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Research Article

Electrocardiogram and Myocardial Enzyme Changes of Acute Myocardial Infarction in Chinese Rural Dogs

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ABSTRACT

This study aims to provide information on the parameter changes in the acute myocardial infarction model of a Chinese Rural Dog. All dogs were fixated in the conscious state and recorded in the prone position. The data were retrospectively analyzed. The P wave interval increased in the first 5 h and then reached the stable phase by the 77th day. T wave interval increased from the 10th to the 12th day, and after 16 days, the T wave interval in the intervention group became lower than that of the control group. Moreover, the amplitude of the T wave peaked at the 10th day and dropped to the preoperative level. The most obvious change of AO was 90 days after surgery. The AMI model of the Chinese Rural Dog was established and the ECG parameters (Pd, Pa, QRSd, QRSa, Td, Ta, PRd, and QTd), and myocardial enzymes (CK, CK-MB, LDH, and AST) changed significantly following AMI.

Key words: Dog, ECG, Six-limb leads, Acute myocardial infarction model

INTRODUCTION

Animal models can replicate some diseases that are not common in clinical settings to provide disease data; moreover, animal models for medical experiments have become important in solving medical problems(Julander and Siddharthan, 2017). In the field of animal models, constructing hardware platforms and developing animal models of various diseases are growing. These have laid the foundation for studying AMI models.

To date, three main methods exist for setting up a model of AMI in dogs: (1) surgical ligation of the left anterior descending coronary artery by using the Jons method, of which the modeling stability is high(Johns and Olson, 1954); (2) micro-embolism ball combined with thrombin powder formulations for coronary artery occlusion, which is a new and effective Modal method with a high success rate, fast recovery, and high survival rate(Jin *et al*., 2017); (3) balloon occlusion method, which makes the model stable and reliable with strong repeatability and easy ischemia-reperfusion(Hideaki *et al*., 2011; Krombach *et al*., 2005). In recent years, a novel model of canine AMI was established by Zhu *et al* using PTCA(Zhu *et al*., 2005); moreover, dogs as experimental animals in cardiovascular disease models have been widely used in studying the cardiovascular physiology conditions pertinent to humans(Bader *et al*., 2000; Jugdutt, 2002). However, the ligation site of Chinese canine AMI model remains to be discussed, that is, the ligation of the left anterior descending coronary artery cannot show the obvious characteristics of AMI, whereas the ligation of the left coronary artery has a positive effect, especially during electrocardiograms (ECG).

Previously, ECG data in Japan was more prevalent than in other countries; however, some differences existed in the basic biological data of different dogs due to germline and breeding environment(Ka-hung, 1989). The broadening of QRSa, small Q wave, towering or inverted T wave, and elevated ST segment are signs of clinical observation of AMI. However, whether these changes occur in dogs has not been explored.

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In the present study, the AMI model was established *via* ligating the left coronary artery in Chinese Rural Dogs. The waveforms of six lead ECG were applied and changes in AO, LA, IVS, LV, LVPW, and EF; the myocardial enzymes of CK-MB, AST, CK, and LDH were analyzed to estimate the changes in the AMI model for cardiac conditions in the biochemical index.

MATERIALS AND METHODS

Reagents and animals

Six healthy, conscious dogs were used for this study. The dogs were situated in a single cage feeding at 20±3°C and humidity 50±20% and provided with complete dog food (Wakduo, China). After fasting for 12 h before operation, intravenous injection of propofol (0.5 ml/kg) for induction of anesthesia was administered in the forearm, and endotracheal intubation was applied through a laryngoscope and connected to the monitor and ventilator with conditions of 101.3 KPa, 2 L/min oxygen flow, and 2% isoflurane to maintain anesthesia.

Experimental animals' grouping

During the experiment, all materials in contact were sterilized by autoclaving and others (plastic products, rubber products) were sanitized with 75% alcohol. The AMI model was established by using surgical methods. Thoracotomy was performed on 6 dogs and divided into preoperative control and postoperative experimental groups. The blood vessel ligated was the left coronary artery.

The animal procedures were approved by the Animal Care and Use Committee of Shandong Agricultural University (Permit number: SDAUA-2017-049) and carried out in accordance with the "Guidelines for Experimental Animals" of the Ministry of Science and Technology (Beijing, China).

