Investigation of the biological effects of artificial perfusion using rat extracorporeal circulation model

Yutaka Fujii, Mikiyasu Shirai, Shuji Inamori, Yoshiaki Takewa and Eisuke Tatsumi

Abstract— **Extracorporeal circulation (ECC) is indispensable for cardiac surgery. Since difficulty in clinical research keeps the knowledge insufficient, it is desirable to have a miniature ECC system for small animals. We aimed to establish a miniature ECC system and apply the system to the rat for investigating biochemical changes. The ECC system consisted of a membranous oxygenator (polypropylene, 0.03 m^2), tubing line (polyvinyl chloride) and roller pump. Priming volume of this system is only 15 ml. Rats were divided into the SHAM group and the ECC group. ECC pump flow was initiated and maintained at 70 ml/kg/min. We measured the serum cytokine levels of tumor necrosis factor-α, interleukin (IL)-6, and IL-10, and biochemical markers (lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase) before, 60, and 120 min after the initiation of ECC. In addition, we measured the wet-to-dry weight (W/D) ratio of the left lung tissues. During ECC, blood pressure and Hb were maintained around 80 mmHg and 10g/dl, the serum cytokine levels and biochemical markers were significantly elevated in the ECC group compared with the SHAM group. The W/D ratio increased significantly more in the ECC group compared with that in the SHAM group. These data suggest that ECC promotes organ damages and systemic inflammatory response. This rat ECC model is considered to be equivalent to the already established human ECC and useful for studying the mechanism of pathophysiological changes during artificial perfusion.**

I. Introduction

Extracorporeal circulation (ECC) is indispensable for cardiac surgery [1]. Despite the fact that ECC is traumatic to blood components and non-physiologic, its influence has not been fully elucidated. Since difficulty in clinical research and animal experiments keeps the knowledge insufficient, it is desirable to have a miniature ECC system for small animals to study the mechanism of pathophysiological changes in the circulation during ECC. Therefore, in this study, we measured the serum cytokine levels of tumor necrosis factor-α, interleukin (IL)-6, and IL-10, and biochemical markers (lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase) before, 60, and 120 min after the initiation of CPB. In addition, we measured the wet-to-dry weight (W/D) ratio of the left lung tissues.

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II. Materials and Methods

A. Animal

The study was approved by the National Cerebral and Cardiovascular Center Research Institute Animal Care and Use Committee, and all procedures met the National Institutes of Health guidelines for animal care.

Sprague-Dawley rats (male 400-450 g) were housed three per cage under a 12-h light-dark cycle with food and water available ad libitum.

B. Anesthesia, surgical preparation, and ECC

The animals were anesthetized with pentobarbital spdium (50 mg/kg body weight, intraperitoneal injection) and placed in the supine position with rectal thermocouple in place. Then, orotracheal intubation was performed using a 14G cannula (Insyte BD Medical, Sandy, UT, USA) and rats were ventilated with a respirator (Model SN-480-7, Shinano Seisakusho Co., Ltd, Tokyo, Japan). Ventilation was volume controlled at a frequency of 70/min, a tidal volume of 8-10 mL/kg body weight, and 40 % of inspired oxygen fraction. Rectal temperature was maintained at 36 ℃ throughout the experiment. Arterial blood pressure was monitored (Model 870, PowerLab system, AD Instruments, Castle Hill, NSW, Australia) via the femoral artery, which was cannulated with polyethylene tubing (SP-31 Natsume Seisakusho Co., Ltd, Tokyo, Japan). The left common carotid artery with a polyethylene tubing (SP-55 Natsume Seisakusho Co.) to serve as the arterial inflow cannula for the ECC circuit. 500 IU/kg heparin sodium was administered after placement of this cannula. A 16 G cannula (Insyte BD Medical) was advanced through the right external jugular vein into the right atrium and served as a conduit for venous outflow .

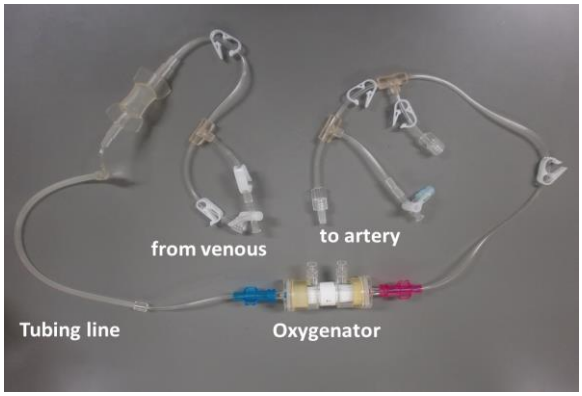

Figure 1. The small animal ECC system.

