African Journal of Biotechnology Vol. 10(72), pp. 16387-16392, 16 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2052
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Heart dysfunction and fibrosis in rat treated with myocardial ischemia and reperfusion

Cheng-Han Huang[1], Yi-Ming Huang[2], Yung-Sheng Tseng[2], Wei-Chi Lee[3], Jui-Te Wu[2], Zhi-Jia Zheng[2] and Hsi-Tien Wu[1]*

[1]Department of Bioagricultural Science, National Chia Yi University, Chiayi, 60004, Taiwan.
[2]Department of Veterinary Medicine, National Chia Yi University, Chiayi, 60004, Taiwan.
[3]Animal Science Technology Service and Pathology Section Head, Animal Production Technical Division, Uni-President Enterprises Corp, Tainan, 72046, Taiwan.

Accepted 30 September, 2011

Because cardiovascular disease remains a serious problem in modern human society, the aim of this study was to establish the rat model animal and to compare the heart dysfunction and fibrosis with SD and LE rats when treated with myocardial ischemia and reperfusion operation. A 20-minute thoracotomy was performed on the rat at the left anterior descending coronary artery occlusion; then the perfusion was carried out. The left ventricular diastolic diameter and left ventricular systolic diameter (LVEDd and LVEDs) were both reduced in LE and SD rats after surgery. Compared with the sham group, the performance of the left ventricular fractional shortening (LVFS) significantly decreased, indicating systolic dysfunction was affected after surgery, and SD was significantly higher than LE at LVFS decreasing rate. The significant areas of collagen fibers were detected by Masson's tri-chrome staining after surgery. These results suggest that SD rat is more suitable than LE rat for successful establishment of the model of myocardial ischemia and reperfusion. Also, the rat model can provide good experimental materials for regenerative medicine and drug testing to enhance research results in the future.

Key words: Myocardial infarction, myocardial ischemia and reperfusion, animal model.

INTRODUCTION

Cardiovascular diseases, especially coronary artery disease, are the leading cause of mortality and morbidity worldwide (Samadikuchaksaraei, 2006; Venardos et al., 2007). The effect of myocardial infarction (MI) primarily caused by coronary artery obstruction results in heart damage and death associated with myocardial cells (Thygesen et al., 2007). Even if other heart tissue is not affected by coronary artery blockage, heart failure may still result, perhaps because the burden is finally too great (Camici and Crea, 2007). Because of scar- and ischemia-related postinfarction events, clinical manifestations are enormous and heterogeneous. The damaged left ventricle undergoes progressive “remodeling” and chamber dilation with myocyte slippage and fibroblast proliferation. These events reflect an apparent lack of effective intrinsic mechanisms for myocardial repair and regeneration (Minguell and Erices, 2006).

Atherosclerosis with thrombus accumulated in the coronary artery causes MI (Hansson, 2005; Insull, 2009). Because of its high metabolic rate, the myocardium undergoes irreversible injury within 20 min of ischemia, which begins in the inner layers and moves toward the outer layers, resulting in the death of large numbers of myocardial cells over a 3 to 6 h period (Michael et al., 1995). Although, cardiomyocytes are the most vulnerable cells, ischemia also kills vascular cells, fibroblasts, and nerves in the tissue. Myocardial necrosis causes severe inflammation, and millions of marrow-derived leukocytes

*Corresponding author. E-mail: htwu@mail.ncyu.edu.tw. Tel: +886-5-2717767. Fax: +886-5-2717755.

Abbreviations: **LVEDd**, Left ventricular diastolic diameter; **LVEDs**, left ventricular systolic diameter; **LVFS**, left ventricular fractional shortening; **MI**, myocardial infarction; **NRC**, United States National Research Council; **LAD**, left anterior descending artery; **FS**%, fractional shortening.

enter the infarct region (Dobaczewski et al., 2010). The macrophages phagocytose the necrotic cell debris and lead to the subsequent healing of the wounds. Before they become scabs, they form a granulation tissue, which contains a proliferation of fibroblasts and endothelial cells, around the infarct area and the surrounding tissue. Ultimately, granulation tissue remodels to form densely collagenous scar tissue (Laflamme and Murry, 2005). The repair process of myocardial infarction in humans requires 2 months to complete; in small experimental animals, such as mice or rats, the process occurs much faster.