Measurement of the ECG

Six limbs leads were applied in this study. ECG was measured once every hour (0–12th h post-operation), once every 2 hours (12–24th h), once every 6 hours (24–72nd h), once every day (4–11th day), once every 2 days (12–32nd day), once every 4 days (33–57th day), and once every 7 days (58–92nd day).

Color echocardiography

The AO, LA, IVS, LV, LVPW, and EF levels were detected by using LOGIQ3 (GE, American general, American). The color Doppler ultrasound was performed to observe the internal structure of heart abnormalities, changes in atrioventricular size, speed, and direction of blood flow, with or without hypertrophy or neoplasm, which can highly influence the heart disease diagnosis.

Pathological sections

The infarction areas were separated and cut into a size of 0.5 × 0.5 × 1 cm, and then fixed with 10% formaldehyde; the transparent agent in the tissue was replaced by paraffin wax; the paraffin wax was sliced with a microtome and the paraffin sections were tiled into water at 40 to 45°C and extended with water tension naturally; the wax was slipped to the middle of the glass slide, and the slide was placed in an incubator at 60 to 65°C for 15–30 minutes; moreover, HE staining, dewaxing, benzene removal, rehydration, dyeing, dehydration, transparent, and sealing were performed. Paraffin sections of the infarcted areas were observed with an Olympus microscope (CX41, Olympus, Japan). The samples of 40 and 90 days post-operation were analyzed.

Statistical analysis

Statistical analysis was performed using SPSS (Version 21.0, SPSS Inc., USA). All values were expressed as mean ± SEM. All measurements were replicated thrice. The differences were considered significant if $P<0.05$ or $P<0.01$. All column charts were drawn by using GraphPad Prism 5.

RESULTS

ECG

As shown in Figure 1A, from 1–3 days after AMI, the ECG changes were found in the five periods of ischemia, ischemic injury, injury infarction, ischemic infarction, and recovery of infarction. The detailed ECG changes are presented in Figure 1B.

Originlab 9.0 was used to describe the postoperative ECG of the 14 groups. All postoperative waves and intervals were significantly different from the controls (Pd, Pa, QRSd, QRSa, Td, Ta, STd, and QTd, $P<0.01$). Moreover, the trends of P wave interval, T wave interval, T wave amplitude, PR interval, and QT interval were the same: The P wave interval increased in the first 5 h and reached the stable phase at the 77th day. The T wave interval increased from the 10th to 12th day, and after the 16th day, the T wave interval was lower than control. The amplitude of the T wave peaked on the 10th day and dropped to the preoperative level. Moreover, the PR interval and QT interval remained high within 5 h, and then the PR interval was maintained at 0.05–0.06 s, and the QT interval was maintained at 0.1–0.13 s (Figure 2).

CK, CK-MB, AST and LDH levels

GraphPad Prism 5 was used to characterize the CK, CK-MB, AST, and LDH levels in the 14 groups (Figure 3). Compared with those in the control group, all 4 myocardial enzymes changed significantly after AMI. Moreover, AST rose at the 6th hour and was steady until the 72nd hour; whereas the LDH and CK-MB levels rose immediately after AMI and reached a high level at approximately 12 hours, and from the 24th or 48th hour they began to decline back to normal. Furthermore, CK rose sharply from the 1st hour after AMI, peaked at the 6th hour, and then went down gradually but remained higher than normal.

Color doppler ultrasound parameters

Color doppler ultrasound was carried out to observe the internal structure of the abnormal heart. The results showed that, 90 days after operation, the heart changed with weakened left ventricular walls, ventricular septal abnormalities, and had no ventricular enlargement and tumors (Figure 4).

