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● *Original Contribution*

ASSESSMENT OF RETROGRADE CORONARY VENOUS INFUSION OF MESENCHYMAL STEM CELLS COMBINED WITH BASIC FIBROBLAST GROWTH FACTOR IN CANINE MYOCARDIAL INFARCTION USING STRAIN VALUES DERIVED FROM SPECKLE-TRACKING ECHOCARDIOGRAPHY

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Abstract—Speckle-tracking echocardiography was used to assess retrograde coronary venous infusion of mesenchymal stem cells (MSCs) combined with basic fibroblast growth factor (bFGF) in a canine model of acute myocardial infarction (AMI). AMI was induced by ligation of the left anterior descending coronary artery. Coronary venous retroperfusion was performed at 1 wk after AMI. Twenty-eight animals were randomized into four groups: saline, bFGF+saline, saline+MSCs and bFGF+MSCs. Echocardiography was performed before AMI, at 7 d post-AMI and 40 d after retroperfusion. Apoptotic cardiomyocytes in the border zone of the ischemic region were evaluated by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling. Vascular endothelial growth factor and factor VIII concentrations were measured by western blotting. The left ventricular end-systolic volume increased significantly, whereas the left ventricular ejection fraction and global and segmental strain values decreased significantly after AMI. After retroperfusion, the strain values of the infarct zone, but not conventional echocardiographic parameters, were significantly different between control and bFGF+MSC groups. Cardiomyocyte apoptosis decreased, whereas vascular endothelial growth factor and factor VIII concentrations were higher in the bFGF+MSC, bFGF and MSC groups. Cardiomyocyte apoptosis was well correlated with the strain values. Although retrograde coronary venous infusion of bFGF and MSCs promoted neo-vascularization of the infarcted myocardium and inhibited apoptosis, there was only a slight strain improvement without a substantial increase in global cardiac functions. (E-mail: Yangya99@hotmail.com) **© 2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Retrograde coronary venous infusion, Mesenchymal stem cells, Canine, Speckle-tracking echocardiography, Strain analysis, Acute myocardial infarction.**

INTRODUCTION

Cell transplantation has emerged as a promising treatment for cardiac repair after myocardial infarction (Francis et al. 2013; Tongers et al. 2011). Efficient cell delivery to the target myocardium is crucially important for this strategy. Several techniques have been used to deliver therapeutic progenitor cells to the heart (Dib et al. 2010), but they are limited by poor survival and engraftment of the transplanted cells and an increased risk of complications (Fukushima et al. 2007; Grossman et al. 2002; Wu et al. 2011). In contrast, retrograde coronary venous infusion is an attractive alternative to the current delivery options because it is minimally invasive, can be repeated easily and may achieve increased cell retention (Jain et al. 2006; Siminiak and Lipiecki 2008; Wu et al. 2011). Clinical cardiologists have aimed to improve myocardial functions in patients with ischemic cardiomyopathy. Therefore, an objective, accurate and sensitive evaluation method is necessary for optimal treatment.

Two-dimensional speckle-tracking echocardiography (STE) has been developed to quantify myocardial deformation (strain; Amundsen et al. 2006; Leitman et al. 2004; Mor-Avi et al. 2011; Pirat et al. 2008) and

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can provide a less-subjective assessment of global and segmental functions (Mor-Avi et al. 2011; Reisner et al. 2004; Zuo et al. 2015). STE may also be capable of detecting much subtler degrees of functional impairment in ischemic tissue, such as those detected by myocardial perfusion imaging. Global and regional strains have been shown to correlate well with visual wall motion scores in patients with acute myocardial infarction (AMI), non-coronary chest pain or dilated cardiomyopathy (Liel-Cohen et al. 2010). In this study, we used an over-the-wire balloon catheter to deliver mesenchymal stem cells (MSCs) combined with basic fibroblast growth factor (bFGF) *via* the coronary vein in a canine infarct model and evaluated myocardial functions using strain values derived from STE.