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The ECC circuit consisted of a membranous oxygenator (Senko Medical Co., Ltd, Osaka, Japan), tubing line (Senko Medical Co., Ltd) and roller pump (Micro tube pump MP-3 Tokyo Rikakikai Co., Ltd, Tokyo, Japan). The ECC circuit was primed by 14 ml of Ringer's solution bicarbonate and 1 ml (1000 IU) of heparin, total priming volume was 15 ml (Fig.1). Figure 2 shows the small animal ECC model schema.

C. *Experimental design*

The animals were divided into two groups: SHAM group (n=5), ECC group (n=7). The SHAM group received surgical preparation only without ECC. ECC pump flow was initiated and maintained at 70 mL/kg/min. Arterial pressure of carbon dioxide (PaO_2) and arterial pressure of oxygen (PaO_2) were maintained at 35-45 mmHg and 300-400 mmHg, respectively. Blood samples were collected at three defined time points, before ECC (pre-ECC), 60 min after initiation of ECC and 120 min after initiation of ECC (end-ECC).

To evaluate the inflammatory responses, TNF-α, IL-6, and IL-10 were measured (ELISA kit, R&D Systems, Minneapolis, MN, USA). The biochemical markers for evaluating organ damage (17), LDH, AST, and ALT were measured (DRI-CHE M 7000, Fujifilm, Kanagawa, Japan). Blood gases, pH, hemoglobin concentration, and electrolytes were also measured. Animals in which the hemoglobin level declined to less than 7 g/dL at any point were excluded from the study. All animals were sacrificed at the end of ECC by myocardial potassium injection and the left lung was harvested and divided into three parts. The superior third was used for the calculation of W/D ratio. The lung block was weighed before and after desiccation for 72 h in a drying oven at 70°C.

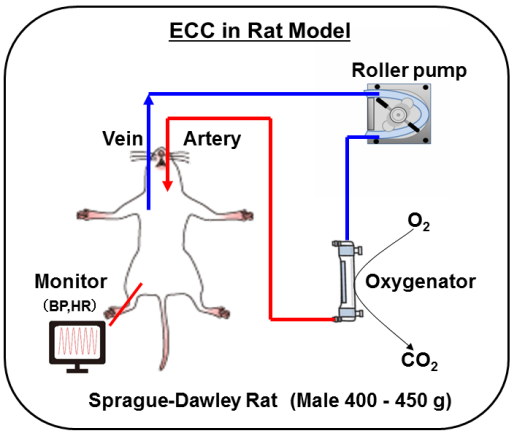

Figure 2. The small animal ECC model schema.

D. *Statistics*

All data are expressed as mean ± standard deviation. Comparison among groups was performed using analysis of variance. Fisher Protected Least Significant Difference post hoc test was used for subsequent comparison between groups at the same time. All statistical analyses were performed using Stat-View 5.0 (Abacus Concepts, Berkeley, CA, USA). Significance was set at $P < 0.05$.

III. RESULTS

Table 1 shows the changes in hemodynamic variables, Hb concentration and PaO_2 and $PaCO_2$ in SHAM and ECC groups during experiments. Mean arterial pressure (MAP) and Hb were significantly decreased during experiment in ECC groups.

TABLE I. HEMODYNAMIC VARIABLES, HB AND BLOOD GAS PARTIAL PRESSURES BEFORE AND DURING ECC

	Group	Pre-ECC	ECC 60 min	ECC 120 min
MAP (mmHg)	SHAM	103 ± 3	100 ± 5	104 ± 3
	ECC	105 ± 5	80 ± 3 †	76 ± 3 †
HR (beat/min)	SHAM	385 ± 15	385 ± 11	381 ± 7
	ECC	406 ± 9	358 ± 8	363 ± 8
PaO2 (mmHg)	SHAM	113 ± 8	106 ± 7	105 ± 6
	ECC	103 ± 8	464 ± 17 †	461 ± 16 †
PaCO2 (mmHg)	SHAM	38 ± 1	37 ± 1	40 ± 1
	ECC	40 ± 1	37 ± 1	36 ± 1
Hb (mg/dL)	SHAM	15.3 ± 1.0	15.2 ± 0.5	14.5 ± 0.4
	ECC	15.4 ± 0.2	10.1 ± 0.5 †	9.8 ± 0.4 †

Variables are expressed by mean ± standard error.
† $P < 0.05$ versus SHAM group at the same time.

