Good Recovery after Nontransthoracic Cardiopulmonary Bypass in Rats

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ABSTRACT

Background. Cardiopulmonary bypass (CPB) has been shown to be associated with systemic inflammatory response leading to postoperative organ dysfunction. Unwanted side effects of CPB are well known but poorly understood due to the absence of a stable recovery animal model that is easy to handle and reduces experiment cost and time. The purpose of this study was to establish a good recoverable rat model of CPB to study the pathophysiology of these potential complications.

Materials and Methods. Twenty adult male Sprague-Dawley rats weighing 480 ± 20 g were randomly divided into either the CPB group (n = 10) or the sham group (n = 10). All rats were anesthetized, intubated, and ventilated. The carotid artery and jugular vein were cannulated. The blood was drained from the right atrium via the right jugular and further transferred by a miniaturized roller pump to a hollow fiber oxygenator and back to the rat via the left carotid artery. Priming consisted of 8 mL of homologous blood and 6 mL of colloid. The surface of the hollow fiber oxgenator was 0.075 m^2. Rats were catheterized and brought on bypass for 120 minutes at a flow rate of 100-120 mL/kg per minute. Oxygen flow/perfusion flow was 0.8 to 1.0, and the mean arterial pressure remained 60 to 80 mmHg. Blood gas analysis, lactate dehydrogenase, and survival rate were examined subsequently.

Results. All CPB rats recovered from the operative process without incident and recovery remained uneventful in follow-up at 1 week. Normal cardiac function after successful weaning was confirmed by electrocardiography and blood pressure measurements. Mean arterial pressure remained stable. The results of blood gas analysis at different times were within normal range. No significant hemolysis could be detected with the use of lactate dehydrogenase during bypass.

Conclusions. The rat model of CPB can in principle simulate the clinical setting of human CPB. The nontransthoracic model is easy to establish and is associated with excellent recovery. This reproducible model may open the field for various studies on the pathophysiological process of CPB and systemic ischemia-reperfusion injury in vivo.

BACKGROUND

Despite new minimally invasive techniques, cardiopulmonary bypass (CPB) is still necessary for many major operations in the field of cardiac surgery and many other surgical procedures [Paparella 2002]. However, CPB causes severe stress that can provoke a systemic inflammatory response syndrome. The excess inflammatory reaction leads to postoperative organ dysfunction [Hayashi 2004]. Unwanted side effects of CPB are well known [Baines 2002] but poorly understood due to the absence of a stable recovery animal model that is easy to handle and reduces experiment cost and time. The rat model of CPB can reduce the cost of animals and equipment, and there is a large availability of assays [Cocchetto 1983]. Since 1970, several attempts to achieve rat CPB models have been described and have been successful to some extent [Subramanian 1968; Proctor 1977; Alexander 1983; Sasaki 1996; Senra 2001; Gourlay 2002]. But most of these models involved partial bypass or depended on large quantities of priming, resulting in pulmonary edema and cardiac arrest after a relatively short perfusion period. Few studies reported a clinically relevant true model of complete CPB. The purpose of this study was to establish a good recoverable CPB model in rats for use in the study of the pathophysiological process of CPB.

MATERIALS AND METHODS

Animal Care

Adult male Sprague-Dawley rats weighing 480 ± 20 g were used in the present study (Table 1 [Cochetto 1983]). All rats received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" prepared by the United States National Institutes of Health (NIH Publication No. 86-23, revised 1996).

Table 1. Comparison of Physiological Parameters of the Cardiovascular System in Rats and Human Adults

	Rats	Human Adults
Heart rate, beats/min	330-480	60-86
Blood pressure, mmHg		
Systolic	88-184	110-140
Diastolic	58-145	68-100
Stroke volume, mL	1.3-2.5	68-100
Cardiac output, mL/min	0.01-0.08	3.7-9.4
Cardiac index, mL/min/m^2	1.5	3.9
Blood volume, mL/kg	64.1	69
Respiratory rate, beats/min	66-114	16-20

Figure 2. Heart rate during cardiopulmonary bypass (mean values ± standard deviation).

Group Classification

Twenty rats were randomly divided into the CPB group (n = 10) and the sham group (n = 10). The experimental preparation, anesthesia, orotracheal intubation, ventilation, cannulation, and heparinization were identical during the entire experiment in both groups. Only the CPB group was connected to the perfusion circuit and underwent bypass.

