Laboratory Research

Effect of angiopoietin-related protein 2 on coronary angiogenesis and myocardial function in a porcine model of acute myocardial ischemia

Shu Meng¹, Changqian Wang¹, Fei Wang¹, Renjian Zhou², Fangbao Ding³, Fuxin Chen¹

¹. Department of Cardiology; ². Department of Nuclear Medicine; ³. Department of Cardiac Surgery, Xinhua Hospital, Shanghai Jiaotong University, Shanghai 200092, China

Background Our previous studies have suggested that angiopoietin-related protein 2 (Arp2) may improve rat cardiac function after acute myocardial infarction (AMI) by accelerating angiogenesis. We want to study the efficacy of the adenoviral vector-mediated gene transfer of Arp2 (Ad.Arp2) in inducing angiogenesis and in improving the myocardial perfusion and function in a porcine acute myocardial ischemic model. Methods The minipigs underwent ligation of the proximal circumflex coronary artery (LCx) and were randomly assigned to treatment with Ad.Arp2, adenoviral vectors with no transgene (Ad.Null) or PBS. Four weeks later, the animals were evaluated using echocardiography, cardiac perfusion imaging and pathologic observation. Results Four weeks after treatment, the Arp2 protein was revealed in the myocardium of Ad.Arp2 animals, but was not found in the Ad.Null or PBS animals. Also, a significant revival of myocardial perfusion was found in the ischemic area in Ad.Arp2-treated animals, whose global and regional myocardial function was greatly improved. The quantitation of new capillaries was much greater in the Ad.Arp2 group than in the Ad.Null or PBS groups. Conclusion Treatment with Ad.ARP2 offers the obvious advantage of greatly improving the blood supply and the heart function. (J Geriatr Cardiol 2008; 5:230-234)

Key words myocardial ischemia; angiogenesis; pig; gene therapy

Atherosclerosis-induced coronary artery disease (CAD) is the leading cause of morbidity and mortality in Western societies and is increasing at an alarming rate in developing countries.¹ Many patients with advanced diffuse CAD who are not eligible for conventional revascularization, such as percutaneous coronary intervention (PCI) or coronary artery bypass grafts (CABG) are often referred to as ‘no option’ patients.² Therapeutic angiogenesis is a promising modality for such patients. It is the up-regulation of endogenous angiogenic factors by gene or protein therapy, and can increase blood vessel formation and improve perfusion to ischemic tissues.³ Our previous studies have demonstrated that angiopoietin-related protein 2 (Arp2) may improve rat cardiac function after acute myocardial infarction (AMI) by accelerating angiogenesis.⁴ However, there have been no previous studies using large animal models of acute ischemia to investigate the effect of Arp2 after AMI. Thus, our aim was to study the efficacy of adenoviral vector-mediated gene transfer of Arp2 in a porcine AMI model.

Corresponding author: Dr. Changqian Wang, Department of Cardiology, Xinhua Hospital, Shanghai Jiaotong University, Shanghai 200092, China (Tel 021-65790000-7058. Email: wcqian@hotmail.com).

Methods

Pig MI model and growth factor implantation

the adenoviral vector-mediated gene transfer of Arp2 (Ad.Arp2) and the adenoviral vectors with no transgene (Ad.Null) were constructed by our lab and stored at -70°C.⁵ Thirty male Chinese minipigs (quality eligible certificate: SYXX 2003-0031) weighing between 25-30 kg, were obtained from the Experimental Animal Center at Xinhua Hospital. All animal care and experimental protocols complied with the animal management rules of the Ministry of Health of the People’s Republic of China (document No.55, 2001) and the Xinhua Hospital guidelines for the care and use of laboratory animals. Animals were randomly divided into three groups (n =10 pigs per group). Before all experimental procedures, all animals were anesthetized by intramuscular administration of ketamine hydrochloride (2mg/kg, Hongrui Medicine, Jiangsu, China), together with an IV injection of succinyl chloride (1.5 mg/kg, Libang Medicine, Xian, China) and were mechanically ventilated with a volume respirator (Zejiang Medical College Instrument Experimental Factory). Median sternotomy was performed to expose the heart, followed by incision of the pericardium. Acute myocardial infarction was produced by ligation of the proximal LCx using a 7.0 prolene suture. Immediately after the procedure, 10
Echocardiographic analysis

