Cardiopulmonary resuscitation in a rat model of chronic myocardial ischemia

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Fang, Xiangshao, Wanchun Tang, Shijie Sun, Lei Huang, Yun-Te Chang, Zitong Huang, and Max Harry Weil. Cardiopulmonary resuscitation in a rat model of chronic myocardial ischemia. J Appl Physiol 101: 1091–1096, 2006. First published June 22, 2006; doi:10.1152/japplphysiol.01487.2005.—Our group has developed a rat model of cardiac arrest and cardiopulmonary resuscitation (CPR). However, the current rat model uses healthy adult animals. In an effort to more closely reproduce the event of cardiac arrest and CPR in humans with chronic coronary disease, a rat model of coronary artery constriction was investigated during cardiac arrest and CPR. Left coronary artery constriction was induced surgically in anesthetized, mechanically ventilated Sprague-Dawley rats. Echocardiography was used to measure global cardiac performance before surgery and 4 wk postsurgery. Coronary constriction provoked significant decreases in ejection fraction, increases in left ventricular end-diastolic volume, and increases left ventricular end-systolic volume at 4 wk postintervention, just before induction of ventricular fibrillation (VF). After 6 min of untreated VF, CPR was initiated on three groups: 1) coronary artery constriction group; 2) sham-operated group, and 3) control group (without preceding surgery). Defibrillation was attempted after 6 min of CPR. All the animals were resuscitated. Postresuscitation myocardial function as measured by rate of left ventricular pressure increase at 40 mmHg and the rate of left ventricular pressure decline was more significantly impaired and left ventricular end-diastolic pressure was greater in the coronary artery constriction group compared with the sham-operated group and the control group. There were no differences in the total shock energy required for successful resuscitation and duration of survival among the groups. In summary, this rat model of chronic myocardial ischemia was associated with ventricular remodeling and left ventricular myocardial dysfunction 4 wk postintervention and subsequently with severe postresuscitation myocardial dysfunction. This model would suggest further clinically relevant investigation on cardiac arrest and CPR.

Sprague-Dawley rat; cardiac arrest; ischemic heart disease

MATERIALS AND METHODS

This protocol was approved by the Institutional Animal Care and Use Committee of the Weil Institute of Critical Care Medicine. All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication 0-309-05337-3, Revised 1996). The Weil Institute of Critical Care Medicine is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Study design. Experiments were carried out in male Sprague-Dawley rats (body wt, 450–550 g). The experimental procedure is diagrammed in Fig. 1. In the first stage, we developed a rat model of chronic myocardial ischemia. In the second stage for investigation of CPR, we measured the alteration of myocardial function 4 wk after surgical intervention, and subsequently resuscitability, postresuscitation myocardial dysfunction, and duration of postresuscitation survival. Three groups totaling 15 animals were included in this study: 1) a coronary artery constriction group, in which animals underwent left coronary artery constriction 4 wk before the induction of ventricular fibrillation (VF); 2) a sham-operated group, in which animals received a sham operation without coronary artery constriction 4 wk before the induction of VF; and 3) a control group, in which no surgery was performed before induction of VF.

Coronary artery constriction. Rats underwent left coronary artery constriction using techniques similar to those previously described by Capasso and colleagues (1, 2, 6, 7) with the modification of using a probe 350 μm in diameter instead of 275 μm. The animals were anesthetized by intraperitoneal injection of pentobarbital sodium (45 mg/kg). The trachea was orally intubated with a 14-gauge cannula mounted on a blunt needle with a 145° angled tip (Abbocath-T, Abbott Hospital, North Chicago, IL) as previously described (18–20).

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The animals were mechanically ventilated with room air. Electrocardiogram (ECG) lead II was continuously monitored. After measurements of baseline myocardial function using noninvasive transthoracic echocardiography were obtained, a thoracotomy via the third left intercostal space was performed. The atrial appendage was elevated, the left coronary artery was located, and a suture was positioned around the vessel near its origin. Subsequently, a probe 350 μm in diameter was held in contact with the wall of the exposed coronary artery. The entire vessel and the probe were ligated, and the probe was quickly removed to allow expansion of the vessel and avoid complete occlusion. The chest was closed, and the animals were allowed to recover in separate cages for 4 wk. Postoperative pain was controlled by giving subcutaneous injections of the analgesic ketorolac (0.4 mg/kg). Successful constriction of left coronary artery was characterized by ECG changes consisting of ST segment elevation immediately after surgery. The Sprague-Dawley rats were compared with sham-operated rats, which were subjected to the same procedure except that the ligation around the coronary artery was not tied, and control rats, which were subjected to the anesthesia and echocardiographic measurements without any surgery.