Various animal studies have shown that myocardial ischemia after reperfusion generates a complex series of inflammatory reactions (Michael et al., 1995). The heart function can be recovered through heart reperfusion when the ischemia occurs in less than 20 min. This step will not lead to myocardial necrosis but may cause temporary heart stunning. Cell necrosis amplifies from the subendocardium to subepicardium continuously when the coronal artery occlusion lasts longer than 20 min (Virmani et al., 1992). Myocardial ischemia and reperfusion in the myocardium lead to serious inflammation in the cardiac tissue, damaging and weakening the ventricular function and possibly leading eventually to ventricular hypokinesis, akinesis, or dyskinesis (Pfeffer and Braunwald, 1990).

Medical scientists have studied coronary artery disease for decades, yet deaths caused by MI and related cardiovascular diseases continue to have serious impact in modern society (Venardos et al., 2007). Thus, choosing a suitable animal model provides good test material for MI and helps in clinical studies that may be needed before the development of pharmaceutical preparations. Appropriate animal model can also provides good material for regenerative medicine and cell therapy research in MI and reperfusion. Thus, the aim of this study was to establish the rat model animal and to compare the heart dysfunction and fibrosis with SD and LE rats after myocardial ischemia and reperfusion operation.

MATERIALS AND METHODS

Animals used in the experiment

Eight-week-old male rats were used to establish the MI model animal. SD rats (Bltw:SD, BioLASCO Taiwan Co., Ltd) and LE rats (Narl:-LE, National Laboratory Animal Center, NARL Taiwan) were used for the experiment. The animals were kept in a clean conventional animal room under air, temperature, and light control. All animals were maintained, handled, and treated following NRC guidelines (1996); and the Animal Experimental Ethics Committee at National Chia Yi University approved all experiments.

Myocardial ischemia and reperfusion operation in the rat

The myocardial ischemia and reperfusion operations were performed according to procedures described by Michael et al. (1995) and Patten et al. (1998) with modification. Rats were anesthetized using a mixture of Zoletil (25 mg/kg) and Xylazine (10 mg/kg) via intramuscular injection. With the rats in the supine position, endotracheal intubation was performed, and they were ventilated with a small animal respirator (A.D.S. 1000, Engler Engineering Co., Florida, USA). The chest was opened by a lateral cut with tenotomy scissors along the left side of the sternum, cutting through the 4th left ribs to approximately midsternum. The left anterior descending artery (LAD) ligation proceeded with a sterile 7-O silk (UNIK surgical sutures mfg. Co., Ltd., Taiwan) slipknot suture passed with a tapered needle. The blocking time was 20 min.

After occlusion for a prescribed period (20 min), reperfusion occurred by unblocking the slipknot. This allowed release of the occlusion and reperfusion of the formerly ischemic bed. The chest walls were retracted by using 4-O silk monofilament suture (Ethicon, Auneau Co., France). The catheter (24G SURFLO® IV catheter, TERUMO, Co., Philippines), which was connected with the 10 ml syringe and inserted before the chest was sutured, pulled out the residual air from chest; the respirator helped the animal breathe. The chest wall was then closed, sutured through one layer of the chest wall and muscle and a second layer through the skin and subcutaneous material. The rat was kept on a warm pad until awake. The control (sham) group underwent thoracic surgery but not vascular ligation of the LAD. The rats were placed in separate cages in an air conditioned animal room with clean water and diet for postoperative care.

Histological section and Masson's trichrome stain

Two weeks after the MI and reperfusion operation, rat hearts were collected and fixed with 4% paraformaldehyde, followed by treatment with 70 to 100% ethanol and xylene. Specimens were embedded in paraffin and sectioned at a thickness of 6 µm via a microtome. Two slides were stained for each specimen, one with hematoxylin and eosin, and the other with Masson's trichrome stain. Slides were observed with a Leica DM2000 microscope and digitally photographed (Wood et al., 2010).

