

Moderate Hypothermic Circulatory Arrest Model in Rats: A New Model with Hyperkalemia-Induced Cardioplegia

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ABSTRACT

Background: Moderate hypothermic circulatory arrest (MHCA) is a safe and effective method of cardiopulmonary bypass (CPB). However, most present rat models involve a deep hypothermic circulatory arrest, which cannot exactly reflect the clinical situation. The aim of this study was to establish a novel and safe rat model of MHCA with hyperkalemia-induced cardioplegia to study the pathophysiology of potential complications.

Methods: Ten adult male Sprague–Dawley rats (age, 16–18 weeks; weight, 450–550 g) were used. The entire CPB circuit consisted of a reservoir, peristaltic pump, membrane oxygenator, heat exchanger, and hemoconcentrator, all of which were connected via silicon tubing. The prime solution was approximately 19 mL. The right jugular vein, right femoral artery, and left femoral artery were cannulated. Blood was drained from the right atrium through the right jugular vein and perfused to the rats via the left femoral artery. CPB was commenced at a full flow rate. The rats were cooled to a rectal temperature of 25°C, and cardioplegia was induced by systemic hyperkalemia. After that, MHCA was carried out for 30 min. At the same time, system self-ultrafiltration was carried out to decrease the concentration of potassium by a hemoconcentrator. The circulatory arrest was followed by reperfusion and over 30 min of rewarming. CPB carefully was terminated. Blood in the circuit slowly was centrifuged for autotransfusion. Blood gas and hemodynamic parameters were recorded at each time point before CPB, before MHCA, at 10 min after the initiation of rewarming, and after CPB.

Results: All CPB and MHCA procedures successfully were achieved. One rat died of respiratory failure. Cardioplegia with systemic hyperkalemia was induced by 1 mL of 10% potassium

chloride injected into the reservoir, and the concentration of potassium was maintained at 17 ± 3 mmol/L. Cardiac function and blood pressure were stable after the operation.

Conclusions: A novel and safe rat model of MHCA with hyperkalemia-induced cardioplegia successfully was established.

INTRODUCTION

Deep hypothermic circulatory arrest (DHCA) is a common method of cardiopulmonary bypass (CPB) that has been widely used for complicated pediatric congenital heart diseases and aortic arch surgery [Stoicescu 2017; Alkhatib 2021]. However, with the introduction of antegrade cerebral perfusion, deep hypothermia has become nonessential for neuroprotection, especially considering that it may cause many negative side effects, such as coagulopathy and organ dysfunction [Livesay 1982; Sun 2009]. The inevitable extension of CPB for cooling and rewarming might cause adverse outcomes [Kazui 2001]. Therefore, moderate hypothermic circulatory arrest (MHCA) increasingly has been used for aortic arch surgery [Numata 2012; Urbanski 2012].

Large-size animal models, such as dogs, piglets, and sheep, have been used for CPB and DHCA studies, because their anatomical structure and size are similar to those of humans. Recently, rat models of DHCA have replaced large animal models due to their advantages, such as low price, similar anatomical structure to the human cardiovascular system, and abundant detection methods at the gene and protein levels [Waterbury 2011]. However, most of the rat models have been based on DHCA, and cardioplegia has been induced by hypothermia or/and ischemia [Jiang 2017; Liu 2018], which cannot simulate the recent clinical practice.

Here, we reported a new rat model simulating MHCA by hyperkalemia-induced cardioplegia, which better reflects clinical practice and is suitable for research on organ dysfunction, such as liver, kidney, and spinal cord, during MHCA.

METHODS

All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals

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published by the National Institutes of Health. This study was approved by the animal care committees of the General Hospital of Northern Theater Command. Ten male Sprague–Dawley (SD) rats (age, 16–18 weeks; weight, 450–550 g) were obtained from Changsheng Laboratory, Benxi, Liaoning, China.