Echocardiography was performed immediately after transplantation and at four weeks after transplantation using an echocardiography system (HP 5500, America) with a 5-MHz transducer. The animals were sedated with isoflurane (2%) via inhalation and placed in the left lateral decubitus position. Parasternal long- and short-axis views were obtained with both M-mode and 2-dimensional echo images. Left ventricle dimensions (end-diastolic diameter [LVEDD] and end-systolic diameter [LVESD]), left ventricular wall thickness at end-diastole (DWT) and left ventricular wall thickness at end-systole (SWT) were measured perpendicular to the long axis of the ventricle at the mid choral level. Fractional shortening (%) was calculated as (LVESD-LVEDD)/LVEDD×100. The left ventricular ejection fraction (LVEF) (%) was calculated automatically by the echocardiography system by the equation: LVEF=(LVEDV-LVESV)/LVEDV×100, where LVEDV=7.0×LVEDD/(2.4+LVESD) and LVESV=7.0×LVESD/(2.4+LVESD). Left ventricular systolic wall thickening (WT %) was calculated as WT%= (SWT-DWT)/DWT×100%.

Spect

To determine myocardial perfusion, myocardial single photon emission computed tomography (SPECT) was performed at both one (baseline) and four weeks (endpoint) after transplantation. For the SPECT 99mTc-sestamibi (Xinke Medicine, Shanghai, China) scans, about 370 MBq were administered to the pigs and the hearts were imaged 1 h later with a gamma camera. The dual head gamma camera (GE, America) with a low energy high resolution collimator with a 20% energy window was set to a 140 eV gamma peak. Thirty-two projection images per 25 seconds in a 64×64 matrix were achieved by using a 180°-rotation arc from the 45-right and anterior section to the 45-left and anterior sector. SPECT images were scored semi-quantitatively using a 5-point scale (0=normal uptake, 1=mild hypoperfusion, 2=moderate hypoperfusion, 3=severe hypoperfusion and 4=no uptake). Ischemia was defined as summed difference score ≥ 2. We used the myocardial count ratio method. The short axis comprises the anterior, lateral, inferior or posterior segments and septal; the horizontal axis comprises the apex, anterior, inferior, or posterior segments. Myocardial count ratios were obtained from regions of interest, including the ischemic segment and the whole left ventricle. The varying levels of myocardial perfusion between treatment periods and among the 3 groups were analyzed.

Histological analysis of myocardium

Animals were then terminated under anesthesia. The heart excised, the target area was then scored with a blade on the epicardial surface and the heart cut into five transverse sections from apex to base. A 3 mm thick transmural section was removed from the fourth transverse ring, fixed in 4% paraformaldehyde and placed in phosphate-buffered saline prior to fixation in a paraffin block. Three 5 μm sections, separated by 50 μm intervals, were stained with hematoxylin and eosin (HE) to assess morphology and inflammation. The proportion of the myocardium showing inflammation was assessed using an Olympus BH-Z Japan light microscope fitted with an eyepiece graticule containing 100 crosses (10×magnification). The data were expressed as number of capillaries per field.

Protein and immunohistochemistry assays

The 5 μm sections, separated by 50 μm intervals of the fourth ring, were immunostained using mouse anti-human Arp2 monoclonal antibody for the target protein immunohistochemistry assay. To further validate the presence of newly formed capillaries, Arp2-treated ischemic myocardiums were immunologically stained for the factor VIII antibody. The number of capillaries was assessed using a squared eyepiece graticule to avoid repeat counting (>200 magnification). The data were expressed as number of capillaries per field.

Statistical analysis

The results were expressed as mean±standard deviation (SD). The statistical significance of differences was assessed using the Student’s t test. Statistical analyses were carried out with SPSS 10.0. The significance level was set at P<0.05.