Echocardiography

Echocardiographic assessment was performed according to procedures described by Michael et al. (1995) and Patten et al. (1998) with slight modification. Echocardiographs were obtained using the Philips SONOS 7500 Ultrasound system (Koninklijke Philips Electronics N.V.) with 12 MHz frequency transducer, 10 x 13 mm footprint at an image depth of 2 cm. Left ventricular M-mode measurements at the level of the papillary muscles were used to define left ventricular end-diastolic diameter (LVEDd) and left ventricular end-systolic diameter (LVEDs). Fractional shortening (FS %) was defined as (LVEDd - LVEDs) / LVEDd x 100% (Park et al., 2010).

Rats were anaesthetized intraperitoneally with a mixture of Zoletil (25 mg/kg) and Xylazine (10 mg/kg). The hair around the chest was shaved while the rat was supine and the limbs were fixed by tape. Echocardiographic assessment was performed 3 days before MI and reperfusion surgery (baseline) and 2 weeks after surgery (after treatment).

Statistical analyses

All values of echocardiography are listed as means ± SEM. The statistical analysis was evaluated with Student's t-test to measure difference among means.

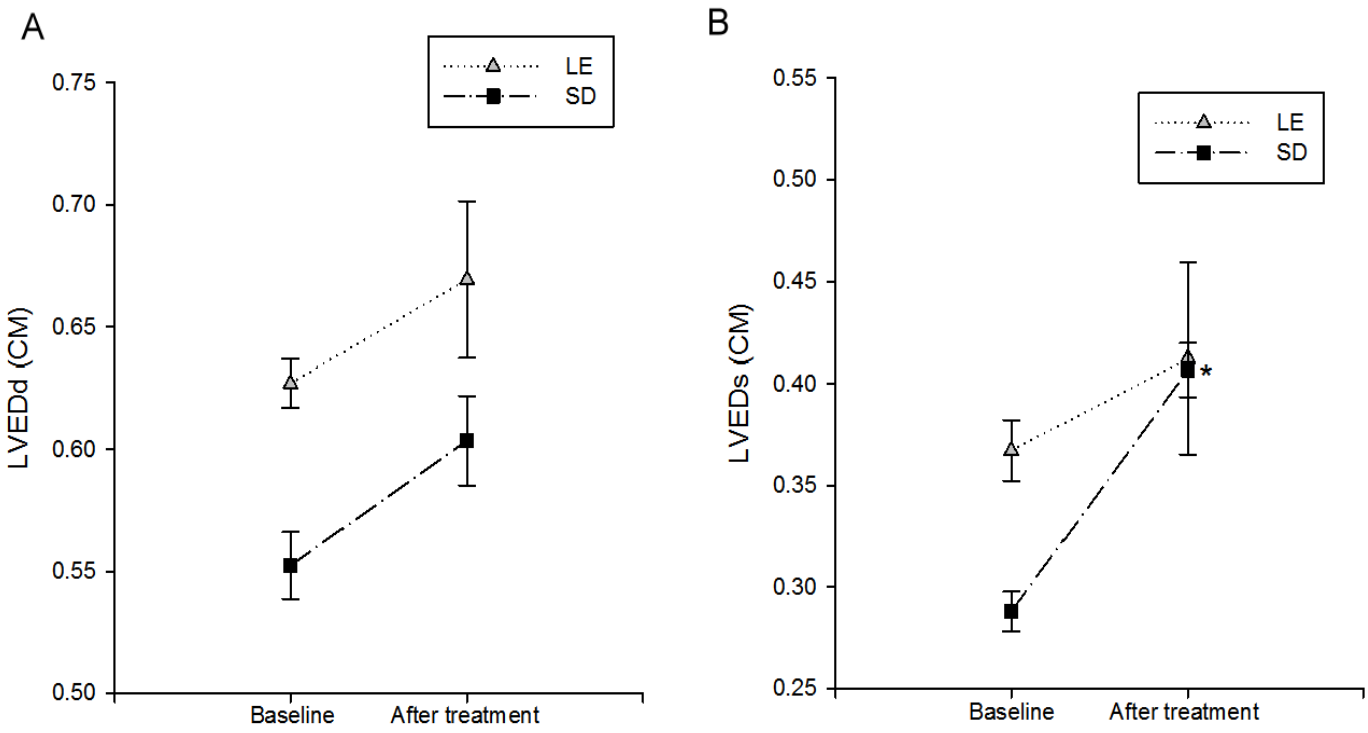

Figure 1. Change of left ventricular end diastolic diameter (LVEDd, A) and left ventricular end systolic diameter (LVEDs, B) before and after myocardial ischemia and reperfusion surgery. The baseline indicated 3 days before surgery. The after treatment indicated 2 weeks after surgery. The triangle line as LE rats (N=6), the black square line as SD rats (N=6). Data represented centimeters (CM).

