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● *Original Contribution*

ECHOCARDIOGRAPHIC STRAIN ANALYSIS FOR THE EARLY DETECTION OF MYOCARDIAL STRUCTURAL ABNORMALITY AND INITIATION OF DRUG THERAPY IN A MOUSE MODEL OF DILATED CARDIOMYOPATHY

Minjuan Zheng,* Feng Pan,† Ying Liu,‡ Zhenzhou Li,§ Xiaodong Zhou,* Xin Meng,* Liwen Liu,* and Shuping Ge*§

*Department of Ultrasound, Xijing Hospital, Fourth Military Medical University, Xi'an, China; †Department of Orthodontics, Stomatology Hospital, Xi'an Jiaotong University, Xi'an, China; ‡Department of Radiology, Xijing Hospital, Fourth Military Medical University, Xi'an, China; and §Pediatric Cardiology, Drexel University College of Medicine, Philadelphia, Pennsylvania, USA

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Abstract—This study aimed to evaluate the role of echocardiography-based strain analysis in the early diagnosis and guidance for management of dilated cardiomyopathy (DCM). Muscular dystrophy mice (which spontaneously develop DCM) and control (C57 BL/6 J) mice were sequentially evaluated by ultrasound biomicroscopy, conventional left ventricle (LV) measurement, two-dimensional (2-D) strain analysis and myocardial histologic analysis for 12 consecutive months. Significant alternation of LV remodeling and dysfunction could be detected by conventional echocardiography after 9 mo, by strain analysis after 5 mo and by histologic analysis after 4 mo. The global longitudinal systolic peak strain (PK) was the most sensitive strain marker for early detection of myocardial structural abnormality in the subclinical stage. Moreover, losartan administration before the PK decrease was associated with significantly preserved LV function. These results suggest that myocardial strain analysis (particularly longitudinal PK) is sensitive for the early detection of LV dysfunction in mice with dilated cardiomyopathy. (E-mail: 1243519584@qq.com) Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology.

Key Words: **Dilated cardiomyopathy, Speckle tracking imaging, Ultrasound biomicroscopy, Losartan.**

INTRODUCTION

Dilated cardiomyopathy (DCM) has been recognized as one of the most common clinical syndromes caused by pathogenesis-related factors, such as viral infection, hereditary and metabolic disorders, that eventually lead to the deterioration of left ventricle (LV) function and subsequent congestive heart failure (HF) (Merlo et al. 2016). Clinically, echocardiography has been considered as the most accepted non-invasive examination for DCM diagnosis.

Interestingly, mounting evidence suggests that myocardial dysfunction appears before echocardiography parameter changes such as decreased ejection fraction (EF), ventricular wall motion or LV remodeling (Finsterer and Stollberger, 2016; Merlo et al. 2016). Moreover, although medications against LV dysfunction are thought to be effective in preventing LV functional deterioration during DCM pathogenesis (Parent et al. 2016), early administration of such medications may be more efficacious for patients at risk for DCM. However, this is difficult in clinical practice because of the delayed detection of LV abnormality by conventional echocardiography.

Myocardial strain analysis is a well-established echocardiographic technique for detecting myocardial dysfunction. Strain is a dimensionless parameter that represents deformation of the myocardium fibers relative to their original dimension (Haland et al. 2016; Smith et al. 2016). Moreover, speckle tracking imaging has been recognized as a useful method for angle-independent quantification of myocardial strain (Smith et al. 2016), applied as a sensitive marker for early myocardial dysfunction before the appearance of LV remodeling (Haland et al. 2016; Smith et al. 2016). In particular, global longitudinal strain has been proposed as a

Corresponding author: Shuping Ge, Drexel University College of Medicine and St. Christopher's Hospital for Children, 3601 A Street, Philadelphia, PA 19134, USA. E-mail: 1243519584@qq.com

potential marker of early detected LV dysfunction in patients with DCM, diabetic cardiomyopathy, peripartum cardiomyopathy and HF with preserved EF (Briasoulis et al. 2016; Flores-Ramirez et al. 2016; Minoshima et al. 2016). However, to the best of our knowledge, changes in myocardial strain parameters (compared with echocardiographic index changes of LV dysfunction) and myocardial histologic changes during the development of DCM have not been systematically evaluated. Moreover, it remains to be determined whether early administration of medications against LV remodeling—such as angiotensin II receptor blockers (ARBs) (Ikeda et al. 2016; Moxley et al. 2010)—before the changes in myocardial strain parameters could better preserve LV function.

