Artificial
Organs

Insufflation of Hydrogen Gas Restrains the Inflammatory Response of Cardiopulmonary Bypass in a Rat Model

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Abstract: Systemic inflammatory responses in patients receiving cardiac surgery with the use of the cardiopulmonary bypass (CPB) significantly contribute to CPB-associated morbidity and mortality. We hypothesized that insufflated hydrogen gas (H_2) would provide systemic anti-inflammatory and anti-apoptotic effects during CPB, therefore reducing proinflammatory cytokine levels. In this study, we examined the protective effect of H_2 on a rat CPB model. Rats were divided into three groups: the sham operation (SHAM) group, received sternotomy only; the CPB group, which was initiated and maintained for 60 min; and the CPB + H_2 group in which H_2 was given via an oxygenator during CPB for 60 min. We collected blood samples before, 20 min, and 60 min after the initiation of CPB. We measured the serum cytokine levels of (tumor necrosis factor-α, interleukin-6, and interleukin-10) and biochemical markers (lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase). We also measured the wet-to-dry weight (W/D) ratio of the left lung 60 min after the initiation of CPB. In the CPB group, the cytokine and biochemical marker levels significantly increased 20 min after the CPB initiation and further increased 60 min after the CPB initiation as compared with the SHAM group. In the CPB + H_2 group, however, such increases were significantly suppressed at 60 min after the CPB initiation. Although the W/D ratio in the CPB group significantly increased as compared with that in the SHAM group, such an increase was also suppressed significantly in the CPB + H_2 group. We suggest that H_2 insufflation is a possible new potential therapy for counteracting CPB-induced systemic inflammation. **Key Words:** Cardiopulmonary bypass—Rat cardiopulmonary bypass model—Systemic inflammatory response—Hydrogen gas.

Extracorporeal life support devices, such as the cardiopulmonary bypass (CPB), preserve the patient's life by providing adequate oxygen supply and blood flow to vital organs (1,2). However, cardiac surgery with the use of CPB is often accompanied by a systemic inflammatory response, contributing significantly to the morbidity and mortality during CPB (3–5).

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Possible factors responsible for the inflammatory response are the blood contact with the surface of the extracorporeal circulation unit, endotoxemia, surgical trauma, ischemic reperfusion injury, and blood loss (6,7). The increase in cytokines, such as interleukins, necrosis factor, and bradykinin (8,9), aggravates the inflammatory response during CPB (10–12).

Recent studies have shown that drinking hydrogen enhanced water prolongs survival of cardiac allografts and may protect cardiac allografts from allograft vasculopathy in rats model (13). The inhalation of hydrogen gas (H_2) has been shown to reduce infarct size in the rat model of myocardial (14) and cerebral (15) infarction through antioxidant effects.

We hypothesized that insufflation of H_2 would attenuate the systemic inflammatory response with a

reduction of inflammatory cytokine levels, providing protective effects against organ tissue damage during CPB. Therefore, in this study, we investigated the effect of H_2 insufflation on levels of serum cytokines, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL-10), and biochemical markers lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in a rat CPB model. In addition, we measured wet-to-dry weight (W/D) ratio of the lung.

MATERIALS AND METHODS

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.

Anesthesia, surgical preparation, and CPB

The animals were anesthetized with pentobarbital sodium (50 mg/kg body weight intraperitoneal injection), placed in the supine position with electrocardiograph monitoring and rectal thermocouple in place. Then, a tracheostomy and intratracheal 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 60/min, a tidal volume of 8–10 mL/kg body weight, and a positive end expiratory pressure of 3 cm H_2O. Rectal temperature was maintained at 36°C throughout the experiment. Arterial blood pressure was monitored (Model 870, PowerLab system, AD Instruments, Castle Hill, 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., Ltd) served as the arterial inflow cannula for the CPB circuit. Heparin sodium 500 IU/kg was administered after placement of this cannula. A 16G cannula (Insyte BD Medical) was advanced through the right external jugular vein into the right atrium and served as a conduit for venous outflow.

The CPB 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) primed by 8 mL of Ringer's solution, 3 mL of mannitol, 3 mL of sodium bicarbonate, and 1 mL (1000 IU) of heparin.

