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Full Length Research Paper

A comparison of efficacy between conventional and modified methods of the chronic myocardial ischemia/reperfusion model

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The objective of this study is to develop and compare the efficacy of a modified versus conventional rat model of chronic myocardial ischemia/reperfusion. Sixty Sprague Dawley (SD) rats were randomly divided into two groups, a modified group (mask respiratory support and short-time chest-opening) and a conventional group (tracheal intubation and long-time chest-opening). Operation time, surgical success rate, survival rate and infarct size were investigated. In addition, the post-operative living state of the rats was observed. In the perioperative period, the surgical success rate was greater in the modified model ($P < 0.05$ vs. conventional model). Both chest-opening time and spontaneous respiration recovery time were significantly shorter in the modified group versus the conventional model ($P < 0.001$). Postoperative resumption of normal behaviors and activities was quicker in the modified surgical group, which demonstrated a statistically significant mortality benefit compared to the conventional group ($P < 0.001$). Infarct size, assessed via triphenyltetrazolium chloride staining, was without statistical difference between the 2 groups ($P > 0.05$). The modified method offers advantages of simplicity, efficiency and independent conduct. Its employment enhances the success rate of the chronic rat myocardial ischemia/reperfusion model.

Key words: Rat, chronic, ischemia/reperfusion model, modified.

Background

Extensive animal research on experimental myocardial infarction (MI) has yielded a large body of knowledge regarding pathophysiology, potential treatment strategies and relevant pharmacological interventions applicable for acute and chronic myocardial ischemia and infarction (Kloner and Braunwald, 1980; Verdouw and Den et al., 1998). Clinical coronary artery thrombosis with subsequent thrombolytic-induced reperfusion is often simulated in the research animal model by a predetermined period of coronary artery ligation with reperfusion initiated by ligature release. Conventional animal MI studies utilize open-chest models with direct surgical coronary artery ligation requiring long perioperative periods, oral intubation with respiratory assistant devices, prolonged anesthesia and chest cavity exposure, resulting in epicardial cooling and exsiccation (Grund et al., 1998). As a result, conventional animals models of ischemia/reperfusion injury continue to suffer high mortality and low success rates. Futhermore, the conventional MI model does not approximate the clinical myocardial infarction event in all aspects. Clinically, patients are not anesthetized at the time of infarct, nor do they undergo thoracotomy, which are factors cited to influence infarct size, as well as local metabolic and inflammatory response (Duncker et al., 1996; Schwartz et al., 1997; Hale et al., 1997).

The aim of the present study was to establish a modified rat model of myocardial ischemia/reperfusion. Relying on a mask delivery method of anesthesia for rapid induction within minutes, swift thorax closure after

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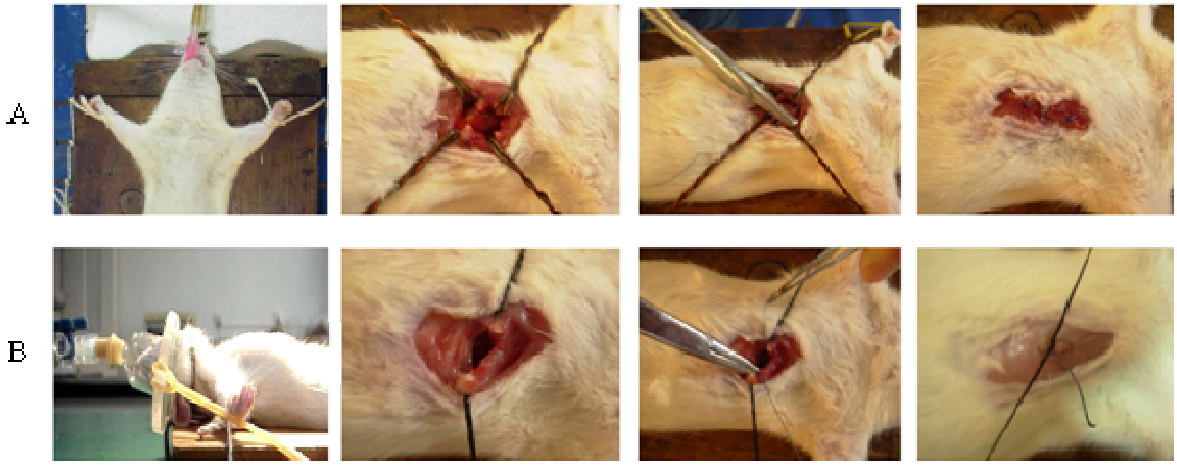

Figure 1. Surgical procedure. A. Conventional operation procedure with tracheal intubation. B. Modified operation procedure with respiratory mask of original design.

