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Original Article

Modeling of acute myocardial infarction in swine by percutaneous coronary artery occlusion and dynamic observation of H-FABP

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Abstract: This study aims to establish the reference for the early diagnosis of AMI by building acute myocardial infarction (AMI) model in Chinese miniswine with percutaneous coronary artery occlusion. Sixteen Chinese miniswine were subjected to general anesthesia followed by left femoral artery puncture. AMI was confirmed by coronary angiography and ECG. The serum levels of heart fatty acid binding (H-FABP) were detected before modeling and at 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, 12 h, 24 h and 48 h after modeling, respectively. We found that serum H-FABP began to rise 0.5 h after AMI and reached the peak at 8 h. It began to decline to normal level after 24 h. In summary, modeling of AMI in Chinese miniswine by percutaneous coronary artery occlusion using gelfoam had a high success rate and the procedures were easy to implement. Dynamic monitoring of serum H-FABP after AMI provided important data for early treatment of AMI.

Keywords: Acute myocardial infarction, heart fatty acid binding, animal model, Chinese miniswine

Introduction

Acute myocardial infarction (AMI) is a heart disease featured by sudden onset, high mortality and high morbidity. Timely intervention of AMI is crucial for improving the prognosis. But so far there are no widely recognized indicators for early diagnosis of AMI [1], especially for non-ST elevation myocardial infarction (NSTEMI) and atypical myocardial infarction [2]. Myoglobin was once used as the enzymatic indicator for early diagnosis of AMI but is no longer used clinically due to its high content in muscles, which leads to low specificity [3]. Troponin is the more common indicator of AMI at present. However, with a large molecular weight [4], low sensitivity and long period, troponin does not increase until 4-6 h after AMI [4-6]. Therefore, troponin level is not an ideal diagnostic indicator of AMI either. The indicator of myocardial necrosis at an ultra-early stage of AMI remains to be identified. Building an animal model of AMI is an important method to detect the serum biochemical indices. We dynamically observed the changes of serum H-FABP by building an AMI model in Chinese miniswine through coronary artery occlusion using gelfoam. The best time to perform thrombolysis and PCI can be determined based on the observations for AMI patients.

Materials and methods

Subjects

Sixtent Chinese miniswine weighing 15-20 kg were provided by Taizhou Taihe Biotechnology Co., Ltd and passed the quarantine inspection (quarantine certificate No. 3205197447, license No. SCXK (Jiangsu) 2011-0002). Half of the Chinese miniswine were males and the other half were females. Swine having poor health, bad appetite, abnormal ECG results and diseased recently were excluded.

Materials

Materials Anesthetics: diazepam, ketamine, lidocaine; contrast agent: iopromide; Alternate

drugs: heparin, adrenalin, dopamine, nikethamide, atropine, potassium chloride, trinitroglycerol; Materials for blood vessel occlusion: gelfoam; defibrillator; digital subtraction angiograph (DSA); ECG monitor; Blood pressure monitor; suction machine; Centrifuge machine; Microplate reader; interventional materials: 6F arterial sheath, 6FJR, microtube, guide wire, J-shaped tip guide wire (all being medical wastes that had been disinfected by epoxyethane); H-FABF ELISA kit.