Surgical Procedure

Rats were anesthetized by intraperitoneal administration of urethane (1 mg/kg) and placed in the supine position. During surgery, the rat was ventilated with a 14-gauge cannula (FIO_2 1.0, frequency 65, tidal volume 10 mL/kg body weight) by the use of a rodent ventilator (rodent respirator DH-150; China). Ventilation was finely adjusted to keep an arterial carbon dioxide tension (PaCO_2) of 35 to 40 mmHg. During CPB, additional urethane was inflated into the oxygenator to maintain anesthesia. The left femoral artery was cannulated by a 22-gauge heparinized catheter (Intima-2 integrated catheter; BD Biosciences, China) for continuous pressure measurement and to collect arterial blood gas analysis (GEM Premier 3000, blood gas analyzer; Instrumentation Laboratory, Lexington,

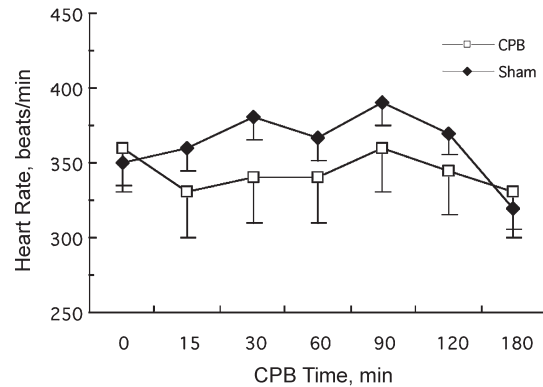Figure 1. The rat model of nontransthoracic cardiopulmonary bypass.

MA, USA). The homolateral femoral vein was cannulated with a 20-gauge catheter for liquid replacement. An 18-gauge catheter was inserted into the right jugular vein and advanced to the right atrium. This position resulted in good drainage. Subsequently, the left carotid artery was exposed and cannulated with a 22-gauge catheter placed into the aortic arch, which served as the arterial perfusion line for the extracorporeal circuit. For anticoagulation, heparin (300 IU/kg) was administered; half of the dose was given directly via the left femoral catheter, the other half via the extracorporeal circuit. A nontransthoracic rat model of CPB is shown in Figure 1.

Perfusion Circuit Preparation

The CPB circuit was composed of a roller pump (Polystan A/S, Vaerlose, Denmark), a hollow fiber oxgenator (surface area 0.075 m^2, specially made by Xijing Medical Instrument, Xijing, China), a poikilothermy water tank (Sarns Machine, Mt. Clemens, MI, USA), a venous reservoir (20-mL injection syringes; BD Biosciences, China), and sterile tubing with an inner diameter of 4 mm for the venous line and 1.6 mm for the arterial line. The high siphon level was 40 cm. The blood was drained from the right atrium via the right jugular by gravity and siphoned and further transferred by the roller pump to the hollow fiber oxgenator and back to the rat via the left carotid artery. Central temperature was measured by a rectal temperature probe; temperature was maintained between 32° and 38° using a heat exchanger under the operating table and also an integrated heat exchanger in the oxygenator. Priming consisted of 8 mL of homologous blood obtained from a donor rat immediately before the experiments and 6 mL of colloid. During CPB, mean arterial pressure (MAP) was regulated at 60 to 80 mmHg. The hematocrit was approximately 25% to 30%. With an inspired oxygen fraction of 100%, 50 mL/min gas flow was sufficient to achieve adequate oxygenation and to maintain PaCO_2 at 35 to 40 mmHg. At the initiation of CPB, the flow rate was gradually adjusted to a level that could sustain MAP near 80 mmHg; at this point, ventilation was terminated and CPB was stably performed at 100 to 120 mL/kg per minute for about 120 minutes. After weaning from the circuit,
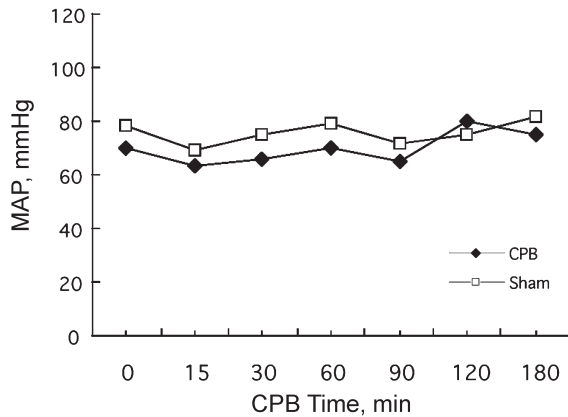
Figure 3. Mean arterial pressure (MAP) during cardiopulmonary bypass (mean values ± standard deviation).

the cardiac function was retained with heart beating and pulsation. The remaining priming solution was infused gradually when the main blood pressure was lower than 60 mmHg.