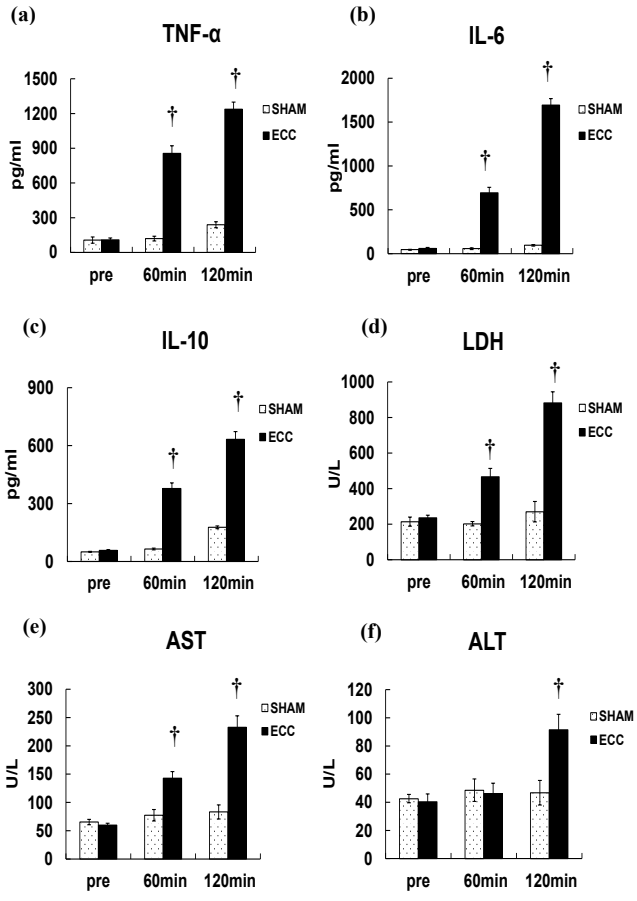

Figure 3. Serum TNF-α (a), IL-6 (b), IL-10 (c), LDH (d), AST (e), ALT(f).
† $P < 0.05$versus SHAM group at the same time periods.

The PaO2 level was much higher in the ECC group (～460 mmHg) than in the SHAM group (～130 mmHg), while no statistical difference was found in the PaCO2 level between these groups.

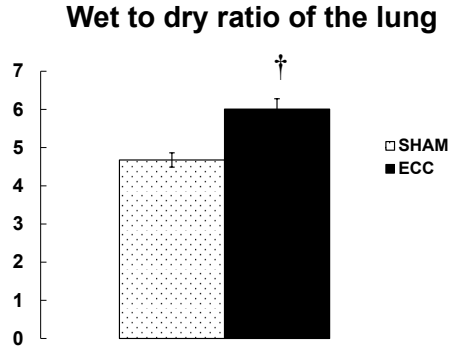

Figure 4. Wet to dry ratio of left lung at the end of ECC.
† P < 0.05versus SHAM group

Before ECC, the serum levels of inflammatory and biochemical markers were not statistically different among the SHAM and ECC groups. Serum inflammatory and biochemical markers remained unchanged during experiment periods in the SHAM group. In the ECC group, the cytokines and increased significantly, reaching a maximum (TNF-α : 1237 ± 62 pg/ml , IL-6 : 1695 ± 73 pg/ml, IL-10: 632 ± 40 pg/ml) at the end of ECC (Fig. 3a-c)

In the ECC group, the levels of biochemical markers significantly increased (LDH : 447±48 U/L, AST : 143±12 U/L, ALT : 46±7 U/L) 60 min after the ECC initiation and increased further (LDH : 882±62 U/L, AST : 233±20 U/L, ALT : 92±11 U/L) 120 min after the ECC initiation (Fig. 3d-e).

The ECC groups showed significantly higher W/D ratio than the SHAM group. (SHAM group : 4.68±0.08, ECC group : 6.01±0.10) (Fig.4).