Anesthesia and preoperative preparation: The rats fasted for 6 h before the operation but were allowed to drink freely. The rats (age, 16–18 weeks; weight, 450–550 g) were anesthetized with 30 mg/kg 2% sodium pentobarbital (Sigma-Aldrich; Merck KGaA) and underwent tracheal intubation (16 G trocar) using a light transmission method. Intraoperative maintenance was conducted by intermittent administration of sodium pentobarbital and 1% rocuronium (Zhejiang Xianju Pharmaceutical Co., Ltd.). Vital signs, including the heart rate, pulse oximetry, and body temperature, were monitored.

Cardiopulmonary bypass and moderate hypothermic circulatory arrest: Rat models of CPB and MHCA were established and modified in accordance with our previous study [Fan 2021], as illustrated in Figure 1. Catheter insertion (24 G) was performed by puncturing the left femoral artery, and pressure was measured. The needle (22 G) was inserted into the right femoral vein as a channel for fluid supplementation. Another needle (22 G) was inserted into the right femoral artery for CPB perfusion. Catheter insertion (18 G home-made, multi-stage, venous return cannula) was performed via the right jugular vein, and the catheter was placed at the level of the right atrium to be used as a drainage end. The CPB circuit consisted of a venous reservoir, peristaltic pump, membrane oxygenator (< 4 mL prime volume) for rats (Xijian, Xi'an, China), heat exchanger (< 4 mL prime volume) for rats (Xijian, Xi'an, China), and hemoconcentrator with 5 mL prime volume (Medica, Medolla MO, Italy). The height difference between the heart of the rats and the venous reservoir was approximately 25 cm. The entire circuit was primed with 19 mL, and the priming solution was prepared using 6 mL of succinylated gelatin, 11 mL of lactated Ringer's solution, 1 mL of 20% mannitol, 250 IU/kg heparin, and 1 mL of 5% sodium bicarbonate and 10 mg/kg furosemide. Systemic heparinization (400 IU/kg) was done via the left femoral vein once the activated clotting time reached 480 s. Arterial blood gases were analyzed using a GEM Premier 3000 blood gas/electrolytic analyzer (model 5700, Instrument Laboratories, Inc., Lexington, MA, USA). Physiologic data, including blood pressure, body temperature, and blood gas analysis, were collected (Figure 1).

CPB was commenced at a flow rate of 150 to 180 mL·kg⁻¹·min⁻¹ and decreased by half during the cooling period. The animals were cooled to a rectal temperature of 25°C for 10 min by using the heat exchanger. After reaching 25°C, cardioplegia was induced by 1 mL of 10% potassium chloride injected into the reservoir, then, the arterial perfusion stopped and the animals were subjected to MHCA. Venous blood carefully was drained into the reservoir, and then 30 mL of substitute fluid (100 mL of 0.9% sodium chloride containing 5 mL of 5% sodium bicarbonate) was injected into

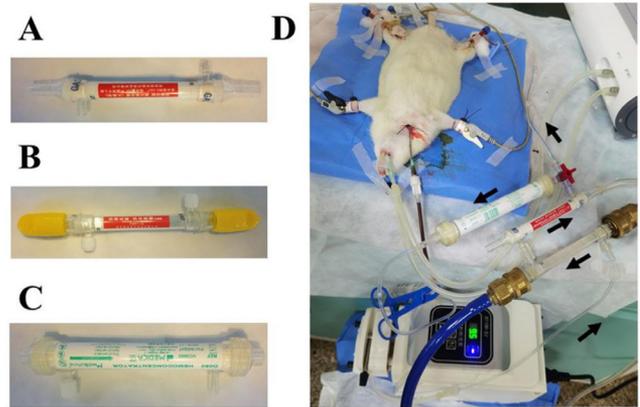


Figure 1. The practicality image of the circuit of the rat CPB and MHCA. A) membrane oxygenator; B) heat exchanger; C) hemoconcentrator; D) The entire CPB and MHCA circuit consisted of a reservoir, peristaltic pump, membrane oxygenator, heat exchanger, and hemoconcentrator and silicon tubes connected. The arrow showed the blood flow direction. CPB, cardiopulmonary bypass; MHCA, moderate hypothermia circulatory arrest.