Results

Improvement of global and regional myocardial function

The basal cardiac dysfunction was indistinguishable among all groups immediately after treatment. Four weeks later, echocardiography was performed to monitor the global and regional myocardial function of the remaining animals (Ad.Arpe2 group: 10 pigs; Ad.Null group: 6 pigs; PBS group: 6 pigs). Significant improvements of LVEF, FS and WT% were observed in the Ad.Arpe2 group compared with the Ad.Null and PBS groups (Fig 1).

Improvement of regional myocardial perfusion and wall motion

The basal myocardial perfusion of the lateral and inferior segments of the left ventricle was indistinguishable
among 3 groups 1 week after treatment. Four weeks later, ischemic level and area of Ad.Arp2 was significantly revived. However, there were no obvious changes in the Ad.Null and PBS groups. (Fig 2a-c, Fig 3).

Inflammation within the myocardium

Mild inflammation was detected in all of the hearts, but there was no significant difference among the 3 groups.

Protein expression and angiogenesis analysis

The protein of Arp2 was revealed in the myocardium of Ad.Arp2 animals, but was not found in Ad.Null or PBS animals. The quantitation of new capillaries was much greater in the Ad.Arp2 group than in the other 2 groups (Fig. 4, Fig. 5a-b).

Discussion

Therapeutic angiogenesis constitutes a potential alternative approach for patients with advanced CAD who are not candidates for conventional revascularization techniques. Delivery of proangiogenic factors to the ischemic myocardium to stimulate collaterogenesis and to improve myocardial perfusion and function is a straightforward idea which has been proposed for several years. Arp2 (angiopoietin-related protein 2 or Angptls 2) was discovered and cloned by Kim et al.\textsuperscript{10} It belongs to a family of proteins structurally similar to the angiogenic regulating factors angiopoietins. Encoded by seven genes, Angptls 1
angiogenesis. We have demonstrated that Arp2 could transfect Arp2 to an ischemic myocardium and up-regulate its Arp2 expression. We also found that the quantitation of new blood vessels was much greater in the Arp2 group than in the other 2 groups. Four weeks after treatment, Arp2 protein was revealed in the myocardium of Ad.Arp2 animals, but not in Ad.Null or PBS animals. This means that construction of a recombinant Ad vector was an efficient and reliable method which could transfect Arp2 to an ischemic myocardium and up-regulate its Arp2 expression. We also found that the quantitation of new blood vessels was much greater in the Arp2 group than in the other 2 groups. Four weeks after vector or PBS administration, SPECT images demonstrated a significant revival of myocardial perfusion in the ischemic area of Ad.Arp2-treated animals compared with Ad.Null- and PBS-treated animals. Meanwhile, echocardiographs documented a significant improvement of regional myocardial function as well as global myocardial function in Ad. Arp2-treated animals compared with Ad.Null and PBS controls. Of course, mild inflammation was detected in all of the hearts, but there was no significant difference among the 3 groups, meaning that a $2 \times 10^{11}$ pfu adenovector is safe for pig myocardial angiogenesis.

Unlike former studies using a 17-segment model of the left ventricle semi-quantitatively image interpretate ischemic myocardium area, we used the method of myocardial count ratio between the ischemic segments to the whole left ventricle, analysis the varying levels of myocardium perfusion between 1 week to 4 weeks after treatment that greatly decreased random errors.

The present study was an example of applying Arp2 to the pig acute ischemic myocardium to establish new capillary networks. This led to a remarkable improvement of both myocardial perfusion and myocardial function. These findings may open new avenues and serve as a guideline for developing future proangiogenic therapies for the clinical treatment of MI.

Acknowledgments: We thank Professor Yongwen Qin (Department of Cardiology, Changhai Hospital, Second Military Medical University) and research technician Shengdong Huang (Department of Thoracic Cardiac Surgery, Changhai Hospital, the Second Military Medical University) for their technical assistance and helpful discussion.

References