RESULT

Echocardiography analysis

In order to evaluate the structure and function of cardiac contractility, this test was conducted 3 days before MI and reperfusion surgery and 2 weeks after surgery, using the cardiac ultrasound M-mode. Results showed that the LVEDd and LVEDs of LE and SD rats increased after surgical treatment (Figure 1). LE rats, LVEDd, baseline, 0.63±0.01 cm; after treatment, 0.67±0.03 cm; SD rats, LVEDd, baseline, 0.55±0.01 cm; after treatment, 0.60±0.02 cm (Figure 1A). LE rats, LVEDs, baseline, 0.38±0.02 cm; after treatment, 0.41±0.05 cm; SD rats, LVEDs, baseline, 0.32±0.02 cm; after treatment, 0.40±0.01 cm, $P<0.05$ (Figure 1B). Compared with the pseudo operation group (sham group), the left ventricular fractional shortening (LVFS) decreased obviously both at SD and LE after MI and reperfusion operation (Figure 2). Sham rats, LVFS, baseline, 47.66±2.02%; after treatment, 49.59±1.20%. LE rats, LVFS, baseline, 41.44±1.90%; after treatment, 38.76±4.20%; SD rats, LVFS, baseline, 47.80±1.50%; after treatment, 40.57±2.54%, $P<0.05$. The SD showed obviously decreasing rate than LE at LVFS. The results showed that the contractility of rat heart decreased after the operation and was in accordance with the purpose of the MI and reperfusion operation in rat myocardial damage.

Histological results on rat heart tissue

In addition to the use of echocardiography, the study also used histological observation on pathological damage. The Masson's trichrome stain is commonly used in studies related to myocardial damage and to identify the regional distribution of collagen fibers and fibrosis in heart tissue. Compared with the pseudo-surgery group, after the MI and reperfusion operation caused an increase in left ventricular diameter and a reduction in the thickness of the left ventricular wall (Figure 3), the area of collagen fibrosis also increased along with the phenomenon of inflammation and numbers of macrophages and lymphocytes infiltration into the myocardial tissue (Figure 4). The results demonstrate the surgery we performed had created a myocardial infarction. With the echocardiography results confirmed, the internal structure and function of the rat heart changed after the MI and reperfusion operation.

DISCUSSION

Rats are the appropriate animal for cardiovascular operations in medical research (Bader, 2010). The purpose of this study was to establish the rat as an animal model for a stable operation of myocardial ischemia and reperfusion, and compared the heart dysfunction and