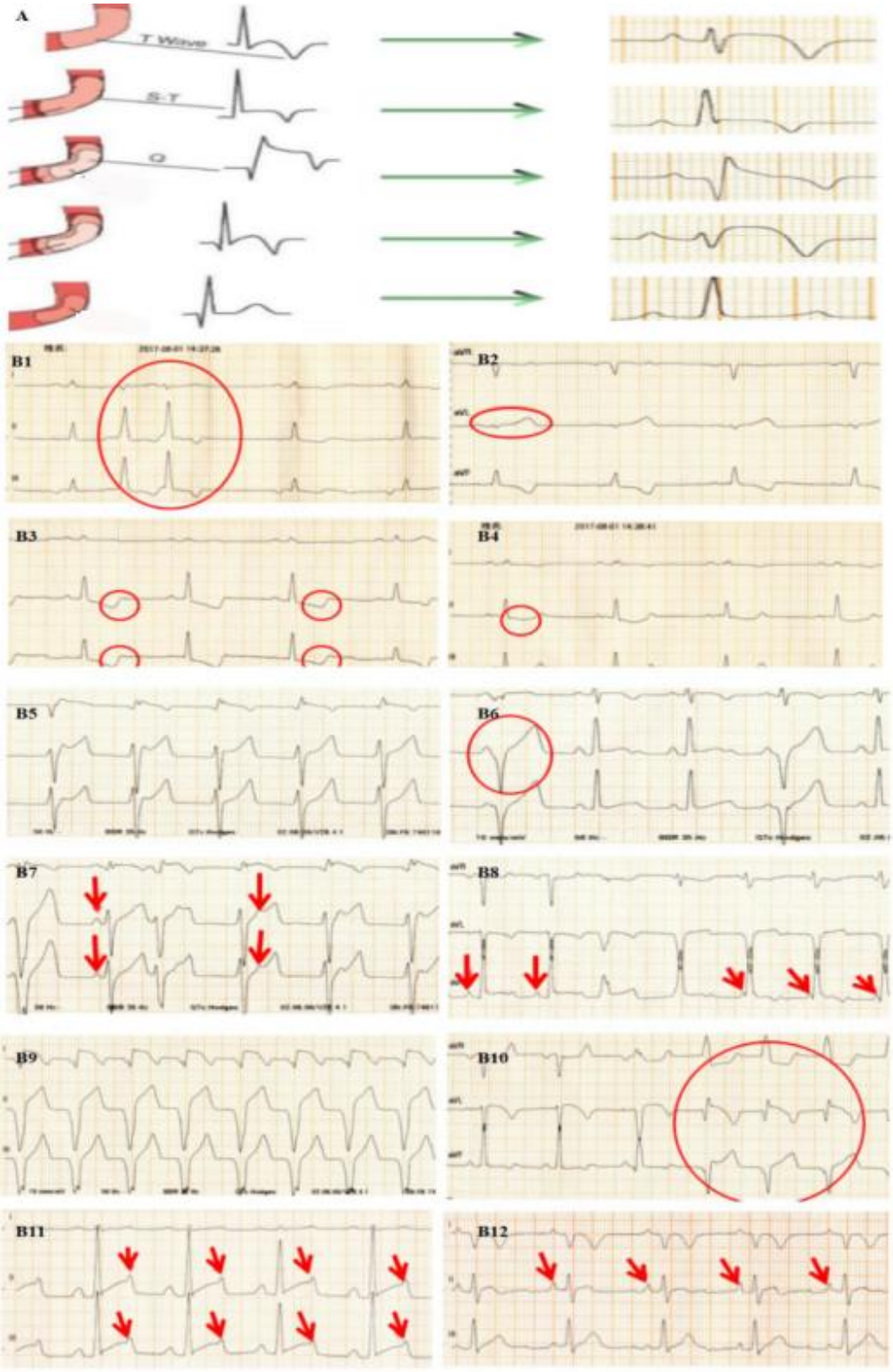
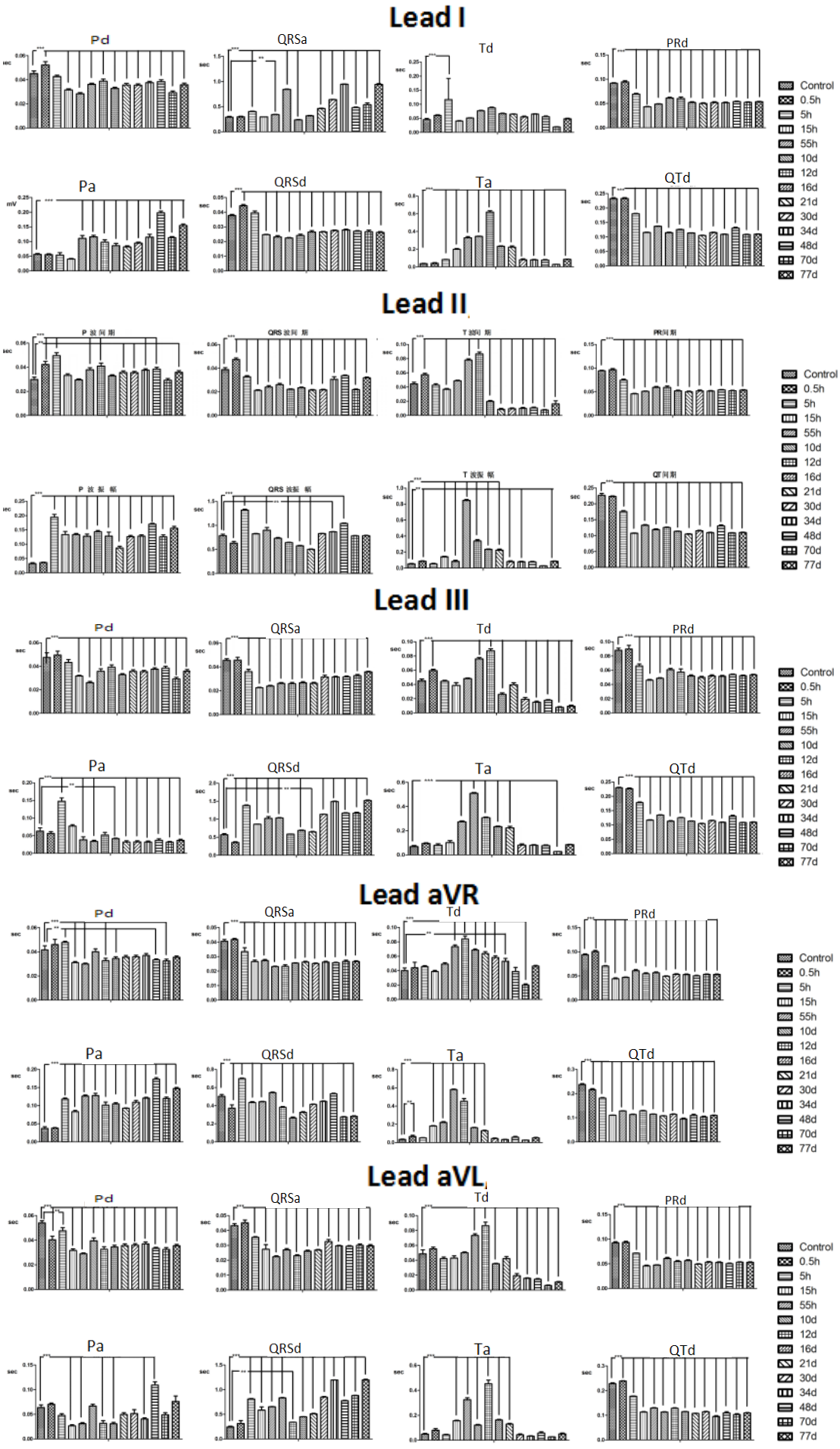