Experimental design

The animals were divided into three groups: Sham operation (SHAM, negative control), CPB (positive control), and CPB + H_2 groups. The SHAM group ($n = 5$) received sternotomy only. In the CPB group ($n = 7$), CPB was initiated and maintained for 60 min. In the CPB + H_2 group ($n = 7$), hydrogen gas was added into the oxygenator during CPB at a concentration of 14 000 ppm (O_2 flow : H_2 flow = 1:1) for 60 min.

CPB was initiated and maintained at 60 mL/kg/min. Arterial pressure of carbon dioxide ($PaCO_2$) and arterial pressure of oxygen (PaO_2) were maintained at 35–45 mm Hg and 300–400 mm Hg, respectively. Blood samples were collected at three defined time points, before CPB (pre-CPB), 20 min after initiation of CPB and 60 min after initiation of CPB (end-CPB).

To evaluate the inflammatory responses (16), 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-CHEM 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 CPB 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.

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 StatView 5.0 (Abacus Concepts, Berkeley, CA, USA). Significance was set at $P < 0.05$.

RESULTS

Before CPB, the serum levels of inflammatory and biochemical markers were not statistically different

TABLE 1. *Hemodynamic variables, Hb and blood gas partial pressures before and during CPB*

	Group	Pre-CPB	CPB 20 min	CPB 60 min
MAP (mm Hg)	SHAM	119 ± 10	100 ± 13	107 ± 11
	CPB	115 ± 16	96 ± 18	73 ± 19*
	CPB + H_2	111 ± 18	92 ± 14	67 ± 11*
HR (beat/min)	SHAM	387 ± 39	374 ± 38	389 ± 26
	CPB	396 ± 29	379 ± 37	341 ± 55
	CPB + H_2	390 ± 34	365 ± 23	340 ± 23
PaO_2 (mm Hg)	SHAM	102 ± 11	101 ± 9	99 ± 10
	CPB	100 ± 3	383 ± 30*	362 ± 29*
	CPB + H_2	99 ± 9	370 ± 35*	351 ± 47*
$PaCO_2$ (mm Hg)	SHAM	38 ± 4	40 ± 5	36 ± 6
	CPB	41 ± 2	34 ± 6	35 ± 5
	CPB + H_2	41 ± 4	36 ± 3	38 ± 4
Hb (mg/dL)	SHAM	15.3 ± 2.1	15.2 ± 1.0	14.5 ± 0.9
	CPB	14.3 ± 1.3	9.9 ± 1.1*	9.4 ± 1.0*
	CPB + H_2	15.0 ± 1.7	9.8 ± 1.5*	9.5 ± 0.9*

Variables are expressed by mean ± standard deviation.
* $P < 0.05$ versus SHAM group at the same time.
MAP, mean arterial pressure.

among the SHAM, CPB, and CPB + H_2 groups. During CPB, systemic arterial blood pressure and heart rate were unaffected by H_2. Table 1 presents the changes in hemodynamic variables, hemoglobin (Hb) concentration and PaO_2 and $PaCO_2$ from both CPB and SHAM groups during experiments.

Serum inflammatory and biochemical markers remained unchanged during experiment periods in the SHAM group. In the CPB group, all the systemic inflammatory markers increased significantly, reaching a maximum (TNF-α: 1347 ± 199 pg/mL, IL-6: 1763 ± 297 pg/mL, IL-10: 1208 ± 228 pg/mL) at the end of CPB. However, in the CPB + H_2 group, the increase in the levels was significantly suppressed by 53~57% compared with the CPB group (Fig. 1a–c).

In the CPB group, the levels of biochemical markers significantly increased (LDH: 916 ± 263 U/L, AST: 128 ± 42 U/L, ALT: 60 ± 17 U/L) 20 min after the CPB initiation and increased further (LDH: 1210 ± 289 U/L, AST: 201 ± 30 U/L, ALT: 147 ± 43 U/L) 60 min after the CPB initiation as compared with the other groups. In the CPB + H_2 group, the elevated levels of biochemical markers were significantly suppressed by 55~65% 60 min after the CPB initiation as compared with the CPB group (Fig. 1d–f).

The CPB groups showed significantly higher W/D ratio than the SHAM group. However, the increase in W/D ratio was significantly suppressed in CPB + H_2 group (SHAM: 4.67 ± 0.19, CPB: 5.59 ± 0.18, CPB + H_2: 5.04 ± 0.21) (Fig. 2).