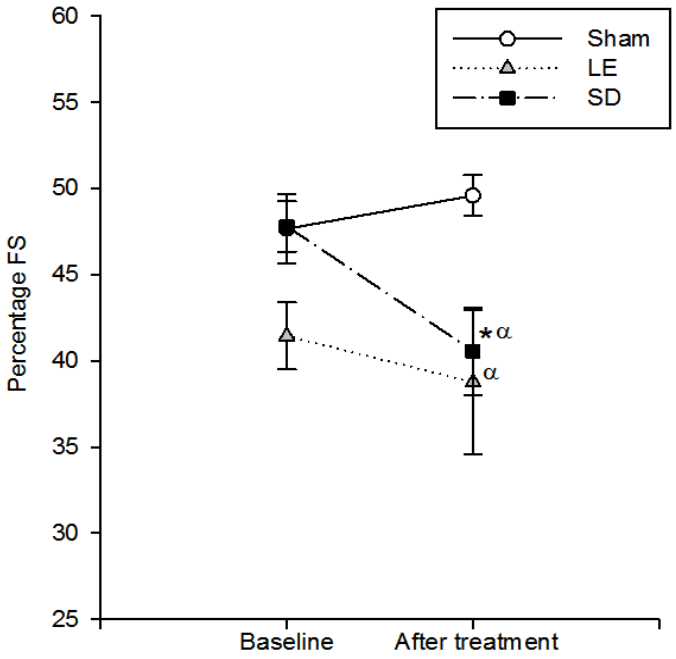

Figure 2. Change of left ventricular shortening fraction (LVFS) before and after myocardial ischemia and reperfusion surgery. The baseline indicated 3 days before surgery. The after treatment indicated 2 weeks after surgery. The circle line as sham operation group (N=5). The triangle line as LE rats (N=6), the black square line as SD rats (N=6). Data represented percentage.

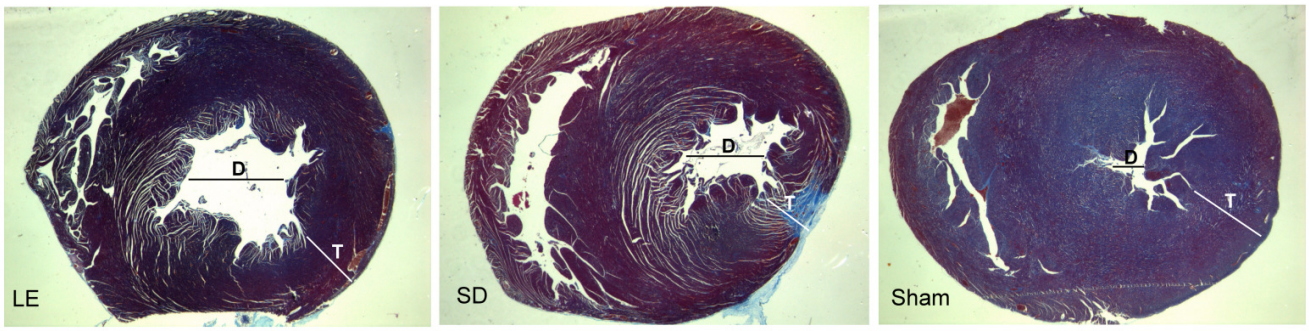

Figure 3. Change of left ventricle area after myocardial ischemia and reperfusion surgery. The increased of left ventricular inside diameter (D) and the loosed of ventricular wall thickness (T) were seen at LE and SD rat that compared with sham group. Cardiac sections were stained with Masson's trichrome staining. All images were captured at 6.8 x magnification.

fibrosis with SD and LE rats when treated with myocardial ischemia and reperfusion operation. For accessibility and stability of performance after the experiment, it is necessary to find the appropriate rats for the study at the outset. Two outbred rats, LE and SD, were chosen for this study to evaluate the individual differences before and after the operation.

Both SD and LE rats can enter into a stable anesthesia period under appropriate doses of anesthetics and can maintain 2 to 3 h until the emergence from anesthesia, which is enough time to complete the operation (Zheng and Hu, 2006). Using the M-mode on the echocardiography combined with appropriate ane-sthetic can stabilize the measurement of cardiac diastolic and systolic performance (Migrino et al., 2008). After myocardial infarction, the left ventricular structure changed as a result of the generated mechanical stretch and led to increased ventricular wall stress. The result was a decrease in left ventricular contractility and loss of its ability to eject blood (Sutton and Sharpe, 2000). After surgical treatment, compared with LE, SD rats had obvious differences in left ventricular diastolic and