the reservoir for ultrafiltration to reduce the concentration of potassium to a normal level. (Figure 2)

After 30 min of MHCA, CPB was restarted, and the animals were rewarmed to a rectal temperature of 36°C for over 30 min by using the heat exchanger while gradually increasing the flow rate to 150 to 180 mL·kg⁻¹·min⁻¹. (Figure 3)

Then, the animals were weaned from CPB. The blood in the circuit was centrifuged (1000g, 4 min) and slowly transfused to raise the level of hematocrit. Then, 80,000 U penicillin was intraperitoneally injected to prevent infection, but protamine was not used to reverse heparin-induced

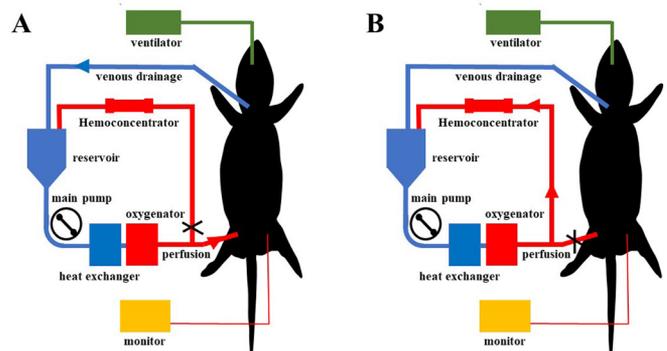


Figure 2. Schematic overview of the rat CPB apparatus. A) CPB carried out during cooling and warming. Oxygenator blood was delivered to the femoral artery of rat via perfusion line, while the right jugular vein was cannulated for venous drainage. The hemoconcentrator line was clamped. B) System self-ultrafiltration was carried out during MHCA. The perfusion line was clamped, while the hemoconcentrator line was opened. The hyperkalemia blood flow was delivered into hemoconcentrator to decrease.

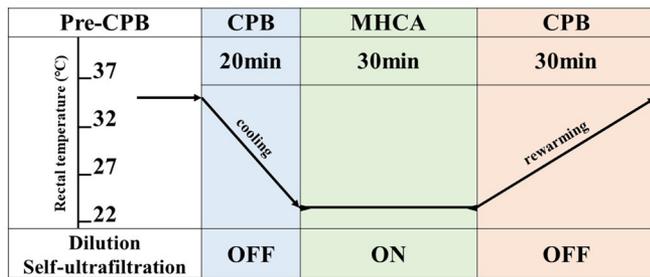


Figure 3. Manipulation procedure for MHCA with CPB. CPB was commenced at a full flow rate. When the rats were cooled to a rectal temperature of 25°C, cardioplegia was induced by systemic hyperkalemia. After that, MHCA was carried out for 30 min. At the same time, system self-ultrafiltration was carried out to decrease the concentrate of potassium by a hemoconcentrator. Then, circulatory arrest was followed by reperfusion and over 30 minutes of rewarming. Finally, CPB was terminated carefully. CPB: cardiopulmonary bypass; MHCA: moderate hypothermia circulatory arrest.

anticoagulation. After spontaneous breathing had resumed, these rats were extubated and allowed to recover in an oxygen-enriched and warm box for 12 h, with available water and food ad libitum. The animals were returned to the hole board cages on the first postoperative day. All of the rats were sacrificed using sevoflurane overdoses. Blood samples, livers, and kidneys were obtained for further experiment.

Arterial blood gas analysis: Arterial samples were collected for blood gas analysis (pH, pO₂, pCO₂, hematocrit, lactate levels, concentration of potassium) at the following four-time points: before CPB, before MHCA, at 10 min after initiation of rewarming, and after CPB.