DISCUSSION

The present data showed that during CPB the serum cytokine levels (TNF-α, IL-6, and IL-10) and biochemical markers (LDH, ALT, and AST) were significantly elevated in the CPB group compared with the SHAM group, indicating that a systemic inflammatory response and organ damage occurred in our rat CPB model. During CPB, blood pressure and Hb were maintained around 80 mm Hg and 10 g/dL, respectively. From these data, our rat CPB model is considered to be equivalent to the established human CPB, which is often associated with systemic inflammation and organ damage (5,18,19).

Possible factors responsible for the inflammatory response during CPB are blood contact with the surface of the extracorporeal circulation unit, endotoxemia, surgical trauma, ischemic reperfusion injury, and blood loss (6,7). Many studies showed the walls of the CPB circuit activate white cells, platelets, and the complement system. Activated leukocytes release cytotoxic agents and reactive oxygen species (ROS) associated with the systemic inflammation and organ damage (20–22). The increase in cytokines, such as interleukins and necrosis factor (8,9), aggravates the inflammatory response (10–12). These complex interactions during CPB lead to further inflammation (10–12).

In this study, we used H_2 that selectively reduces the hydroxyl radical, the most cytotoxic of ROS, and effectively protected cells (15). H_2 is known to have advantages as a potential antioxidant: it rapidly

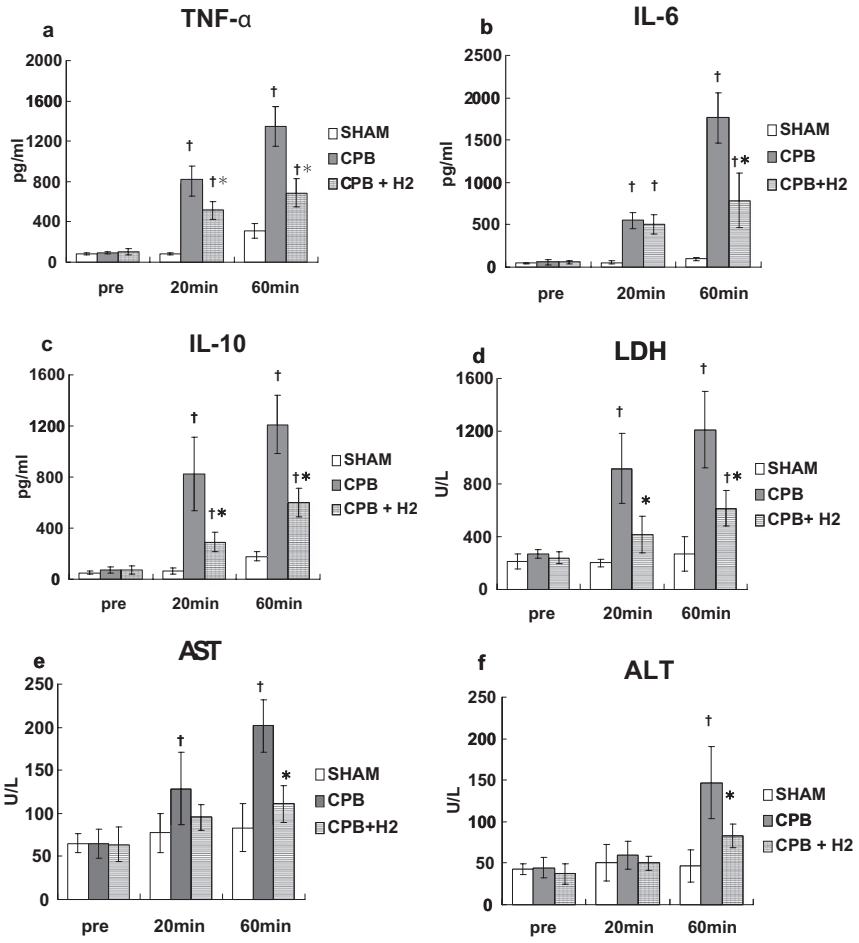

FIG. 1. Serum tumor necrosis factor (TNF)-α (a), interleukin (IL)-6 (b), interleukin (IL)-10 (c), lactate dehydrogenase (LDH) (d), aspartate aminotransferase (AST) (e), and alanine aminotransferase (ALT) (f). †$P < 0.05$ versus SHAM group, *$P < 0.05$ versus CPB group at the same time periods.