Fig. 1: ECG changes within three days of AMI. Five abnormal period appeared after AMI. In the ischemic period, T wave began to reverse; in the ischemic injury period, the "J" began to go up, with ST segment above the equipotential line; in the injury infarction period, QRS wave widened, the speed of ventricular depolarization slowed down; when the ischemic infarction period happened, Q waves significantly deepened; and when the infarct recovery phase happened, T wave's direction returned to normal. (B1) Half an hour after surgery, two consecutive VPC named "dual law" appeared. (B2) ST segment of leads II and III declined slightly. (B3) Forty minutes after operation, ST segment of leads II and III declined obviously. (B4) ST segment of aVL lead elevated. (B5) Ten hours after surgery, occasional accelerated ventricular self-rhythm appeared. (B6) Then, multiple single VPC appeared. (B7) After 11 hours, III degree atrioventricular block appeared, P wave joined together with QRS wave, the atrial excitation cannot be transmitted to the ventricular. (B8) After 12 hours, the VPC of aVF appeared, P wave and QRS wave were separated, showing ventricular self-rhythm. (B9) After 17 hours, paroxysmal sustained ventricular tachycardia became normal, HR reached to 100 b/min. (B10) After 21 hours, temporary VT appeared. (B11) The next day, AMI began to affect the right coronary branch, showing steeple-crowned T wave in leads II, III, aVF, aVL. (B12) The amplitude of P wave in lead II was higher than one third of QRS wave, suggesting pulmonary P wave, which may be related to the transient increase or decrease of right atrial load.


