of chronic heart failure (CHF).[5,6] Many studies have confirmed that proinflammatory cytokines contribute to cardiomyocyte loss by apoptosis and play a role in the remodelling in CHF.[7,8] Moreover, secretory acid sphingomyelinase (S-SMase), which hydrolyzes sphingomyelin into ceramide, was found to be upregulated in patients with CHF, and might contribute to regulatory processes that lead to CHF pathophysiology.[9] However, to date no one has documented the levels of ceramide itself in CHF or determined its potential prognostic value. These were the goals of the current study, in which we hypothesized that ceramide levels are upregulated in patients with CHF, and associated with the severity of symptoms and mortality.

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Corresponding authors: Dr Xingxuan He, Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA. Tel.: +1- 212-659-6824; fax: +1-212-849-2508.

E-mail: xingxuan.he@mssm.edu

Dr Guogang Zhang, Department of Cardiovascular Medicine, Xiangya Hospital, Central South University, Changsha 410008, China. Tel.: +086-731-84327695; fax: +086-731-84327695.

E-mail: xyzgg2006@sina.com

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patients died. The optimal threshold value of ceramide was 6.05 ng/mL. Ceramide levels as continuous and as dichotomous variables are risk factors for mortality in CHF (adjusted hazard ratio, 1.31; 95% confidence interval, 1.16-1.47; $P < 0.001$ and adjusted hazard ratio, 2.07, 95% confidence interval, 1.53-2.81; $P < 0.001$, respectively). When ceramide levels were combined with conventional CHF risk factors, the area under the curve increased from 0.68 (0.63-0.72) to 0.72 (0.68-0.76); $P = 0.047$. The continuous net reclassification index and integrated discrimination improvement index were 17.2% (5.0-29.9%; $P = 0.027$) and 0.04 (0.01-0.08; $P = 0.020$), respectively.

Conclusions: Plasma ceramide levels were increased and correlated with the severity of CHF, and were an independent risk factor of mortality in patients with CHF and reduced left ventricular systolic function. Ceramide levels might provide additional predictive value after conventional risk assessment.

6,66 ± 2,61 ng/ml). Au cours d'un suivi moyen de 4,4 ans (écart interquartile: 3,5-5,3 ans), un total de 200 patients atteints d'ICC sont décédés. La valeur du seuil de céramide optimal est de 6,05 ng/ml. Les niveaux de céramide en tant que variable continue ou dichotomique sont des facteurs de risque de mortalité en ICC (rapport de risques ajustés, 1,31; intervalle de confiance à 95 %, 1,16-1,47; $P < 0,001$ et rapport de risques ajustés, 2,07, intervalle de confiance à 95 %, 1,53-2,81; $P < 0,001$, respectivement). Lorsque les niveaux de céramide ont été combinés avec des facteurs de risque classiques d'ICC, l'aire sous la courbe est passée de 0,68 (0,63-0,72) à 0,72 (0,68-0,76); $P = 0,047$. L'indice continu « Net Reclassification » et l'indice « Integrated Discrimination Improvement » étaient de 17,2 % (5,0-29,9 %; $P = 0,027$) et de 0,04 (0,01-0,08; $P = 0,020$), respectivement.

Conclusions : Les niveaux de céramide plasmatique ont été augmentés et corrélés avec la gravité de l'ICC, et ont été un facteur de risque indépendant de la mortalité chez les patients avec ICC et une réduction de la fonction systolique ventriculaire gauche. Les niveaux de céramide pourraient apporter une valeur prédictive supplémentaire à l'évaluation des facteurs de risque conventionnels.

Methods

Study subjects and data collection

The study was carried out between 2008 and 2013, and we consecutively recruited patients from the Department of Cardiovascular Medicine of Xiangya Hospital, Changsha, China with symptoms of heart failure (HF), aged ≥18 years, and with a left ventricular ejection fraction (LVEF) of ≤50%. During the next observation period of at least 3 months after hospital discharge, patients were excluded from the present study if they clinically deteriorated and needed to change medication regimens. Finally, 423 patients who met all inclusion criteria and none of the exclusion criteria were selected into a follow-up period, and data on general health characteristics were collected, and blood samples and echocardiography data for analysis. Inclusion criteria were: (1) men and women aged ≥18 years; (2) LVEF of ≤50%, measured within the past 6 months; (3) stable medical regimen, defined as no major changes in medication in the past 3 months; and (4) physician diagnosis of chronic systolic HF according to European Society of Cardiology guidelines for HF 2008.[10] Exclusion criteria included the presence of severely decreased kidney function (estimated glomerular filtration rate [eGFR] < 30 mL/min/1.73 m^2), acute myocardial infarction, and overt cause of dyspnea, including chest wall trauma or penetrating lung injury. Patients with significant concomitant diseases, such as pulmonary disease, malignancy, autoimmune disorders, neurodegenerative disorders, thyroid disease, or concurrent viral disease, were also excluded from this study. All patients were receiving standard medical treatment as clinically indicated with diuretics, angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists, β-blockers, digitalis, aspirin, and/or warfarin in varying situations. One hundred four healthy volunteers of a similar age and sex with no disease history or current clinical or biochemical sign of cardiovascular disease or other chronic disease were included as healthy controls.

This study conformed to the Declaration of Helsinki. All subjects studied provided informed consent, which was approved by the local ethics committee on human research (Central South University).

Blood sampling

Peripheral venous blood samples were taken from an antecubital vein and were transferred to disposable heparin blood collection tubes, followed by centrifugation at 1000*g* for 10 minutes at 4°C. Aliquots of plasma were immediately stored at −80°C until analyzed.

General characteristics, laboratory assays, and echocardiography

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice with the subject in the sitting position after resting for at least 5 minutes. Height, weight, total cholesterol, creatinine, high-sensitivity C-reactive protein (hsCRP), hemoglobin, and high-sensitivity cardiac troponin I (hs-cTnI) levels were measured in the morning after an overnight fasting. Plasma cholesterol, creatinine, hsCRP, and hs-cTnI were assayed using routine automated laboratory techniques. C18, C20, C22, and C24 ceramide were from Avanti Polar Lipids (Alabaster, AL). The serum tumour necrosis factor (TNF)-α enzyme-linked immunosorbent assay kit was from R&D Systems (Minneapolis, MN). All other biochemical reagents were from Sigma Chemical Co. (Shanghai, China). Blood N-terminal probrain natriuretic peptide (NT-proBNP) levels were immediately measured using an Elecsys 2010 instrument (Roche Diagnostics). All echocardiographic examinations were sent to the core laboratory for assessment of the left ventricular end-diastolic (LVED) and LVEF levels.

Ceramide assay

Plasma (0.5 mL) was added to a tube containing 1.2 mL hexane and extracted at room temperature for 30 minutes.