Animal preparation for CPR. Four weeks after surgical intervention, the animals were again studied. The Sprague-Dawley rats were fasted overnight but received free access to water. The animals were anesthetized by an injection of 45 mg/kg ip pentobarbital sodium. Additional doses of 10 mg/kg were administered at hourly intervals but not within 30 min preceding the onset of cardiac arrest. The chest was closed, and the animals were allowed to recover in separate cages for 4 wk. Postoperative pain was controlled by giving subcutaneous injections of the analgesic ketorolac (0.4 mg/kg). Successful constriction of left coronary artery was characterized by ECG changes consisting of ST segment elevation immediately after surgery. These ischemic rats were compared with sham-operated rats, which were subjected to the same procedure except that the ligation around the coronary artery was not tied, and control rats, which were subjected to the anesthesia and echocardiographic measurements without any surgery.

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End-tidal PCO2 (PETCO2) was measured with a side-stream infrared CO2 analyzer (model 200, Instrumentation Laboratories, Lexington, MA) interposed between the tracheal cannula and the respirator. A 23-gauge polyethylene (PE) catheter (Intramedic PE-50, Becton-Dickinson, Sparks, MD) was advanced from the right carotid artery into the left ventricle for measurement of left ventricular (LV) pressure, the rate of LV pressure increase at 40 mmHg (dP/dt40), and the rate of LV pressure decline (−dP/dt). A 23-gauge PE catheter (PE-50, Becton-Dickinson) was advanced through the left external jugular vein and the superior vena cava into the right atrium. Right atrial pressure was measured with reference to the midpoint with a high-sensitivity pressure transducer (model 42584-01, Abbott Critical Care Systems, North Chicago, IL). A 4-F PE catheter (model C-PMS-401L, Cook Critical Care, Bloomington, IN) was advanced through the right external jugular vein into the right atrium. A precured guide wire supplied with the catheter was then advanced through the catheter into the right ventricle until an endocardial electromgram was confirmed. A PE-50 catheter (Becton-Dickinson) was advanced through the left femoral artery into the thoracic aorta for measurement of aortic pressure with the high-sensitivity pressure transducer. A thermocouple microprobe 10 cm in length and 0.5 mm in diameter (9030-12-D-34, Columbus Instruments, Columbus, OH) was advanced from the right femoral artery into the descending thoracic aorta for measurement of blood temperature. A PE-50 catheter (Becton-Dickinson) was advanced through the left femoral vein into the inferior vena cava for sampling venous blood and for blood transfusion. ECG lead II was continuously recorded. A heat lamp was used to maintain body temperature at 36.8°C (±0.2%) throughout the experiment.

CPR experimental procedure. After baseline measurements but before induction of VF were completed, mechanical ventilation was established at a tidal volume of 0.65 ml/100 g of body wt and a frequency of 100 breaths/min. The inspired O2 fraction (FIO2) was maintained at 0.21. A progressive increase in 60-Hz current to a maximum of 4 mA was then delivered to the right ventricular endocardium. The current flow was continued for 3 min to preclude spontaneous reversal of VF. Mechanical ventilation was discontinued after onset of VF. Precordial compression was begun 6 min after the onset of VF with a pneumatically driven mechanical chest compressor as previously described (18–20). Coincident with the start of precardial compression, mechanical ventilation was resumed. The FIO2 was increased to 1.0. Precordial compression at a rate of 200 min−1 was synchronized to provide a compression-ventilation ratio of 2:1 with equal compression-relaxation duration. Depth of compression was adjusted to maintain a coronary perfusion pressure (CPP) at 25 ± 2 mmHg. This typically yielded a PETCO2 value of 11 ± 2 Torr. Resuscitation was attempted with up to three 2-3 biphasic waveform countershocks (CodeMaster XL, Heartstream Operation, Philips, Seattle, WA) after 12 min of cardiac arrest and 6 min after the start of precardial compression. In unsuccessfully resuscitated animals, precardial compression was restarted and maintained for 30 s before introducing a second sequence of electrical shocks. ROSC was defined as the return of supraventricular rhythm with a mean aortic pressure (MAP) of 60 mmHg for a minimum of 5 min. Animals were monitored for a total of 4 h after successful resuscitation. All catheters, including the endotracheal tube, were then removed. The animals were observed for an additional 68 h, after which they were euthanized with an intraperitoneal injection of pentobarbital sodium (150 mg/kg).