Statistical analysis: Statistical analysis were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). All parametric values were expressed as mean ± SD.

RESULTS

Nine rats survived after the operation (weight, 502.22 ± 18.65 g), while one rat died of respiratory failure. CPB and MHCA successfully were processed. Cardioplegia was induced theoretically by 18–20 mmol/L (actually 14.08 ± 0.59 tested in four samples) systemic hyperkalemia, and the heartbeat recovered automatically when CPB was restarted with a 6.30 ± 0.84 mmol/L concentration of potassium. The parameters around CPB are displayed in Table 1. (Table 1) The concentration of potassium in the reservoir corrected by substitute fluid was 2.67 ± 0.60 mmol/L. Blood pressure after the operation was comparable to blood pressure before the operation. The values of hematocrit began to decline after CPB (21.89% ± 1.66%) and increase after the transfusion of concentrated red blood cells (27.11% ± 1.20%) in the circuit but were still significantly lower than before (39.78% ± 1.87%). Moreover, the levels of lactate increased dramatically after MHCA (7.72 ± 1.09 μmol/L) compared with those before CPB (1.18 ± 0.26 μmol/L) with 30 min MHCA but decreased gradually to 4.57 ± 0.76 μmol/L after CPB with oxygen delivery recovered by CPB.

DISCUSSION

In our study, a novel and clinical analog model of MHCA in rats was successfully established based on a previously published rat model of CPB [Fan 2021], which can be reproduced and is reliable for further research on MHCA.

Recently, an increasing number of studies have shown that MHCA can offer safe and effective organ protection because of selective cerebral perfusion [Jiang 2021; Algarni 2014; Jiang 2020]. Thus, MHCA has been carried out as the main strategy of CPB in aortic arch surgery [Urbanski

Table 1. Hemodynamics and blood gas analysis

	Before CPB	Before MHCA	After MHCA	After CPB
Weight (g)	502.22 ± 18.65			
MAP (mm Hg)	68.0 ± 5.81	42.3 ± 1.49*	42.8 ± 1.54*	72.6 ± 2.84*
Heart rate	279.44 ± 12.63	154.33 ± 15.51*	57.56 ± 21.33*	284.56 ± 12.73
pH	7.41 ± 0.06	7.31 ± 0.022*	7.31 ± 0.015*	7.419 ± 0.013
PaO ₂ (mm Hg)	146.44 ± 8.6	398.11 ± 17.45*	397.44 ± 11.75*	110.33 ± 15.62*
PaCO ₂ (mm Hg)	38.67 ± 5.06	38.33 ± 2.45	32.67 ± 3.43*	40.33 ± 3.13
Rectal temperature (°C)	34.76 ± 0.56	21.08 ± 1.06*	27.26 ± 1.285*	35.83 ± 0.251
Hematocrit (%)	39.78 ± 1.87	21.89 ± 1.66*	19.78 ± 1.55*	27.11 ± 1.20*
Concentration of potassium (mmol/L)	3.23 ± 0.47	3.53 ± 0.29	6.30 ± 0.84*	5.22 ± 0.69*
lactate (μmol/L)	1.18 ± 0.26	1.81 ± 0.54	7.72 ± 1.09*	4.57 ± 0.76*

MAP, mean arterial pressure; CPB, cardiopulmonary bypass; MHCA, moderate hypothermic circulatory arrest. *P < 0.05 vs. before CPB. The concentration of potassium in the reservoir after MHCA was 14.08 ± 0.59 mmol/L (N = 4) with six samples out of the test range on the GEM Premier 3000 blood gas/electrolytic analyzer. In contrast, the concentration of potassium in the reservoir after ultrafiltration was 2.67 ± 0.60 mmol/L (N = 9).