Therefore, in this study, by using a spontaneous DCM mice model, we evaluated the value of strain analysis for the early diagnosis of DCM and assessed the preventive effects of early losartan administration on myocardial dysfunction in a DCM model.

METHODS

Animal and study protocols

Thirty-seven male muscular dystrophy (mdx) mice (C57 BL/10 ScSn-Dmdmdx/J, weight 28.9 ± 6.5 g) and 39 male C57 BL/6 J mice (C57 BL/6 J, weight 29.3 ± 4.7 g) (The Jackson Laboratory, Bar Harbor, ME, USA) were used as the inherited DCM model of gradual–congestive HF and control mice groups, respectively. Mdx mice are a well-accepted animal model for Duchenne mdx, a neuromuscular disorder caused by a mutation in the dystrophin gene on chromosome Xp21.1. Dystrophin is a component of the dystrophin glycoprotein complex, which links the extracellular matrix and cytoskeletal proteins. Dystrophin deficiency leads to myofiber damage, inflammation and fibrosis, eventually causing HF in mice at about 12 mo old. Therefore, mice with DCM are often used as models for congestive HF (Fayssoil et al. 2013).

In this study, both mdx and control mice were observed for 12 mo, and all echocardiographic examination approaches were performed at the end of each mo; 3 or 4 mice were sacrificed for histologic examination at each time point. To acquire imaging data, high-frequency ultrasound biomicroscopy (UBM) was performed with the Vevo 770 High-Resolution *In Vivo* Micro-Imaging System (VisualSonics, Toronto, Ontario, Canada). Briefly, mice were anesthetized using 2% isoflurane and laid in a supine position on a platform with their legs taped to ECG electrodes for ECG recording. Body temperature was monitored *via* a rectal thermometer and maintained at 36–38°C using a heating pad and lamp. Hair was removed from the chest before imaging using a chemical hair remover. Echocardiographic data were acquired 2 or 3 min after initiation of anesthesia. A single-crystal mechanical transducer (RMV 707 B) with a central frequency more than 50 MHz and focal length of 12.7 mm was used. The maximum field of view of 2-D imaging was 20 × 20 mm with a spatial resolution of 115 μm (lateral resolution) and 55 μm (axial resolution), which can provide images with a frame rate at 35–45 frames per second.

The experimental protocols were approved by the Institutional Animal Care and Use Committee of Department of Ultrasound, Xijing Hospital, Fourth Military Medical University (Xi'an, China). Furthermore, all procedures performed in these studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Conventional echocardiography data acquired by ultrasound biomicroscopy

At the end of each month, echocardiography by UBM was performed on mdx and control mice, which were continuously observed for a total of 12 mo. In the UBM procedure, the region of interest was adjusted to maximize resolution and the most suitable image gain, while other parameters such as depth, focus and time-gain compensation were adjusted to delineate all myocardial segments. LV remodeling and global function were assessed by M-mode UBM (Fig. 1a). All measures—including LV end-diastolic dimension (LVEDD), LV end-systolic dimension (LVESD, mm), LV end-diastolic volume (LVEDV, mm), LV end-systolic volume (LVESV, μL), fractional shortening (FS, %) and ejection fraction (EF, %)—were derived from UBM by averaging data from five cardiac cycles. The M-mode calculation formulas were defined as: LV Volume = $(7.0/2.4 + D) \times D^3$, D = LVEDD (for EDV) or LVESD (for ESV); FS = (LVEDD−LVESD)/LVEDD × 100%; and EF = (LVEDV−LVESV)/LVEDV × 100%.

Strain analysis-based speckle tracking imaging

Two-dimensional (2-D) speckle tracking–based strain analyses of the myocardial motion were obtained in both long- and short-axis images (at the midventricular level) (Fig. 1b). All images were acquired for multiple cardiac cycles and stored digitally in a hard drive for offline analysis. Strain analysis by speckle tracking imaging was performed offline, using software (Image Arena v4.0, TomTec, Munich, Germany) based on both the long- and short-axis images, as described previously (Koopman et al. 2011). The endocardial and epicardial borders were detected in the frame-to-frame data from cine loops, tracking the ventricular motion and formed the region of interest of myocardium, which allows for measurements of segmental and global myocardial