Postmortem. At autopsy, organs were inspected for gross abnormalities, including evidence of traumatic injuries consequent to cannulation, airway management, or precardial compression. Correct coronary artery constriction was confirmed by the gross morphological appearance of the heart. Compared with the normal myocardial zone, the relative pallor of the wall of left ventricle, mostly the anterior wall of the left ventricle, indicated the formation of fibrosis. A PE-50 catheter (Becton-Dickinson) with an attached syringe containing the colored dye solution, indocyanine green (1 mg/ml), was then advanced through the ascending aorta into the aortic root before touching the aortic valve, where the coronary ostia arise. The catheter was tightly secured with a ligature. Approximately 2 ml of the colored dye were then slowly injected. Gross visualization showed that the affected myocardium was not initially stained, and ultimately it was only partially stained by the injection of the dye. This presented a striking contrast to the immediate and complete staining in the normal myocardium.

Measurements. The dynamic changes of myocardial mechanical function at baseline before coronary artery constriction and 4 wk after surgery were measured by noninvasive transthoracic echocardiography. Echocardiograms were performed with the aid of Sonos 2500
echocardiographic system utilizing a 7.5-Hz transducer (model 21363A, Hewlett-Packard, Medical Products Group, Andover, MA). The animal was imaged in the parasternal short-axis plane through the anterior chest. At two-dimensional imaging of short-axis view, LV end-systolic volumes (LVESV) and LV diastolic volumes (LVEDV) were calculated by the method of discs (Acoustic Quantification Technology, Hewlett-Packard, Andover, MA). From these, ejection fractions (EF) were computed. These measurements served as quantifiers of left ventricular remodeling and myocardial contractile function.

Aortic, LV, and right atrial pressures, ECG, and PETCO2 values were continuously recorded on a personal computer-based data-acquisition system supported by CODAS hardware and software (DataQ, Akron, OH). CPP was calculated as the difference between aortic and time-coincident right atrial pressures in the interval between chest compressions. A 1.0-ml aliquot of arterial blood from a donor rat of the same colony was transfused into the inferior vena cava immediately after withdrawal of 0.5-ml samples from the aortic and inferior vena cava catheters. At 4 min after the start of precordial compression and at 60 and 240 min after successful resuscitation, this panel of measurements was repeated.

Myocardial function during the second experimental phase was assessed from measurements of LV pressure. The rate of LV pressure increase at 40 mmHg (dP/dt) was measured by analog differentiation as an indicator of isovolumic contractility that is relatively independent of changes in preload and afterload. The rate of LV pressure decline (−dP/dt) was measured as an indicator of myocardial relaxation.

Analyses. The significance of differences among groups was determined by analysis of variance and Scheffé’s multiple-comparison techniques. Comparisons between time-based measurements within each group were performed with analysis of variance for repeated measurements. Measurements are reported as means ± SD. A value of $P < 0.05$ was regarded as significant.

RESULTS

Three groups of five animals were investigated during cardiac arrest and resuscitation 4 wk after surgical intervention. All animals were resuscitated. Although the total energy required for successful defibrillation before ROSC needed in the coronary artery constriction group was numerically greater than that in the other two groups, there were no significant differences in the total number of shocks among the groups. All animals survived the 72-h period (Table 1).

Physiological parameters of global cardiac performance at baseline before surgery and 4 wk after surgery measured by noninvasive transthoracic echocardiography are illustrated in Fig. 2. There were no differences in EF, LVEDV, and LVESV at baseline before surgery among the three groups. Coronary artery constriction provoked significant decreases in EF, increases in LVEDV, and increases in LVESV after 4 wk, when compared with either baseline before surgery within the groups or with those of sham-operated animals and control animals obtained after 4 wk and before onset of VF. No changes were observed for EF, LVEDV and LVESV in sham-operated animals and control animals during the 4-wk period.

Four weeks after surgery, hemodynamic and blood analytical measurements before induction of VF did not differ significantly among the three groups. MAP and heart rate (HR) in the coronary artery constriction group were numerically, but insignificantly, lower than those in the sham-operated and control group (Fig. 3). There were no differences in CPP, PETCO2, and blood-gas measurements among the groups during CPR. There were no differences in hemodynamics (Fig. 3) and blood-gas measurements among the groups after resuscitation.