Method for AMI modeling

Anesthesia: Chinese miniswine were fasted from food and water for 12 h and 8 h, respectively. Anesthesia was induced by intramuscular injection of ketamine at 10 mg/Kg. After cleaning, the four limbs of the Chinese miniswine were fixed to the wood plank and the miniswine were placed on the catheter bed. Oxygen was supplied at the rate of 3 L/min, and skin preparation was performed on the chest and the four limbs. Under continuous ECG monitoring, venous access was made at the marginal ear vein using a cannula needle, and 1 ml diazepam and 10 mg ketamine were injected. Anesthesia was maintained throughout the operation, and defibrillator and suction machine were used when necessary. Seldinger right femoral artery puncture was performed to build the AMI model: Disinfection with iodophor and draping were performed conventionally; At the site of strongest pulse in the right femoral artery, lidocaine was injected for local anesthesia. After puncture, bright fresh arterial blood squirted out from the tail of the needle. Then the guide wire was inserted, and 6F arterial sheath was delivered along the guide wire. Ten milliliter of the blood sample was collected, and 3 ml was used for detecting prothrombin time (PT). The remaining 7 ml of the blood sample was centrifuged and stored in a refrigerator at -80°C. Pellet injection of 10000 U heparin was performed, and 2000 U heparin was given additionally every half hour during the operation; While using the J-shaped tip guide wire, the guide wire was inserted to the root of the ascending aorta. Left and right coronary angiography was carried out in multiple positions. ECG showed sinus rhythm of 160-200 beats/min and blood pressure of 160/130 mmHg. After the distal end of the Runthrough wire reached the proximal end of OM branch, the guide wire was removed. Gelfoam suspension (dissolved in iopromide) was injected via the microtube. Angiography revealed that the blood flow in the distal end of the OM branch disappeared, and AMI was successfully modeled. PT was determined using 3 ml of the blood sample, and the microtube was removed. Vital signs and oxygen saturation were monitored; After operation, 6F arterial sheath was removed, and subclavian vein catheter was indwelled for drawing blood after AMI. The incision of the femoral artery was closed; the needles were sealed with heparin, fixed at the operated site and covered with film. Chinese miniswine were sent back to the breeding center and bred separately. Measures were taken to avoid infection. Blood was drawn via the subclavian vein catheter at 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, 12 h, 24 h and 48 h after modeling, respectively. The blood samples were centrifuged at 50000 r/min and the supernatant was collected.

Detection of serum H-FABP

Serum was sampled at before modeling and at 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, 12 h, 24 h and 48 h after modeling, respectively. The serum level of H-FABP was determined using ELISA method.

Statistical analysis

All data were analyzed by using SPSS 16.0 software. Measurement data were expressed as $\bar{x} \pm s$. ANOVA of repeated measures was adopted to compare mean differences in 2 groups. $P<0.05$ indicated statistically significant difference.

Results

Survival of experimental animals

Coronary angiography and coronary artery occlusion with gelfoam were successfully performed in 16 Chinese miniswine. One swine had ventricular fibrillation and died after ineffective electrical cardioconversion.

Results of coronary angiography before and after AMI

The anatomy of coronary artery in Chinese miniswine is similar to that of human beings. After the occlusion of OM branch by gelfoam, the blood flow at the distal end disappeared,

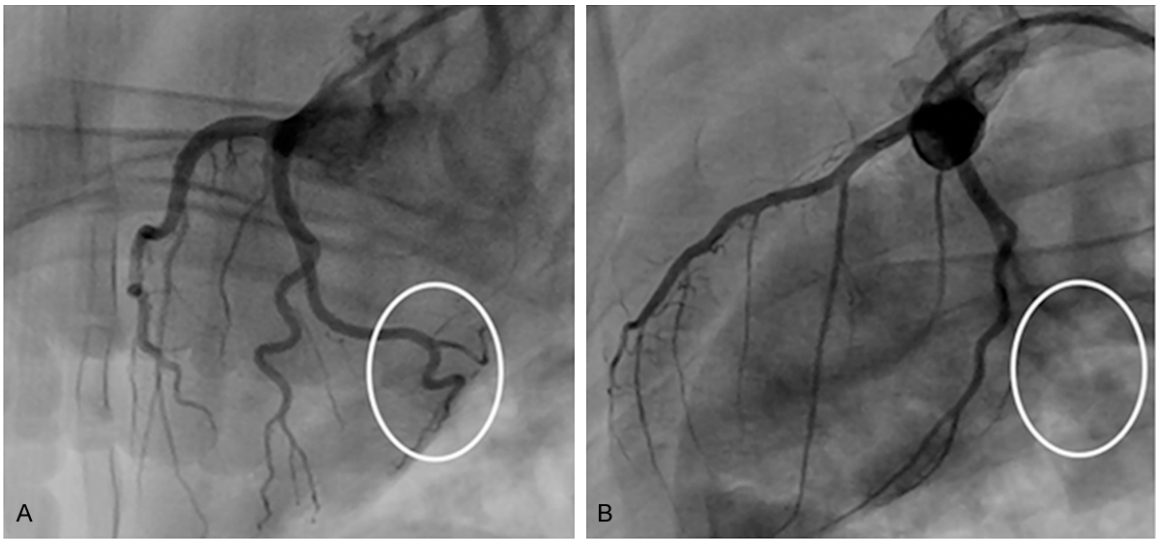

Figure 1. Coronary angiography of Chinese miniswine before and after AMI. A: Coronary angiography in normal conditions; B: Coronary angiography after AMI.