2012; Sun 2013; Luehr 2014]. However, previous rat models of circulatory arrest have involved DHCA [Jiang 2017; Liu 2018; Jungwirth 2006], which induces circulatory arrest at 20°C or below. Thus, research on the pathophysiology and testing of new therapeutic methods in MHCA require an MHCA model. However, large-animal models are expensive and inconvenient, and there are not enough genetic and protein resources for research. Therefore, a rat model of MHCA might be an optimal choice for further studies.

Because the blood volume of rats is small, the prime volume of the CPB circuit should be limited. In previous studies, the CPB circuit often only contained an oxygenator and circuits, in which heat exchange was carried out by a homemade cooling blanket and ice bags [Jiang 2017; Fan 2021; Jungwirth 2006]. Although the method for heat exchange did not increase the prime volume, the temperature modulation was inaccurate and inefficient. To solve these problems, a separate heat exchanger was used in a few studies [Liu 2018; Linardi 2020; Kim 2008], which made heat exchange accurate and efficient with the increased prime volume. In addition, the method of cardioplegia induction rarely has been mentioned in most studies referred to DHCA [Jiang 2017; Liu 2018; Kellermann 2009; Yu 2016], and may involve hypothermia or/and ischemia. Therefore, it remains unclear how to induce cardiac arrest and maintain cardioplegia.

The main innovations of the present study include the use of a separate heat exchanger to modulate temperature exactly to MHCA, and the application of systemic hyperkalemia to induce cardiac arrest, which might simulate clinical situations more reliably in a rat model. During the present experiment, a separate heat exchanger (Xijian, Xi'an, China) combined with ice bags was used for immediate cooling, which increased the prime volume by approximately 4 mL. To solve the difficulty of cardioplegia, systemic hyperkalemia was induced, as in our previous study about profound hypothermia-induced suspended animation for delayed resuscitation [Liu 2017]. Based on a pre-experiment, 1 mL of 10% potassium chloride was directly injected into the reservoir, which increased the concentration of potassium to theoretically 18–20 mmol/L (actually 14.08 ± 0.59 mmol/L tested in four samples). The concentration of potassium was high enough to induce cardiac arrest and maintain cardioplegia. After MHCA, the high concentration of potassium must decrease to a nearly normal level to ensure heart resuscitation. Thus, during MHCA, a hemoconcentrator was used for ultrafiltration to decrease the concentration of potassium by adding 30 mL of substitute fluid into the reservoir, thereby diluting the concentration of potassium to 2.67 ± 0.60 mmol/L. The substitute fluid contained 95 mL of 0.9% sodium chloride and 5 mL of 5% sodium bicarbonate, which were able to dilute the concentration of potassium and modify metabolic acidosis caused by MHCA.

Based on the modification of heat exchange and cardioplegia, the rat model of MHCA was successfully established. However, using a separate heat exchanger and a hemoconcentrator increased the prime volume by nearly 10 mL (heat exchanger: 4 mL; and hemoconcentrator: 5 mL), and

the whole prime volume was approximately 19 mL. The negative pressure generated by a pump or vacuum assistant venous device carried on the reservoir might be beneficial for venous drainage and could reduce the prime volume [Jiang 2017; Lebreton 2012], which will be used in our future studies. In the present experiment, the rats weighing 502.22 ± 18.65 g were enrolled with more auto blood volume to avoid excessive hemodilution, and hematocrit (Hct) levels were maintained at $19.78\% \pm 1.55\%$ without transfusion. After transfusion of centrifuged red blood cells, Hct increased to $27.11\% \pm 1.20\%$, which was still significantly lower than the pre-CPB Hct level.

CONCLUSION

We successfully established a simple, inexpensive, hyperkalemia-induced cardioplegia, and an MHCA model in rats. This model was easy to execute and reduced experimental costs. This model also allowed us to study the pathophysiological process of MHCA and the mechanisms of medium- or long-term post-MHCA multiple organ dysfunctions and possible novel protective strategies.

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