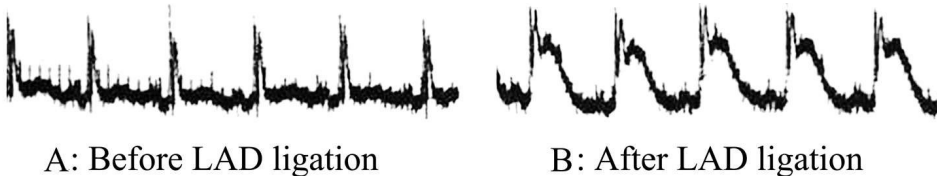

Figure 2. ECG changes before and after coronary ligation. A. Normal ECG before left anterior coronary artery ligation. B. Ambulatory change and J-point elevation immediately after ligation of the coronary artery.

coronary slip-knot ligation and reperfusion initiation via slipknot release while the animal is conscious was done. Our modified model more closely approximates the clinical ischemia/reperfusion process and we present data evaluating its efficacy compared to the conventional MI/R model.

MATERIALS AND METHODS

Animals

Male Sprague Dawley (SD) rats (*n* = 60), each weighing 180 - 200 g, were obtained from the experimental animal center of the Fourth Military Medical University. The animals were randomly allocated into either the modified or conventional MI/R model groups. All experiments were performed in accordance with the National Institutes of Health Guidelines on the use of laboratory animals and were approved by the Fourth Military Medical University Committee on animal care.

Surgical protocol

Rats in the conventional group were anesthetized with 1% pentobarbital sodium solution (60 mg/kg, intraperitoneal injection) and secured in the supine position with cords binding the upper jaw and four extremities (Figure 1A). Tracheal intubation via 24 G IV catheter ensued, with rodent respirator ventilation (Medical Instrument Corporation of Zhejiang University, China) at 120 breaths/min, tidal volume 1mL/breath. Intubation was successful if the respiratory rhythm coincided with that of the artificial respirator. The left chest was shaved and disinfected with 75% ethanol, and skin along the lateral left sternal border was delicately dissected via a 3 cm incision. Anterior left thoracotomy through the fourth intercostal space was performed (Figure 1A). Subcutaneous tissues were detached along the inferior fringe of the left pectoralis major muscles and the right fourth and/or third ribs were dissected to expose the heart. A 6–0 silk suture, threaded through a minute sectioned plastic tube, was placed approximately 2 - 3 mm from the origin of the left anterior descending coronary artery (LAD). After 5 min of stabilization, myocardial ischemia was initiated by clamping the plastic tube against the cardiac surface. After 30 min of ischemia, the snare was released and coronary perfusion was restored for 72 h. Thorax closure was performed layer by layer and tracheal extubation occurred as spontaneous respiratory activity resumed.

In modified MI/R surgery group, rats were anesthetically induced with inhaled 5% isoflurane (Ohmeda, Liberty Corner, NJ) and maintained with 2% isoflurane via a special respiratory mask of original design (Figure 1B). Myocardial ischemia was produced by a swift method of temporary heart exteriorization via a left thoracic incision, placing a 6-0 silk suture slipknot around the LAD artery approximately 2-3 mm from its origin and closing the thorax with a pre-placed thoracic cavity purse string suture while simultaneously evacuating any intrathoracic air. The entire procedure occurred in less than 3 min. Inhaled anesthesia was withdrawn and the animals recovered within 3 min after surgery completition. After 30 min of ischemia, the slipknot was released, reperfusing the myocardium (Liang et al., 2004).

Successful coronary occlusion and reperfusion was verified by typical electrocardiogram (ECG) changes (Figure 2).