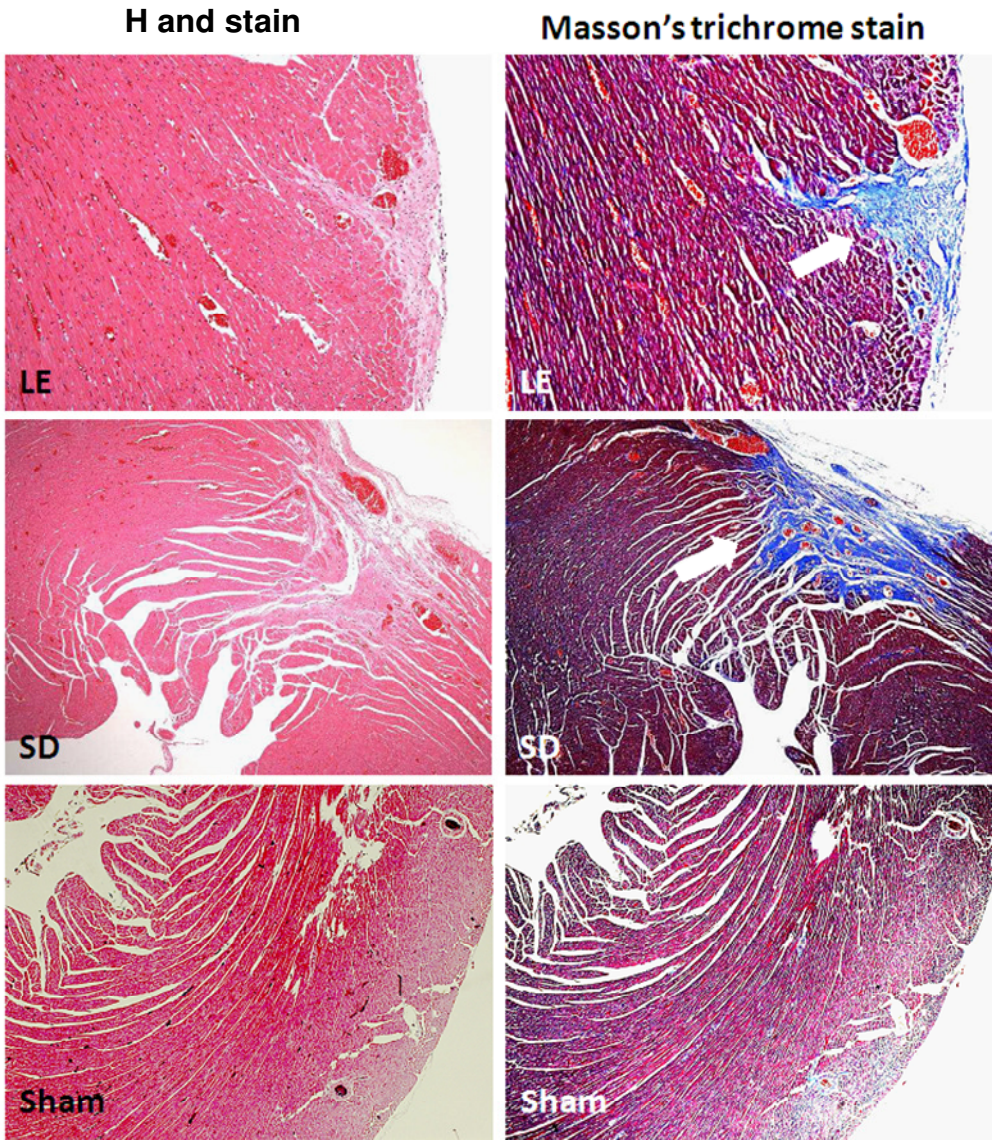

Figure 4. The myocardial fibrosis formation after myocardial ischemia and reperfusion surgery. Pictures on the left were hematoxylin and eosin (H and E) staining results, pictures on the right were Masson's trichrome staining results. Blue stain area presented the formation of myocardial fibrosis (arrow indicated). The orders from top to bottom were LE rat, SD rat and the sham group. All images were captured at 10 X object lens.

systolic diameter. After the MI and reperfusion operation, on both the SD and LE, caused an increase in left ventricular diameter, and a reduction in the thickness of the left ventricular wall. Echocardiography measurements of SD after surgery were significant, the differences in left ventricular fractional shortening (LVFS) were more apparent than in LE. According to the results, the significant performance indicated the SD rats were more suitable for myocardial ischemia and reperfusion surgery.

In accordance with the collagen fibers area by Masson's trichrome staining, infarct size and distribution were also obvious. By contrast, the observation of postoperative ventricular tissue biopsies indicates that the SD and LE rats exhibited fibrous tissue hyperplasia after myocardial self-healing and mild inflammation and macrophage infiltration in myocardial tissue; however, the postoperative inflammation and formation of collagen fibers in SD or LE rats showed no significant difference.