Lead I
Pd
QRSa
Td
PRd
Pa
QRSd
Ta
QTd
Lead II
Lead III
Lead aVR
Lead aVL
Control
0.5h
5h
15h
55h
10d
12d
16d
21d
30d
34d
48d
70d
77d

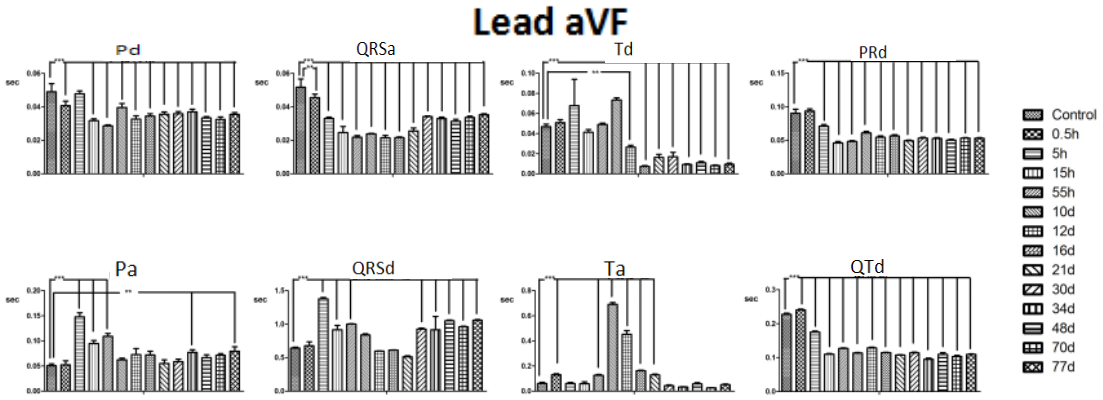


Fig. 2: ECG changes within ninety days of AMI. From the 2D histogram, it is clear that P wave interval, T wave interval, T wave amplitude, PR interval and QT interval in the six leads have the same tendency over the 90 days after AMI. The P wave interval increased in the first 5 h and then reached the stable phase to 77th day. The T wave interval increased from 10th day to 12th day, and after 16th day, the T wave interval was lower than control. The amplitude of T wave peaked on the 10th day and then dropped to the preoperative level.

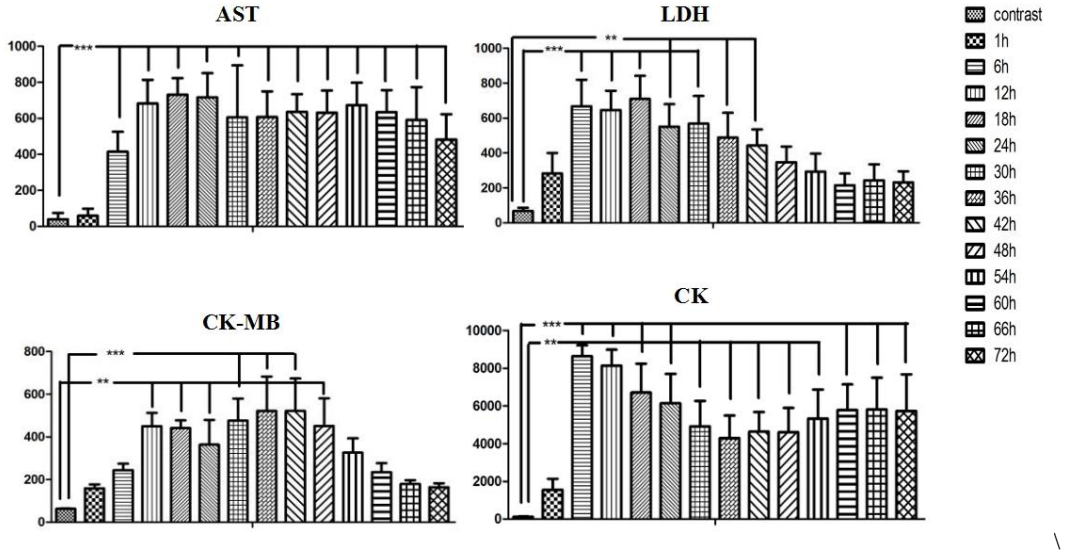


Fig. 3: Changes of CK, CK-MB, AST and LDH within three days after AMI. Myocardial enzymes in myocardial injury or necrosis increased to varying degrees, AST rose at the 6th hour and then kept steady until the 72nd hour. Whereas for LDH and CK-MB, they all rose immediately after AMI and reached a high level at about 12 hours, from 24th or 48th hour they began to decline to normal. With regard to CK, it rose sharply from 1st hour after AMI, reached to the peak at 6th hour, and then went down gradually but remained higher than normal. The units used was IU/L.

