Ultrasound in Med. & Biol., Vol. 33, No. 10, pp. 1561–1571, 2007

0301-5629/07/$–see front matter

doi:10.1016/j.ultrasmedbio.2007.04.016

● *Original Contribution*

CONTRAST ENHANCED ECHOCARDIOGRAPHIC FOLLOW-UP OF CARDIAC REMODELING AND FUNCTION AFTER MYOCARDIAL INFARCTION IN RATS

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(*Received* 25 *October* 2006; *revised* 22 *April* 2007; *in final form* 26 *April* 2007)

Abstract—Echocardiography is a reliable and commonly used method to examine cardiac diseases. Recent employment of modern technologies provides new opportunities to study left ventricular (LV) remodeling after myocardial infarction (MI) also in small rodents. LV volumes as most important prognostic parameters can be estimated by noncontrast enhanced echocardiography in rats from M-mode or single cross sections only. In this study, contrast enhanced echocardiography and volume measurements by the biplane method of discs (Simpson's rule) were applied in rats to monitor remodeling and function after MI. MI was induced in female Sprague-Dawley rats ($n = 26$ for MI, and $n = 16$ for sham). LV remodeling and heart function were serially studied by contrast enhanced echocardiography for 12 to 16 wk. At the end of the observation periods hemodynamic data were additionally measured by left and right heart catheterization. LV end systolic volume (LVESV) measured by biplane method of discs correlated best with LV developed pressure as indicator for severely impaired heart function. Interestingly, LV end systolic area (LVESA) from native short axis view correlated well with LVESV ($R^2 = 0.93$) and was the second best predictor for depressed heart function. Moreover, left atrial size was a powerful indicator of severely impaired heart function whereas ejection fraction or fractional area change were primarily related to infarct size. In conclusion, contrast enhanced echocardiography in rats is feasible and an economical method to study time-dependent LV remodeling and deterioration of contractile function after MI. (E-mail: Alexander.Deten@vetphys.unizh.ch) **© 2007 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Echocardiography, Contrast medium, Myocardial infarction, Animal study, LV remodeling, Heart function.**

INTRODUCTION

Echocardiography is one of the most commonly performed noninvasive diagnostic tests in patients with known or suspected cardiovascular diseases, including myocardial infarction (MI) and heart failure. It provides comprehensive evaluation of cardiovascular structure and function that characterize disease processes as well as therapeutic efficacy of treatment (for an overview see the recommendations of the American Society of Echocardiography [Gottdiener et al. 2004; Lang et al. 2005] and the European Association of Echocardiography [Lang et al. 2006] and references therein).

Left ventricular (LV) remodeling after MI is a well-known pathologic process that results in progressive dilation and distortion of the LV. It is mainly determined by the size and location of the ischemic area. This process can be recognized by many echocardiographic changes, such as an increase in LV size and volume, altered LV geometry, diastolic dysfunction and other hemodynamic changes. It has been shown that LV volume is one of the best prognostic parameters after acute myocardial infarction (Otterstad et al. 2001). Furthermore, therapeutic strategies that minimize the extent of LV remodeling and improve prognosis are echocardiographically characterized by an attenuated increase in LV dimensions. Therefore, it is recommended that characterization of LV remodeling includes accurate sequen-

Video Clips cited in this article can be found online at: http://www.umbjournal.org.

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tial assessment of LV size and volume and diastolic filling patterns (Gottdiener et al. 2004; Lang et al. 2006).

Recent technological developments in echocardiography facilitate the analysis of ventricular remodeling and heart function in small laboratory animals but there are limitations when compared with human echocardiography. Mainly, LV dimensions were estimated either from one-dimensional M-Mode tracings (Sjaastad et al. 2000), area measurements in parasternal views (Coatney 2001) or volume calculations from single cross sections (Francis et al. 2001). These methods have been validated in humans but it is generally recognized that the accuracy of the prolate-ellipse, area-length and truncated ellipsoid methods is limited to normally shaped and sized ventricles, whereas the biplane method of discs is accurate in abnormally shaped ventricles (Gottdiener et al. 2004; Lang et al. 2006).

Delineation of endocardial borders in rats is conventionally not possible in apical views. Therefore, the estimation of LV size in rats had to be modified to use parasternal sections only. These mathematical models require geometric assumptions that are probably not applicable since in rats, shape and size of the LV are also profoundly altered after MI in a time-dependent manner (Pfeffer and Braunwald 1990). In this study, contrast enhanced echocardiography (Cohen et al. 1998) and volume measurements by the biplane method of discs (Simpson's rule) were applied in rats to study time-dependent LV remodeling and deterioration of contractile function after MI. Furthermore, it was investigated whether area measurements from native short axis views also adequately reflect LV remodeling.

METHODS

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85 to 23, revised 1996) and was approved by the appropriate State agency of Saxony.

Myocardial Infarction

Myocardial infarction was induced in female Sprague-Dawley rats (Charles River, Sulzfeld, Germany) by ligation of the left coronary artery under isofluran anesthesia inhaled over a nose mask (Deten et al. 2002). Briefly, the fourth intercostal space was opened, the heart was exteriorized and the pericardium was cut. The left coronary artery was ligated between the left auricle and the pulmonary outflow tract with a monofil thread (Ethicon USP 6/0, Ethicon GmbH, Norderstedt, Germany) while holding the apex of the heart with forceps. Thereafter, the chest was closed. The electrocardiogram was monitored until the rats recovered from anesthesia. In case of ventricular fibrillation, the rats were resuscitated by heart compression and electrical defibrillation. Sham operated rats underwent the same procedure except for the ligature.

Study Design

In a pilot study, 10 rats underwent surgery (six MI, four sham). Echocardiography was performed on the day before surgery and sequentially over 16 wk. Additionally, all rats were examined 6 h after surgery to verify the success of the ligature. Successful induction of MI was assumed when the entire left ventricular free wall appeared akinetic. One rat was excluded because of induction of MI failed. One rat died 8 wk after MI.

In a second study, 32 rats underwent surgery (20 MI, 12 sham). Echocardiography was performed on the day before surgery and 2, 4, 8 and 12 wk after surgery. Two rats were excluded in the echocardiographic selection due to failure of ligation. No rat died.

At the end of the observation period, heart function of all rats was additionally measured invasively by left and right heart catheterization. Infarct size was measured in the MI rats of the pilot study and seven additional MI rats that were used to establish echocardiography. Infarct size was measured in three to six slices after fixation, slicing and photographing as previously described (Pfeffer et al. 1979).

Echocardiography

Echocardiographic measurements were done with a commercially available ultrasound system (GE Vivid 7 equipped with an 11.5 MHz sector scan probe, GE Healthcare, Technologies Norway AS, Oslo, Norway). Examinations were performed in spontaneously breathing animals, under 2% isofluran anesthesia, in left lateral decubitus position. Parasternal short axis view was recorded at the largest round diameter of the left ventricle (Fig. 1, supplementary video 1). For recording of the contrast enhanced loops, a tail vein was punctured and the canula was connected to a catheter (inner diameter 0.5 mm). The contrast medium (Optison®, GE Healthcare Amersham Health, Buckinghamshire, United Kingdom) consisted of a mixture of albumin and gas filled microbubbles. It was slightly shaken and filled into the catheter directly before the examination to prevent unmixing (phase-separation). After adjusting the apical view and reducing the ultrasound beam power, the contrast medium was injected from the prefilled catheter. The volume for the entire examination did not exceed 500 μl.

Criteria for all apical views were to display the mitral valve opening and the LV in its longest cross