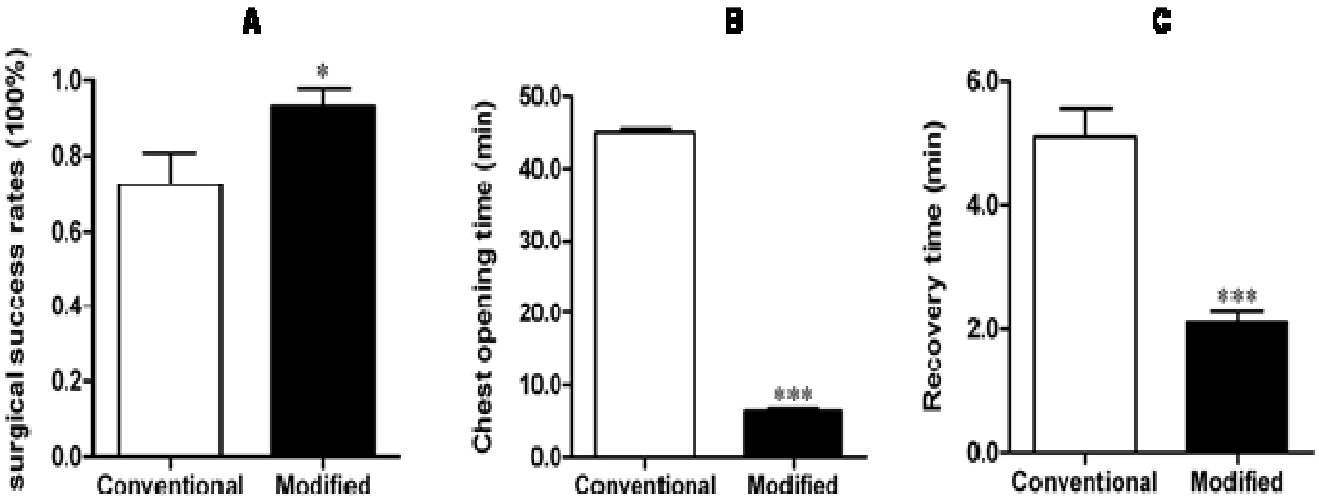

Figure 3. Surgical success rates and length of operation times, as noted. A. Surgical success rates. B. Chest-opening time. C. Time needed for spontaneous respiratory recovery. All data showed as means ± S.E.M. *P < 0.05, ****P* < 0.001, n = 30.

Time calculation

To compare the efficiency of the 2 methods, procedure time was recorded and analyzed in 2 separate periods:

1) Time from skin incision to thorax closure
2) Time for spontaneous respiration recovery.

Arterial oxygen saturation measurement

Arterial oxygen saturation data was acquired via the non-invasive Mouseox system (Starr Life Sciences Inc, USA), utilizing a sensor clip placed on the hind limb or paw. The arterial oxygen saturation was recorded immediately after thoracotomy and concluded after 1 h reperfusion.

Determination of myocardial infarction

To determine myocardial infarct size (IS), all surviving rats at the conclusion of the 72 h reperfusion period were anesthetized and underwent thoracotomy. The LAD was re-tightened, and 3 ml/kg of 1% Evans blue was perfused to stain the area at risk (AAR) via clamped aortic injection. Upon removal, each heart was transversely sectioned into 4 - 5 slices, each of 1 - 2 mm thickness. The Evans blue solution stained the perfused myocardium, leaving the ischemic vascular bed (AAR) uncolored. AAR was calculated as a percentage of total LV tissue (colored non-ischemic tissue and non-colored AAR combined). To distinguish between viable ischemic and dead infarcted tissue, slices were incubated with TTC (10 mg/ml, 20 min at 37°C. TTC stained viable, normal myocardium (with intact dehydrogenase enzyme systems) dark red, while areas of necrosis (lacking dehydrogenase activity) did not stain and color analysis was performed using Optimas 2.0 software. IS was then calculated and expressed as percentage of AAR (Liu et al., 2007).

Survival rates

Upon spontaneous respiration, rats were given food and water *ad libitum*. Any deaths and time of occurrence were recorded. Rat activity was monitored throughout the 72 h postoperative period.