diffuses into tissues and cells and does not affect ROS that function in cell signaling, and thereby, has little adverse effects (15,23). We showed for the first time that H_2 insufflation significantly suppressed the elevated levels of serum cytokines (TNF-α, IL-6, and IL-10) and biochemical markers (LDH, AST, and ALT) during CPB. Possible mechanisms for the decrease in biochemical markers is that H_2 insufflation suppressed the cell damages due to the direct action of the hydroxyl radical (15,23). Because ROS is known to trigger a cytokine cascade initiated by TNF-α release (24), it is also possible that H_2 insufflation suppressed cytokine generation via the ROS-scavenging effect. Notably, a recent study suggested H_2 inhalation reduces infarct size by scavenging ROS in a rat model of myocardial ischemia-reperfusion injury (14). In addition, drinking hydrogen enhanced water protected cardiac and aortic allograft recipients from allograft vasculopathy purportedly via antioxidant and anti-inflammatory effects (13). Considering these previous findings and the present data together, we suggest that H_2 insufflation not only attenuates the direct cell-damaging effect of ROS, but also inhibits the proinflammatory cytokine generation, reducing biochemical markers reflecting organ damage in the rat CPB model.

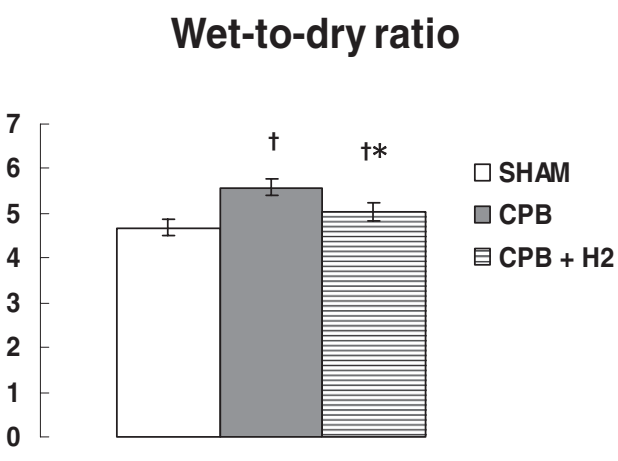

FIG. 2. Wet-to-dry ratio of the left lung. †$P < 0.05$ versus SHAM group, *$P < 0.05$ versus CPB group.

In this study, rat CPB was also maintained under nonphysiological hyperoxic conditions as used in clinical CPB. Lee and Choi (25) previously showed that hyperoxia induces oxidative cell damage by promoting the formation of ROS and the expression of inflammatory cytokines (25). Therefore, it is highly likely that hyperoxia contributed partly to the increase in the serum cytokine and biochemical markers in our rat CPB model. Hence, H_2 insufflation may attenuate the hyperoxia-induced formation of ROS and cytokines through the antioxidant effects.

It is generally known that hemolysis is induced by mechanical stress during CPB (26). Therefore, it is possible that biochemical markers (LDH, AST, and ALT) were not reduced in CPB to the level observed in SHAM rats in part because H_2 insufflation does not reduce the mechanical stress-induced increase in hemolysis.

The present study showed that the W/D ratio of the lung increased during CPB. These data are consistent with a previous study (27) that showed an increase in the W/D ratio of the lung and pulmonary edema in a rat CPB model. Our new finding is that this increase in the W/D ratio was attenuated with H_2 insufflation. Because CPB increases pulmonary vascular permeability (28), it is possible that H_2 insufflation attenuates the injury of pulmonary vascular endothelium by scavenging ROS and reducing the increase in vascular permeability during CPB.

Although the detailed mechanism of the abovementioned anti-inflammatory effects of H_2 insufflation was not elucidated in the present study, this treatment may potentially serve as a novel clinical intervention in reducing the CPB-induced systemic inflammation.

CONCLUSIONS

This study demonstrated that systemic inflammatory response and organ damage including pulmonary edema were induced in the rat CPB model and that H_2 insufflation provided anti-inflammatory and organ-protective effects. We propose that H_2 insufflation could be a potential clinical therapy for counteracting CPB-induced systemic inflammation and organ damage. We consider that this rat CPB model is equivalent to already established human CPB and is useful for studying the mechanism of pathophysiological changes during artificial perfusion.

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