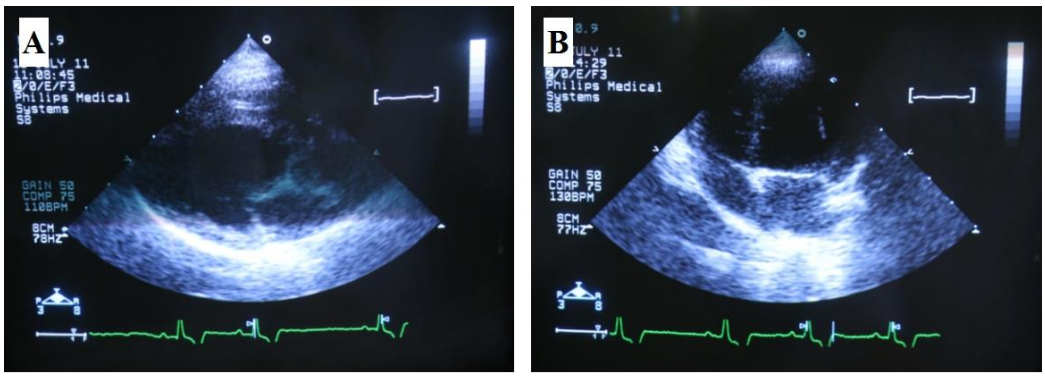


Fig. 4: Changes of color doppler ultrasound parameters at 90th day after AMI. Preoperative: AOD=12, LA=18.3, IVS=4.8, LVD=22**, LVPW=5.4, EF value=71.5%. Postoperative: AOD =14, LA =18, IVS =4.5, LVD=33**, LVPW =4.2, EF value =72%

Histopathology

For the 40-day post-operation sample, a large number of inflammatory cells infiltration, small pieces of fibrosis, and vascular wall thickening mainly appeared in the infarcted area, accompanied with granulation tissue; moreover, the interstitial appearance manifested with glass-like changes. Moreover, 90 days after operation, organization, focal calcification, and scarring in the myocardium appeared, accompanied with large areas of adipose tissue hyperplasia (Figure 5).