Statistical analysis

All data were expressed as means ± S.E.M. Statistical software Graph pad Prism 5.0 was used and all values were analyzed using t-test. Differences were considered statistically significant if P < 0.05.

RESULTS

Success rates and procedure times

Regarding anesthetic complications, one rat in the conventional surgical group overdosed on the anesthesia, resulting in a 96.6% (n = 29) anesthesia success rate. There were no complications in the modified surgical group utilizing facemask ventilation (100%, n = 30 anesthesia success rate). Success in LAD ligature and creating ischemia was defined as J-point elevation in the ECG within a 24 h period postoperatively. A significantly increased surgical success rate was observed in the modified group, as indicated by a 20.9% difference (93.3 vs. 72.4%, P < 0.05) (Figure 3A). The length of surgical chest-opening time was significantly shorter in the modified method group (6.5 ± 2.0 min vs conventional 44.9 ± 2.7 min, P < 0.001) (Figure 3B) and the time needed for spontaneous respiratory recovery was also decreased in the modified surgercial group (2.1 ± 0.9 min vs. 5.1 ± 2.5 min, P < 0.001) (Figure 3C).

Arterial oxygen saturation

Arterial oxygen data revealed during the ischemia period to experiment conclusion, the modified surgical model achieved greater sustained arterial oxygen saturation when compared to the conventional surgical MI/R model (Figure 4). This differential may be attributed to both swift

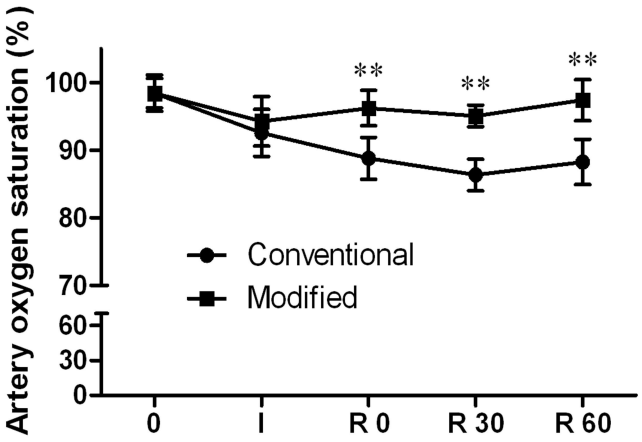

Figure 4. Oxygen saturation levels in remote arterial blood. Data were showed as means ± SEM. X axis labels: I for ischemia and R for reperfusion. **P < 0.01, n = 28.

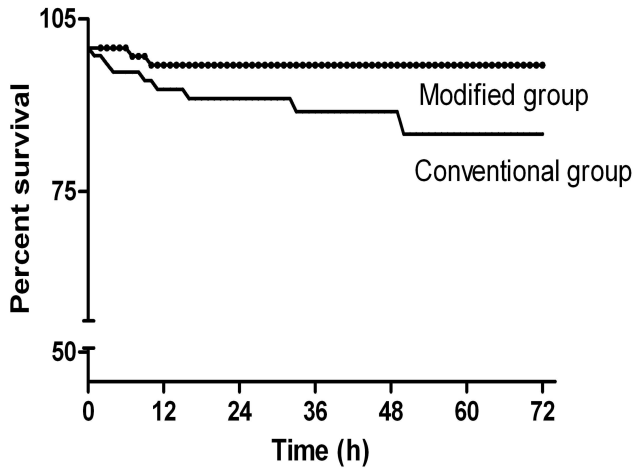

Figure 5. Survival rates over the course of 72 h post-operation. Data showed as means ± S.E.M.

thorax closure and spontaneous respiratory recovery in the modified surgical model.