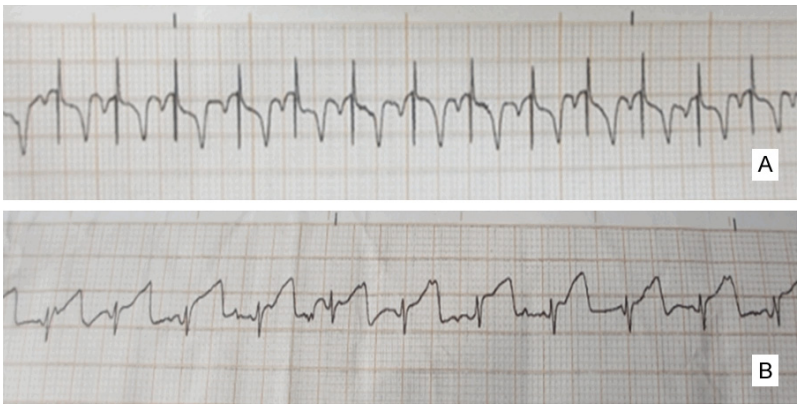

Figure 2. ECG of Chinese miniswine before and after AMI.

indicating successful modeling of AMI (**Figure 1**).

ECG before and after AMI

Figure 2A shows the normal ECG result in Chinese miniswine, with sinus rhythm and heart rate of 167 beats/min. **Figure 2B** shows the ST segment elevation, indicating successful modeling of AMI.

Serum level of H-FABP after AMI

The serum level of H-FABP began to rise at 0.5 h after AMI and reached the peak at 8 h. H-FABP declined after 24 h and the normal level was restored gradually. Dynamic changes of serum H-FABP level (ng/ml) at each time point are shown as follows:

Figure 3 shows that serum H-FABP reached the peak at 8 h in Chinese miniswine and began to drop to normal at 24 h.

Discussion

AMI is a severe threat to human health which may lead to heart failure, shock, malignant arrhythmia and even sudden cardiac death. The mortality and prognosis can be dramatically improved by timely diagonsis and reperfusion therapy. Many previous AMI models are built by thoractomy and coronary artery ligation, which damages the normal anatomy of the thoracic cavity and affects the cardiopulmonary function. Moreover, damages and inflammation caused by surgery interfere with postoperative continuous observation. In recent years, AMI is conveniently modeled by percutaneous coronary artery occlusion using gelfoam. The heart of Chinese miniswine is similar to that of human in terms of anatomy, blood vessel distribution and weight. The coronary artery system of swine has few collaterals and the branches are slender. Therefore the pathogenesis of ischemic heart disease can be satisfactorily simulated in swine. Moreover, the angiogenesis after ischemic heart disease in small swine is

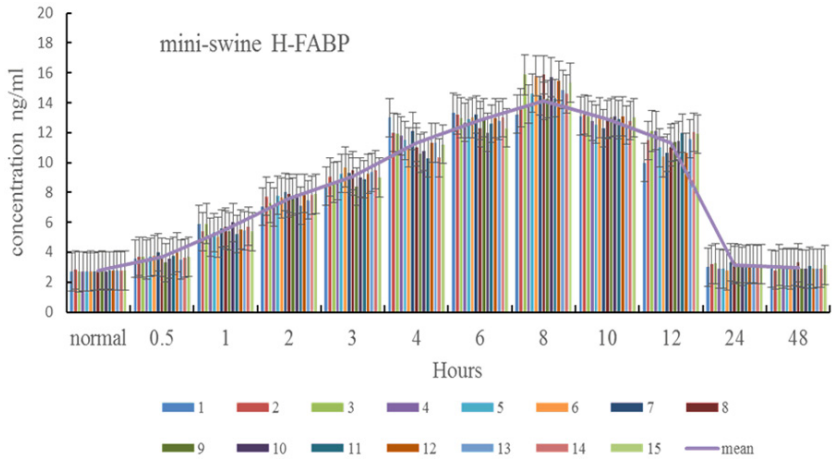

Figure 3. The trend of Serum H-FABP concentration before and after AMI of Chinese mini-swine.

very limited, so AMI can be modeled stably with short modeling cycle and good repeatability. The femoral artery of swine is thick enough to accommodate 6F arterial sheath [7], and the anatomy of the coronary artery in swine is similar to that of human beings. Coronary artery occlusion can be used to simulate AMI that shares many similarities with that in human beings in terms of pathogenesis, thereby enabling the studies on pathophysiology, treatment and drug intervention effects in AMI.