METHODS

Acute myocardial infarction model and retrograde infusion

The protocols conformed to the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health publication 85–23, revised 1985) and were approved by the institutional ethics committee of Capital Medical University, China. All animals received appropriate care during the study. Twenty-eight healthy adult, male or female mongrel dogs (Beijing Luyuan Laboratory Animal Plant) weighing 17.0 ± 1. 9 kg were used in this study.

The experimental animals underwent left thoracotomy under anesthesia induced with xylazine hydrochloride (0.5 mL/kg, intramuscular injection) and sodium pentobarbital (0.3 mL/kg, intravenous injection as needed) together with mechanical ventilation. Electrocardiogram monitoring with clear R wave recognition was recorded. The left anterior descending (LAD) coronary artery was ligated beyond the first diagonal branch to create the myocardial infarction, followed by closing the chest.

At 7 d after infarction, the animals were randomly divided into four groups: bFGF (n = 6), MSCs (n = 6), bFGF+MSCs (n = 8) and saline (n = 8), which received bFGF, MSCs, bFGF+MSCs or saline, respectively. To avoid immunologic rejection, MSCs were derived from canine autologous bone marrow aspirates.

All laboratory animals were anesthetized as described above before examination at 7 d after infarction. The degree of LAD coronary artery occlusion was examined by percutaneous coronary angiography. In addition, a coronary sinus venogram was performed initially to delineate the anatomy and find the target vein (anterior inter-ventricular vein [AIV]). A 0.14-in exchange-length extra support guidewire was advanced to the AIV. Subsequently, an over-the-wire balloon catheter was advanced over the guidewire and positioned in the mid-AIV. The balloon was inflated, occluding the AIV, and a total of 1×10^7 MSCs in 10 mL saline (MSC group) or 40 μL of 200 μg/L bFGF (bFGF group) was injected into the AIV; both were injected into the bFGF+MSC group. In the control group, 10 mL saline-only was infused.

Echocardiography

All echocardiographic images were acquired using an Artida ultrasound unit (Toshiba Medical System, Tokyo, Japan) equipped with a PST-30 SBT transducer (2.5–5 MHz). Examinations were performed before LAD coronary artery ligation, 7 d after LAD coronary artery ligation and 40 d after MSC infusion. Dogs were restrained in the right lateral recumbency position with sedation using acepromazine (0.01 mg/kg, intravenous injection, 10 mg/mL) and buprenorphine (0.0075 mg/kg, intravenous injection, 0.2 mg/mL). Electrocardiogram monitoring with clear R wave recognition was recorded in concurrence with echocardiographic examination using the same ultrasound unit. Conventional M-mode echocardiographic measurements were obtained from the left parasternal long-axis view at the basal level. The left ventricular ejection fraction (LVEF) was calculated by a modified biplane Simpson's method. Early and late diastolic mitral in-flow velocity E and A values were acquired by imaging of the apical four-chamber view.

Regarding strain parameters, two-dimensional imaging (appropriate depth and gain, pulse repetition frequency >40 f/s) of the apical four-, three- and two-chamber views was required for longitudinal and transverse strains, and left parasternal short-axis views at basal, middle and apical levels for radial and circumferential strains. Using 2-D tracking software, one ideal cardiac cycle was selected for subsequent analysis. The endocardial border of each plane in the end-systolic frame was traced manually. A region of interest was then drawn to include the entire myocardium. The software algorithm automatically segmented the left ventricular (LV) plane into six equidistant segments, according to the definition of the left ventricular wall division of the American Society of Echocardiography, and speckles in the region of interest were identified on a frame-by-frame basis to construct a strain-time curve and strain graphs. In general, the LV wall is divided into 16 segments according to the American Society of Echocardiography. We divided the infarct zone into seven segments including the basal segment of the anterior ventricular septum, the middle segments of the anterior ventricular septum and the anterior wall and four apical segments based on the perfusion region of the coronary artery. The border zone included basal segments of the anterior wall, the lateral wall and posterior ventricular