Post-operative states and survival rate

Of the 28 rats randomized to the conventional surgery group, three died within 3 h post-operation, two died within 6 h, 2 died within 12 h, one died of self-inflicted scratching wounds within 24 h and 2 more rats died within 72 h (Figure 5 survival curve). Of the 30 rats randomized to the modified surgical group, three died within 3 h and two died within 12 h (Figure 5 survival curve). Of the surviving rats of either group, all performed activities of daily living 30 min after spontaneous recovery from anesthesia without further respiratory assistance. However, rats in the conventional group displayed varying

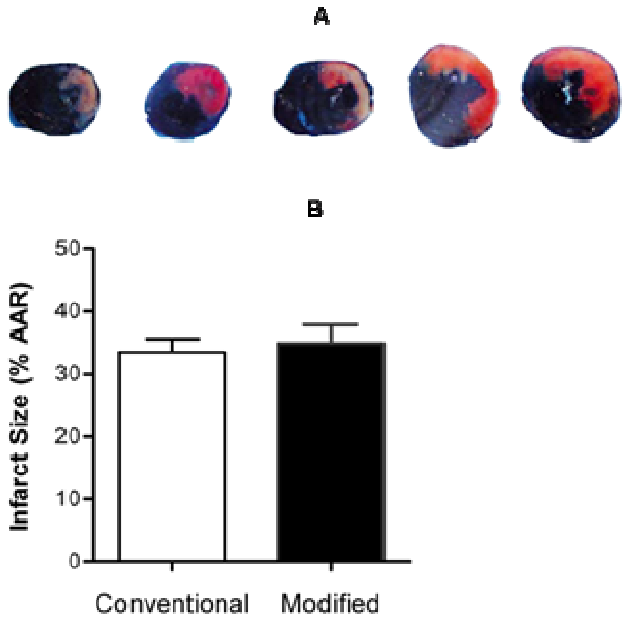

Figure 6. Infarct size after 30 min ischemia after 24 h reperfusion. A. Dark blue is non-ischemia non-occluded cardiac region; dark red is ischemic but viable cardiac region; white is infracted dead tissue. B. Infarct size analysis between the conventional versus modified surgery groups. No statistically significant difference found, P > 0.05 n = 18.

degrees of decreased appetite, decreased activity level, poor grooming behavior and restlessness.

Infarct size

Figure 6A demonstrates various heart cross sections from both study groups. Dark blue stained regions represent non-ischemia, non-occluded regions and dark red regions represent ischemic but viable regions of cardiac tissue and the white areas indicate infracted dead tissue. Colorimetric analysis and comparison of different cardiac tissue regions was performed using Optimas 2.0 software. No significant difference was found in the infarct parameter AAR/LV between groups (data not shown). The infarct area was similar between the conventional and modified surgical group (Figure 6B).

DISCUSSION

A satisfactory research model simulating a physiologic process must have reproducibility and accuracy; its advantages and limitations must be understood so that applicability to the clinical situation can be maximized. The conventional thoracotomy method of inducing ischemia/reperfusion offers the advantages of open access to the heart, direct visualization of successful coronary artery occlusion and ability to measure local

contractile performance (Li et al., 2008; Su et al., 2007). However, airway injury and low success rate limit the method's application in the chronic ischemia/reperfusion model (Zhao et al., 2006).

In the modified myocardial ischemia/reperfusion model, anesthetic induction is achieved using isoflurane, a halogenated volatile agent maintaining general anesthesia by central nervous system depression. Rats can be easily anesthetized in shorter time. Maintenance of anesthesia can be easily adjusted by volume control through a respiratory mask, which mixes room air with isoflurane, causing no harm to the rat. In sharp contrast to the conventional model, this method of anesthetic delivery allows rapid recovery after mask removal. The mask device of original design developed in this study is cost-effective, easy to fabricate and reproduce from readily available components, simple to use and safe. Atop these advantages, the operator of the device needs no special technical training and the learning curve for proficiency is short.

LAD occlusion in small rodents has been proved to be a good model for myocardial ischemia research (Yu et al., 2008;Toyota et al., 2007). However, the conventional ischemia/reperfusion model requires lengthy thoracotomy and aesthesia for the duration of the ischemia period. As a result, animals are at risk for significant hypothermia, if their body temperature is not carefully controlled. In the modified surgical model, animals undergo both thoracotomy and anesthesia for less than 5 min duration, spending the majority of the ischemia period (> 25 min) conscious, with an intact thoracic cavity. The risk of temperature imbalance in the modified surgical model is much less than in the conventional model, better imitating physiological ischemia/reperfusion circumstances.