Protocol and Statistical Analysis

All data were given as mean ± standard deviation. Before starting the experiments, different system parameters such as flow, pressure management, heart rate, electrocardiography, and hemolysis were tested in vitro. Arterial blood gases, taken from the left femoral artery, were determined at 0 minutes, 30 minutes, 60 minutes, 90 minutes, the termination of CPB, and post- CPB 60 minutes. In all CPB group rats, a CPB time of 120 minutes was planned. But due to either technical or surgical problems, some animals stayed either longer or shorter on CPB (mean CPB time, 120 ± 20). Statistical analysis was performed using SPSS 10.0 software (SPSS, Chicago, IL, USA). Comparisons between groups at specific time-points were carried out with the Student t test. A P value less than .05 was considered statistically significant.

RESULTS

There were no technical failures or operative deaths in the animals used in the present study. All CPB rats recovered from the operative process without incident and recovery remained uneventful in the follow-up at one week. Normal cardiac function after successful weaning was confirmed by electrocardiography and blood pressure measurements. No significant difference between hemodynamic parameters was observed. MAP remained stable throughout the experiments. MAP was lower in the CPB group at the start of CPB and heart rate slowed down after the initiation of CPB and remained lower but stable throughout the procedure (Figures 2 and 3).

Table 2 displays the results of blood gas analysis that were within acceptable ranges at different times. Values of hematocrit were significantly lower in the CPB group than the sham group due to hemodilution induced by the priming volume of the CPB circuit ($P < .01$). Compared with the pH value at initiation of CPB, the PH value had an obvious decrease during the perfusion process and post-CPB. In the CPB group PaO_2 values were significantly lower during CPB and post-CPB than at the initiation of CPB ($P < .01$), whereas $PaCO_2$ remained stable. However, in the sham group, there were no changes in PaO_2 or $PaCO_2$. The hemolysis of the system was tested at different flow volumes (60 mL/min and 120 mL/min, Figure 4). Lactate dehydrogenase was used as the hemolysis parameter, and no significant hemolysis could be detected in the given time frame under bypass conditions.

DISCUSSION

CPB has been shown to be associated with systemic inflammatory response leading to postoperative organ dysfunction

Table 2. Results of Blood Gas Analysis at Different Times during Cardiopulmonary Bypass (CPB)

	CPB 0 min	CPB 30 min	CPB 60 min	CPB 90 min	CPB Termination	Post-CPB 60 min
PaO_2, mmHg						
CPB	380 ± 34	280 ± 29*†	260 ± 24*†	275 ± 28*†	245 ± 22*†	330 ± 31*†
Sham	373 ± 30	385 ± 33	379 ± 29	369 ± 27	381 ± 31	376 ± 32
$PaCO_2$, mmHg						
CPB	35.5 ± 1.9	36.2 ± 2.0	38.5 ± 2.3	37.6 ± 1.8	38.2 ± 2.0	37.4 ± 1.7
Sham	38.5 ± 2.1	37.5 ± 1.8	39.5 ± 2.6	37.9 ± 2.0	38.3 ± 2.3	39.0 ± 2.4
PH						
CPB	7.38 ± 0.01	7.34 ± 0.03‡	7.30 ± 0.06*	7.29 ± 0.05*	7.31 ± 0.03*	7.28 ± 0.04*
Sham	7.39 ± 0.01	7.41 ± 0.02	7.43 ± 0.03	7.38 ± 0.02	7.40 ± 0.01	7.38 ± 0.02
Hematocrit, %						
CPB	41.5 ± 2.1	30.9 ± 1.0*†	27.8 ± 0.8*†	29.3 ± 0.9*†	30.9 ± 0.7*†	35.5 ± 1.2*†
Sham	41.3 ± 2.0	41.0 ± 2.1	40.9 ± 1.8	41.0 ± 1.5	40.8 ± 1.8	40.6 ± 1.6

*$P < .01$ versus CPB 0 minutes.