Myocardial function during the second experimental phase as measured by dP/dt0 and −dP/dt was significantly decreased and LVDP was significantly increased in the coronary artery constriction group compared with the sham-operated and control groups at baseline before induction of VF (Figs. 4–6). The significant differences in dP/dt0, ~dP/dt, and LVDP among the three groups consistently persisted after resuscitation. As shown in Fig. 4, 4 h after resuscitation, the dP/dt0 of the coronary artery constriction group returned to ~83% of baseline values before induction of VF, whereas it returned to ~73% in the sham-operated group of baseline values and 76% in control group; however, no significant difference was observed among the groups ($P > 0.05$). As indicated in Fig. 5, the −dP/dt of the coronary artery constriction group returned to

Table 1. Effects of intervention on the ROSC, number of defibrillations, and duration of survival

<table>
<thead>
<tr>
<th>Group</th>
<th>ROSC</th>
<th>Number of Shocks</th>
<th>Duration of Survival, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary artery constriction</td>
<td>5/5</td>
<td>5.2±4.6</td>
<td>72±0</td>
</tr>
<tr>
<td>Sham operated</td>
<td>5/5</td>
<td>3.8±2.6</td>
<td>72±0</td>
</tr>
<tr>
<td>Control</td>
<td>5/5</td>
<td>2.4±1.5</td>
<td>72±0</td>
</tr>
</tbody>
</table>

Values are means ± SD. ROSC, return of spontaneous circulation.
80% of baseline values, 67% in the sham-operated group, and 72% in the control group; also, no significant difference was observed among groups (P > 0.05). Similar changes were observed in LVDP (Fig. 6).

**DISCUSSION**

Our present study indicated that a nonocclusive constriction of the left coronary artery of the rat heart, 4 wk after surgical intervention, and before induction of VF, was associated with LV remodeling and alterations in global cardiac performance and more severe postresuscitation myocardial dysfunction after subsequent resuscitation. This rat model of chronic myocardial ischemia would suggest more closely clinical relevant investigation on cardiopulmonary arrest and CPR.

When favorable results are reported in animal models, the new interventions are often implemented in human victims of cardiac arrest. However, the results obtained in the laboratory may not be reproducible in human trials. For example, randomized controlled trials have failed to prove that high-dose epinephrine improves the outcome of humans suffering from cardiac arrest (4, 17), whereas it has a significantly improved outcome in animal models of cardiac arrest and resuscitation (5, 12). The fact that the patients had underlying ischemic heart disease, whereas the experimental animals were otherwise healthy, might contribute to this discrepancy between animal studies and human trials. Therefore, it is necessary to more closely simulate in these animal models the event of cardiac arrest and resuscitation that occurs in humans.

Clinically, ischemic heart disease is by far the principal cause of VF. In survivors of cardiac arrest, coronary artery disease with vessels exhibiting >75% cross-sectional stenosis are found in 40–86% of patients, depending on age and sex of the population studied (11). Ischemic heart disease usually develops over a time course of years and is often associated with LV remodeling, including changes in geometry, structure, and function. Persistence of the remodeling, although initially adaptive, ultimately precipitates the progression of heart failure. The mechanism responsible for this deleterious transition from adaptive remodeling to dysfunction is not fully understood and is more likely under the influence of the neurohumoral axis, loading conditions of the remaining myocardium, and alteration of the extracellular matrix (9, 10, 13, 16). The effect of these pathological changes on the investigation of cardiac arrest and resuscitation should be taken into account.

Our present investigation adopted a rat model of nonocclusive coronary artery constriction. The extensive investigations

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**Fig. 3.** Effects of intervention on mean aortic pressure (MAP), heart rate (HR) before onset of cardiac arrest and after resuscitation. Values are means ± SD; nos. in parentheses are no. of animals.

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**Fig. 4.** Effects of intervention on rate of left ventricular pressure increase at 40 mmHg (dP/dt40) before onset of cardiac arrest and after resuscitation. Values are means ± SD; nos. in parentheses are no. of animals.

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**Fig. 5.** Effects of intervention on rate of left ventricular pressure decline (dP/dt) before onset of cardiac arrest and after resuscitation. Values are means ± SD; nos. in parentheses are no. of animals.