H-FABP is a low-molecular-weight soluble protein with the greatest abundance in the cytoplasm of myocardial cells [8, 9]. Consisting of 132 amino acids, H-FABP accounts for about 5% of total soluble proteins in the myocardial cells and participates in fatty acid metabolism [9]. Myocardial cells are very sensitive to hypoxia and ischemia and mobilize fatty acids for energy supply through oxidation at early stage of coronary artery occlusion, leading to a sharp rise of H-FABP in the myocardial cells. Cell membrane damage can occur 0-3 h after ischemia and hypoxia with an increasd permeability and early entry of H-FABP into the blood circulation. When the damage caused by hypoxia and ischemia is irreversible, the cell membrane ruptures, releasing the H-FABP into the intercellular matrix. H-FABP may enter the blood circulatin and then is discharged in the urine. Studies show that H-FABP is a more sensitive and more specific early diagnostic indicator of AMI [10], especially for early diagnosis within 3 h after chest pain. H-FABP enters the blood circulation 1-3 h after the damage of myocardial cells, reaching the peak at 4-6 h and finally falling to the normal level at 24 h [2, 9]. Therefore, the monitoring of plasm H-FABP is very important to early diagnosis of AMI [11, 12].

Busch et al. [13] built AMI model in miniature swine by closed-chest coronary balloon occlusion to evaluate the effect of reperfusion on the infarcted myocardium. It was found that this approach better simulated the onset of AMI compared with open-chest model and the results could be directly applied to human beings. Soon et al. [14-17] built AMI model in miniature swine by coronary artery occlusion using balloon and spring ring. They studied the interventional effect of myocardial regeneration and stem cell transplantation on the infarcted myocardium and also the impact of AMI on the microcirculation. The AMI model in miniature swine prepared by this method was stable and produced repeatable and reliable results. We built AMI model in Chinese mini-swine by coronary artery occlusion using gelfoam. The procedures were easy and caused little injury to the coronary artery. The animals lived long enough for observations.

Alhadi et al. [18, 19] found that the sensitivity of H-FABP was higher than that of troponin and CK-BB at different time points after chest pain in AMI and the negative predictive value was obviously higher than sensitivity. It was believed that H-FABP was a highly sensitive biochemical marker for early diagnosis of AMI, which also provided reference for determining the time of blood flow reconstruction. Kleine AH et al. [20] compared 40 AMI patients with 40 healthy subjects and found that serum H-FABP increased obviously 1 h-5 h after AMI. Normal serum H-FABP was restored at 12-24 h. Given the significant correlation with AMI, serum H-FABP can be used as a sensitive indicator for the confirmation and exclusion of AMI or even for the prediction of reinfarction [21]. However, the time point of the occurrence of AMI cannot be

definitely determined due to large interindividual differences, which may affect the measurements of serum H-FABP, leading to bias. AMI was induced by coronary artery occlusion using gelfoam, and the time point of the occurrence of AMI was determined by DSA with certainty in the present study. Having achieved an accurate control of the infarcted site and a stable infarcted area, the dynamic changes of serum H-FABP after AMI were revealed clearly. It was found that serum H-FABP began to rise at 0.5 h after AMI, peaked at 8 h and declined after 24 h until the normal level was restored. Using this model, the time point of the rise of serum H-FABP and dynamic changes of H-FABP after AMI were determined more reliably, providing reference for early diagnosis of AMI.

Serum H-FABP has not been widely applied as an indicator probably due to the high price of H-FABP ELISA kit, low specificity, complex procedures and a need for combined detection of troponin level and other indicators myocardial necrosis. There is much room for improvement. In order to confirm the findings of the present study, a larger sample size is required for future studies.

Disclosure of conflict of interest

None.

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