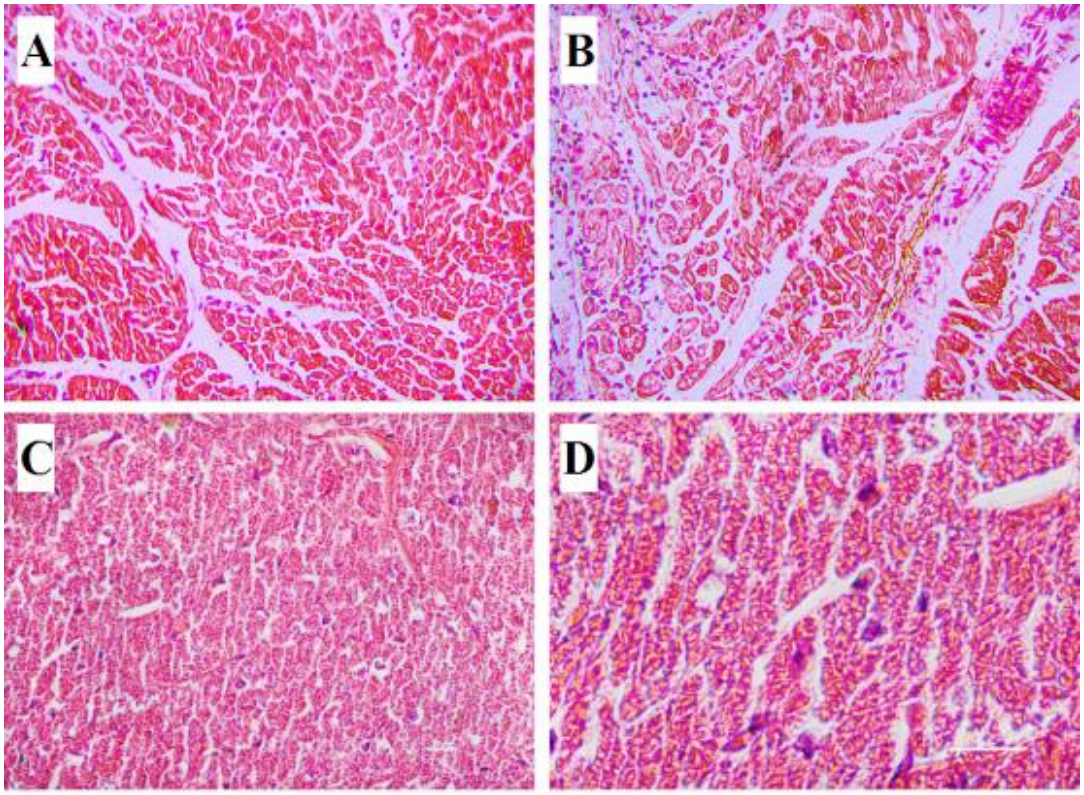


Fig. 5: Pathological changes in the myocardial infarction area. A is normal myocardium; B is 40th day *post*-operation, C and D are 90th day *post*-operation. There mainly appearing with a large number of inflammatory cells infiltration, small pieces of fibrosis and vascular wall thickening in the infarcted area, accompanied with granulation tissue; interstitial appearance appeared Glass-like changes. Whereas 90 days after operation, there occurred organization, focal calcification, scarring in the myocardium, accompanied with large areas of adipose tissue hyperplasia.

DISCUSSION

ECG, as an important means of heart examination, is crucial in acquiring the AMI model data. Distinct from previous descriptions, Wen *et al* established a model of AMI in dogs by using a gelatin sponge and tested the trend of enzyme activity and ECG in the serum within 24 hours(Wen *et al*., 2010). Zhang *et al*. found that resveratrol injection has significant protective effects on the hearts of the dogs with AMI(Zhang *et al*., 2006). By comparing the changes of QT and RR intervals in the 24 short-term ECG before and after AMI, Piccirillo *et al* obtained the variability index (QTeVI, QTpVI and TeVI) of QTe (from Q wave to T wave end), QTp (from Q wave to T wave peak) and Te (from T wave peak to T Wavefront)(Gianfranco *et al*., 2014). However, to obtain the more detailed information, we examined the real-time changes of ECG within 3 days of AMI and compared the differences in the ECG waveforms between the 6 limb leads over a 90 day period in the current study.

Changes in myocardial enzymes are highly significant in the diagnosis of AMI. Lei *et al*(Lei *et al*., 1995) first found that myocardial enzymes increased in the AMI model and then decreased within 0–60 h; however, they did not subdivide the types of myocardial enzymes. In the current study, the changes in the myocardial enzymes CK-MB, AST, CK, and LDH within 0–72 h were evaluated, in which LDH and CK-MB were consistent with the previous conclusion, and showed a trend of initially rising and then decreasing; however, CK and AST showed the same trend with LDH and CK-MB within the first 0–36 h, and then went into a stable period.

Two-dimensional echocardiography combined with Doppler and color Doppler flow imaging is now the preferred technique for the early diagnosis and assessment of complications in "surgical" AMI and may be of importance in AMI models, with regard to atrial and ventricular changes for visual inspection. Color Doppler ultrasound is performed to observe the internal structures of heart abnormalities; moreover, Nicklas *et al* detected local morphological changes after acute transmural AMI by using ultrasonography in dogs(Nicklas *et al*., 1982); additionally, Harrison used color Doppler to detect mechanical complications, such as primary pump failure and ventricular septal rupture, to distinguish left ventricular real aneurysm and pseudoaneurysm(Harrison *et al*., 1989). In the present study, the heart at 90 days post-operation was assessed, and we found that the left ventricular wall weakened significantly, the ventricular septum showed abnormalities, and the ventricle did not show enlargement and vegetation; moreover, the most significant change was seen in the LV diameter, from 22 to 33 mm.

The infarcted area was pathologically examined. Chu Jun *et al* found myocardial fibers that were degenerative and necrotic in the infarcted area in dogs with AMI. Moreover, the fibers of the necrotic myocardium were swollen, ruptured, and homogenized; furthermore, the longitudinal stripes disappeared, bleeding was seen in the infarcted area, hemosiderin was sedimentary and

phagocytic cells showed infiltration (Chu Jun *et al*., 2002). Similar changes were found in our result.

Conclusions

The Chinese Rural Dog of AMI model was established in our study, and it showed that the ECG parameters (Pd, Pa, QRSd, QRSa, Td, Ta, PRd, and QTd), and myocardial enzymes (CK, CK-MB, LDH and AST) may be associated with AMI.

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