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**Fig. 6.** Effects of intervention on left ventricular end-diastolic pressure (LVDP) before onset of cardiac arrest and after resuscitation. Values are means ± SD; nos. in parentheses are no. of animals.
concerning the impaired myocardial function in this rat model of nonocclusive constriction of the left coronary artery have been well performed in the laboratory of Capasso (1, 2, 6, 7) and others (3, 14). Although this model also lacks the aspect of atherosclerosis which is the major cause of the chronic narrowing of human coronary arteries, this model allows a slower, more realistically moderate development of ischemic heart failure than complete coronary artery ligation. Investigations (3, 6, 14) have proved that the reduction of the coronary artery diameter after chronic constriction of the left main coronary artery over variable durations of myocardial ischemia have consistently led to significant decreases in coronary blood flow compared with the sham groups. Capasso et al. (2, 7) reported that the chronic constriction of the left main coronary artery of 1-mo duration resulted in variable reductions in luminal diameter associated with alterations in cardiac performance in combinations with extensive ventricular remodeling characterized by chamber enlargement and thinning of the wall. Although in our present study, aiming to observe the duration of postresuscitation survival, limited us to directly measure the coronary blood flow, postmortem gross visualization with Indocyanine Green indirectly indicated that the reduction of coronary blood flow was present in the ischemic heart. In agreement with the observations of Capasso et al., the coronary artery constriction group in our present study after a 4-wk interval was associated with LV remodeling and alterations in global cardiac performance before onset of cardiac arrest. These were characterized by significant decreases in EF, dP/dt 40, and −dP/dt and with significant increases in LVEDV, LVESV, and LVPD compared with sham-operated animals and control animals. Therefore, we excluded the possibility that the sham intervention would alter the cardiac performance because no changes in EF, LVEDV, and LVESV in the sham-operated animals occurred during a 4-wk period, and there were no differences in dP/dt 40, −dP/dt, and LVPD at baseline between control animals and sham-operated animals before induction of VF.

We had demonstrated that both CPP and PetCO2 are extremely important predictors for ROSC (21). In our present study, both CPP and PetCO2 remained constant, and no differences were observed among the three groups. All the animals were resuscitated. Based on these observations, we concluded that the previous thoracotomy did not alter the effect of chest compression during CPR, which overthrew our original suspicion that the surgical injury on the chest caused by the thoracotomy might affect the efficiency of chest compression. Furthermore, we demonstrated that the more severe postresuscitation myocardial dysfunction in the coronary artery constriction group was caused solely by the simulated ischemic heart disease, because the possible variables, including CPP and PetCO2, were identically controlled, and total energy required for successful defibrillation before ROSC among the three groups had no significant difference. Also, the fact that no differences in both postresuscitation myocardial function and duration of survival between sham-operated animals and control animals further confirmed that the thoracotomy did not affect the postresuscitation outcome.

Several studies of CPR have been done in the pig and dog model of cardiac arrest, in which investigators compared the defibrillation efficacy of electrically induced VF and ischemic-induced spontaneous VF initiated by acute coronary artery balloon occlusion (15, 22). These studies found that defibrillation of spontaneous VF caused by acute ischemia requires significantly more energy than defibrillation of VF caused by the application of 60-Hz current in the absence of ischemia. However, in the present investigation, there were no significant differences in the total number of shocks before ROSC in the coronary artery constriction group compared with those in the other two groups. Therefore, our current data suggest that different mechanisms might be responsible for the different defibrillation efficacy between the acute ischemic heart and chronic ischemic heart.

Although the effects of remodeling and alterations in global cardiac performance after coronary artery constriction, 4 h after resuscitation, were statistically insignificant, the percentage of dP/dt 40 and −dP/dt compared with baseline values in the constriction group was numerically greater than the other two groups. Yet, the mechanism accounting for this interesting phenomenon remained unknown, which might be related to the “chronic myocardial ischemic preconditioning.” This relatively preserved postresuscitation myocardial function in the chronic ischemic heart deserves further investigation.

Even with significantly impaired postresuscitation myocardial function, the duration of postresuscitation survival in coronary artery constriction animals did not differ from those in the sham-operated animals and control animals. The following potential mechanisms might contribute to this phenomenon. First, because ischemic heart disease usually develops over time (8), the animals may still be in the situation of adaptive remodeling, relatively compensated cardiomegaly after a 4-wk period of coronary artery constriction. Second, the global myocardial ischemic duration of cardiac arrest and CPR was 12 min in our present study, and according to our protocol, all the rats were euthanized 72 h after resuscitation. Thus this global ischemic downtime might not be long enough to differentiate short-term outcome among these three groups. Therefore, modification of present protocol either by extending the period of coronary constriction to induction of cardiac arrest in an effort to develop more severely deleterious ventricular remodeling and myocardial dysfunction, or by prolonging the global ischemic downtime, might be able to magnify the effect of potential ischemic myocardial disease on the duration of postresuscitation survival.

In summary, this rat model of chronic myocardial ischemia, 4 wk after surgical intervention, was associated with LV remodeling and myocardial dysfunction and with more severe postresuscitation myocardial dysfunction after subsequent resuscitation. The attractiveness of this model was related to its accurate reflection of pathophysiology during cardiac arrest and resuscitation in patients with ischemic heart disease, which would suggest further closely clinical relevant investigation on cardiac arrest and CPR.

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