†$P < .01$ versus the sham group.
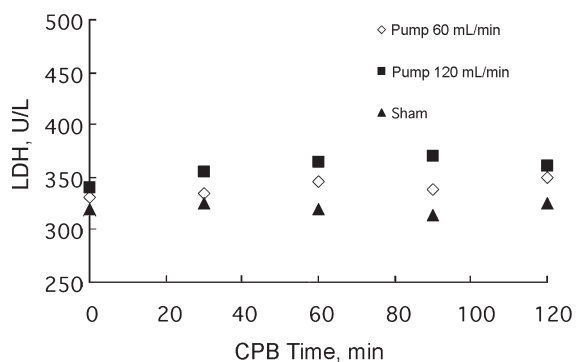
‡$P < .05$ versus CPB 0 minutes.

Figure 4. No remarkable blood hemolysis could be detected using lactate dehydrogenase (LDH, standard range, 240-480 U/L).

and postperfusion syndromes [Baines 2002; Paparella 2002; Haashi 2004]. Despite excellent improvements in modern CPB, the manifestations of side effects have been repeatedly reported [Kazuo 2005]. The study of the underlying mechanisms and development of protective strategies for the pathophysiological processes of CPB were more important than CPB technology itself in clinical investigation [Kawahira 2003]. Earlier experimental studies with the aim of achieving experimental CPB have been limited to large animals. These models are very expensive, need to utilize very complex instruments, and require tedious performance and intensive labor. Furthermore, they have significant individual differences because of the lack of pure breeding in the animals. Therefore, investigators cannot satisfactorily conduct studies with larger experimental groups.

The use of the rat fits the needs of a CPB model because it reduces experiment cost and time. Furthermore, rats and humans have similar physiological parameters in the cardiovascular system, and there is a large availability of assays [Cocchetto 1983]. In the past, a few investigators have established CPB in rats. The fist model was reported by Subramaniam et al [1968]. In this model, perfusion was established between the right atrium and the femoral artery. The priming volume was as high as 120 mL. Proctor [1977] developed an experimental bubble oxygenator with a priming volume of 25 mL. In our model, a bypass time of 120 minutes was chosen because it seemed long enough for complex surgical procedures in clinical patients. The cannulation technique was easily achieved, and the cannula was safely removed from blood vessels by compression. Minimal surgical aggression by jugular-carotid cannulation without sternotomy was achieved to minimize systemic inflammatory response syndromes due to surgical trauma. Gu and associates [1999] reported that surgical access (sternotomy only), despite the absence of CPB, induced complement activation in patients who underwent off-pump coronary artery bypass grafting.

In our CPB model, we used a hollow fiber oxygenator specifically designed with a surface area of 0.075 m^2, which is a miniature version of the ones used in human cardiac surgery. It was specially tailored for use in rodents, and we were able to reduce the total priming volume to 14 mL,

proportionally similar to the priming volume commonly used in clinical practices. In addition, the very small circulating blood volume does not lead to increased hemolysis as assessed by lactate dehydrogenase measurements (Figure 4), underlining the good mechanical properties of the CPB circuit system. The results of blood gas analysis indicated that the hollow fiber oxygenator could supply adequate oxygenation for 120 minutes of bypass. To solve another key issue, venous blood drainage, a 40-cm high siphon level, and an inner diameter of 4 mm for the venous outflow tract were utilized to overcome the resistance of the venous line.

Hemodynamic results showed that MAP was lower at the time of cannulation. In the CPB group, the time required to prepare the extracorporeal circuit led to a different level of anesthesia, which could explain the difference in these results despite a similar anesthesia procedure. However, full-flow CBP was stably performed and MAP remained stable at 60 to 80 mmHg. Moreover, the heart rate slowed down after the initiation of CPB and remained lower but stable throughout the procedure because systemic arterial pressure remained pulsatile and the heart must contribute to the blood pressure. In our CPB models, both the PH value and PaO_2 level had obvious decreases during the perfusion process and post-CPB in the femoral artery; these phenomena indicate a metabolic acidosis and lung perfusion, which are common in clinical practice [Palanzo 1997]. In our experiment, all CPB rats recovered from the operation without incident and their recovery remained uneventful at the follow-up at 1 week. After successful weaning, normal cardiac function was confirmed by electrocardiography and blood pressure measurements. The results of blood gas analysis at different times were in normal range.

In conclusion, this study demonstrates the technical feasibility of a rat model for nontransthoracic CPB with a good recovery rate. This model is easy to handle and reduces experiment cost and time and therefore allows for small animal studies with larger experimental groups. Because rats tolerate CPB and subsequent weaning very well, this reproducible model will also allow for the study of the pathophysiological process of CPB in vivo and will extend this knowledge with methods of molecular biology.

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