Though the modified model had limitations (due to slip knot malfunction, two rats had to undergo re-thoractomy to initiate reperfusion), prolonged surgical time and internal organ manipulation translated to lower mean survival rate post-operatively in the conventional MI/R technique group. For a chronic MI/R investigative study, conventional techniques are simply not suitable. The modified surgical model, by avoiding endotracheal intubation and causing only minimal chest-opening time, resulted in speedy recovery from anesthesia to resume spontaneous behavior and activities such as feeding.

In conclusion, the modified surgical technique can be practiced with ease in a laboratory with few additional resources, a high success rate and tools that are easily manipulated and independently mastered. Moreover, the modified model is not only applicable to the establishment of acute MI/R models, but also provides a means for the establishment of chronic infarction/reperfusion models in the rat.

ACKNOWLEDGMENT

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REFERENCES

Kloner RA, Braunwald E (1980). Observations on experimental myocardial ischaemia. Cardiovasc Res. 14(7): 371-395.

Verdouw PD, Den Doel MA V, De Zeeuw S, Duncker DJ (1998). Animal models in the study of myocardial ischaemia and ischaemic syndromes. Cardiovasc Res. 39(1): 121-35.

Grund F, Sommerschild HT, Kirkeboen KA, Ilebekk A (1998). A new approach to normalize myocardial temperature in the open-chest pig model. J. Appl. Physiol. 84(6): 2190-2197.

Duncker DJ, Klassen CL, Ishibashi Y, Herrlinger SH, Pavek TJ, Bache RJ (1996). Effect of temperature on myocardial infarction in swine. Am. J. Physiol. 270(4 PT 2): H1189-1199.

Schwartz LM, Verbinski SG, Vander H, Reimer KA (1997). Epicardial temperature is a major predictor of myocardial infarct size in dogs. J. Mol. Cell Cardiol. 29(6): 1577-1583.

Hale SL, Dave RH, Kloner RA (1997). Regional hypothermia reduces myocardial necrosis even when instituted after the onset of ischemia. Basic Res. Cardiol. 92(5): 351-357.

Liang F, Gao E, Tao L, Liu H, Qu Y, Christopher TA, Lopez BL, Ma XL (2004). Critical timing of L-arginine treatment in post-ischemic myocardial apoptosis-role of nos isoforms. Cardiovasc Res. 62(3): 568-577.

Liu HT, Zhang HF, Si R, Zhang QJ, Zhang KR, Guo WY, Wang HC, Gao F (2007). Insulin protects isolated hearts from ischemia/reperfusion injury: cross-talk between pi3-K/akt and jnks. Sheng Li Xue Bao. 59(5): 651-659.

Li J, Zhang H, Wu F, Nan Y, Ma H, Guo W, Wang H, Ren J, Das UN, Gao F (2008). Insulin inhibits tumor necrosis factor-alpha induction in myocardial ischemia/reperfusion: role of akt and endothelial nitric oxide synthase phosphorylation. Crit. Care Med. 36(5): 1551-1558.

Su H, Sun X, Ma H, Zhang HF, Yu QJ, Huang C, Wang XM, Luan RH, Jia GL, Wang HC, Gao F (2007). Acute hyperglycemia exacerbates myocardial ischemia/reperfusion injury and blunts cardioprotective effect of gik. Am. J. Physiol. Endocrinol. Metab. 293(3): E629-635.

Zhao X, Wu N, Zhou J, Yang Y, Fang Y, Cheng W, Ma R, Tian Y, Huang L (2006). A technique for retrograde intubation in mice. Lab. Anim. (NY). 35(3): 39-42.

Yu QJ, Si R, Zhou N, Zhang HF, Guo WY, Wang HC, Gao F (2008). Insulin inhibits beta-adrenergic action in ischemic/reperfused heart: A novel mechanism of insulin in cardioprotection. Apoptosis, 13(2): 305-317.

Toyota E, Kawaguchi Y, Ogasawara Y, Watanabe N, Neishi Y, Kawamoto T, Okura H, Yoshida K (2007). Novel rat model of ischemic cardiomyopathy induced by repetitive myocardial ischemia/ reperfusion injury while conscious. Circ J. 71(5): 788-795.