IV. Discussion

In this study, our small animal ECC system was able to maintain adequate levels of blood gases ($PaCO_2$:35-45 mmHg, PaO_2: 300-400 mmHg), Hb (around the 10 g/dl level) and blood pressure (Mean arterial pressure more than 70 mmHg). Previous models have required high priming volumes to achieve acceptable hematocrit concentrations during the experiment. On the other hand, our model offers the advantage of a low priming volume not requiring transfusion in ECC group rats. Most previous research was performed in isolated heart models (e.g., Langendorff's method) [2]. By using our small animal ECC model, due to its minimal invasiveness and ease of recoverability, short- and long-term effects of ECC time, temperature (hypothermic condition), blood contact surface area and potentially also direct gene transfer on myocardial function and histological outcomes can be assessed better than in isolated heart models. While these models allow investigating the immediate effects of therapeutic interventions or different cardioplegia solutions, they preclude the assessment of long-term histological, biochemical, or functional outcomes. Survival studies using dogs or pigs [3,4] have been performed but are limited due to sample size and costs.

The present data showed that during the serum cytokine levels (TNF-α, IL-6 and IL-10) and biochemical markers (LDH, ALT, AST) were significantly elevated in the ECC group compared with the SHAM group, indicating that organ damage and a systemic inflammatory response occurred in our rat ECC model. During ECC, blood pressure and Hb were maintained around 80 mmHg and 10 g/dl, respectively. From these data, our rat ECC model is considered to be equivalent to the established human ECC, which is often associated with systemic inflammation and organ damage [5-7].

The significant systemic inflammatory responses occurred, reaching a maximum at the end of ECC. Additionally, the biochemical markers reflecting organ damages significantly increased 60 min after the ECC initiation and increased further 120 min after the ECC initiation. The significant increase in the W/D ratio which suggests pulmonary edema [8] is consistent with the previous clinical data [9]. From these data, our rat ECC model is considered to be equivalent to the established human ECC, which is often associated with systemic inflammation and organ damage [10].

It has been suggested that the factors responsible for the inflammatory response during ECC are blood contact with the surface of the extracorporeal circulation unit, endotoxemia, surgical trauma, ischemic reperfusion injury, and blood loss [11]. Many studies showed the walls of the ECC circuit activate white cells, platelets and the complement system. The increase in cytokines, such as interleukins and necrosis factor [12], aggravates the inflammatory response [13]. These complex interactions during ECC lead to further inflammation [13]. In our rat ECC models, the insufflation of hydrogen which selectively reduces the hydroxyl radical could decrease the levels of serum cytokines and biochemical markers, and the W/D ratio of the lung, suggesting that this radical contributes toward promoting the systemic inflammatory responses and organ damages during ECC [8].

Our previous study showed the selective reduction of hydroxyl radical with hydrogen gas attenuates both pro- and anti-inflammatory cytokines, suggesting that this radical acts to non-selectively increase these cytokines [8]. In addition, our new finding is that this increase in the W/D ratio was attenuated with hydrogen gas insufflation. Because ECC increases pulmonary vascular permeability, it is possible that hydrogen gas insufflation attenuates the injury of pulmonary vascular endothelium by scavenging reactive oxygen species and reducing the increase in vascular permeability during ECC. Although the detailed mechanism of the abovementioned anti-inflammatory effects of hydrogen gas insufflation was not elucidated in the previous study[8], this treatment may potentially serve as a novel clinical intervention in reducing the ECC-induced systemic inflammation. Solution of the inflammation mechanism during ECC require future research.

We have to study of due to its minimal invasiveness and ease of recoverability, short- and long-term effects of ECC time, temperature (hypothermic condition), blood contact surface area and potentially also direct gene transfer on myocardial function and histological outcomes. In addition, the model allows for the investigation of unique animal strains with varying susceptibility to myocardial injury depending on either their genetic background or disease (e.g., diabetes, old age, hypertension).

There are some limitations to this current model. Although our model closely resembles current clinical standards with respect to the ECC circuit, a number of potentially important differences to the clinical setting are present. Median sternotomy, direct surgery on the heart involving aortic cross-clamping, and cardiac arrest with the use of cardioplegia were not performed. Similarly, the absence of significant atheromatous disease and the complex comorbidities seen in patients undergoing coronary artery bypass graft surgery are limitations.

V. Conclusion

In this study, we developed a miniature ECC model and applied the system to the rat. In our rat ECC models, we demonstrated that adequate levels of blood gases and Hb, and blood pressure were maintained and that the systemic inflammatory response and organ damages including pulmonary edema were induced associated with the production of cytokines. We considered that our rat ECC model is equivalent to the established human ECC, which is often associated with systemic inflammation and organ damage. This miniature ECC model could be a very useful approach for studying the mechanism of pathophysiology during ECC and basic assessment of the ECC devices.

Acknowledgment

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