Conclusion

The study compared the heart dysfunction and fibrosis with SD and LE rats when treated with myocardial ischemia and reperfusion. From the successful rat model established, we found out that SD rat is a suitable model

animal than LE for myocardial ischemia and reperfusion studies. Rat heart systolic function weakened significantly after postoperative recovery and obviously in myocardial collagen fiber proliferation. This method to establish the model animal can be used to study drug treatment to improve the condition of myocardial ischemia, stem cell therapy research, and regenerative medicine applications, helping to improve research in cardiac medicine.

ACKNOWLEDGEMENTS

This work was supported by Nation Science Council, Taiwan (NSC 97-2313-B-415-005-MY3).

REFERENCES

Bader M (2010). Rat models of cardiovascular diseases. Methods Mol. Biol. 597: 403-414.

Camici PG, Crea F (2007). Coronary microvascular dysfunction. N. Engl. J. Med. 356: 830-840.

Dobaczewski M, Gonzalez-Quesada C, Frangogiannis NG (2010). The extracellular matrix as a modulator of the inflammatory and reparative response following myocardial infarction. J. Mol. Cell. Cardiol. 48: 504-511.

Hansson GK (2005). Inflammation, atherosclerosis, and coronary artery disease. N. Engl. J. Med. 352: 1685-1695.

Insull Jr. W (2009). The pathology of atherosclerosis: plaque development and plaque responses to medical treatment. Am. J. Med. 122: 3-14.

Laflamme MA, Murry CE (2005). Regenerating the heart. Nat. Biotechnol. 23: 845-856.

Michael LH, Entman ML, Hartley CJ, Youker KA, Zhu J, Hall SR, Hawkins HK, Berens K, Ballantyne CM (1995). Myocardial ischemia and reperfusion: a murine model. Am. J. Physiol. 269: H2147-2154.

Migrino RQ, Aggarwal D, Konorev E, Brahmbhatt T, Bright M, Kalyanaraman B (2008). Early detection of doxorubicin cardiomyopathy using two-dimensional strain echocardiography. Ultrasound Med. Biol. 34: 208-214.

Minguell JJ, Erices A (2006). Mesenchymal stem cells and the treatment of cardiac disease. Exp. Biol. Med. 231: 39-49.

Park K, Chang SA, Kim HK, Park HE, Na SH, Kim YJ, Sohn DW, Oh BH, Park YB (2010). Normal ranges and physiological changes of midwall fractional shortening in healthy korean population. Korean Circ. J. 40: 587-592.

Patten RD, Aronovitz MJ, Deras-Mejia L, Pandian NG, Hanak GG, Smith JJ, Mendelsohn ME, Konstam MA (1998). Ventricular remodeling in a mouse model of myocardial infarction. Am. J. Physiol. 274: H1812-1820.

Pfeffer M, Braunwald E (1990). Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. Circulation, 81: 1161-1172.

Samadikuchaksaraei A (2006). Stem cell therapy for acute myocardial infarction. Hellenic. J. Cardiol. 47: 100-111.

Sutton MG, Sharpe N (2000). Left ventricular remodeling after myocardial infarction: Pathophysiology and therapy. Circulation, 101: 2981-2988.

Thygesen K, Alpert JS, White HD (2007). Universal definition of myocardial infarction. Circulation, 116: 2634-2653.

Venardos KM, Perkins A, Headrick J, Kaye DM (2007). Myocardial ischemia-reperfusion injury, antioxidant enzyme systems, and selenium: a review. Curr. Med. Chem. 14: 1539-1549.

Virmani R, Kolodgie FD, Forman MB, Farb A, Jones RM (1992). Reperfusion injury in the ischemic myocardium. Cardiovasc. Pathol. 1: 117-129.

Wood A, Docimo S, Elkowitz DE (2010). Cardiovascular disease and its association with histological changes of the left stellate ganglion. Clin. Med. Insights Pathol. 3: 19-24.

Zheng X, Hu SJ (2006). Effects of simvastatin on cardiohemodynamic responses to ischemia-reperfusion in isolated rat hearts. Heart Vessels, 21: 116-123.