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CONTINUOUS VENOVENOUS HEMOFILTRATION IMPROVES ENDOTOXIN INDUCED LUNG INJURY IN PIGS -EVALUATION WITH ULTRASONOGRAPHY-

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Abstract To investigated the effect of high volume continuous hemofiltration (HVHF) on the lung lesion observed with transesophageal echocardiography (TEE) in endotoxin-induced acute lung injury in piglets. Piglets weighing 20-30kg. Interventions: After endotoxin of 20 μg/kg intravenous administration, animals were randomly assigned to control group (n=7) and HVHF group (n=7). In both group, filtration was performed for five hours and zero balanced. Filtration flow was 150 ml/hr in HVHF group and 0ml/hr in control group. The area of the lesion, resistive index and maximal blood velocity in regional pulmonary artery were estimated using TEE. High mobility group B1 protein (HMGB1), cortisol and catecholamine concentrations were measured in plasma at endotoxin 5 hours. Measurements of blood gas, hemodynamics and TEE images were obtained every hour. The value of PaO_2, density area and resistive index were 70.0±8.8 mmHg, 10.7±4.5 cm^2 and 0.61±0.23 in control group and 120.4±20.3 mmHg, 3.3±2.0 cm^2 and 0.88 ±0.22 in HVHF group at endotoxin 5 hours respectively (mean±SD). PaO_2 and resistive index increased, and density area decreased in HVHF group than those of control group significantly ($p<0.05$, respectively). There was significant relationship between PaO_2 and density area ($r=0.76$, $p<0.01$). There was no significant difference in hemodynamics, HMGB1, cortisol and catecholamines concentration in plasma and morphometry between control group and HVHF group. In conclusion, these results suggest that non-specific blood purification with HVHF improves arterial oxygenation and lung image observed with TEE in endotoxin-induced acute lung injury in piglets.

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Introduction

Sepsis is a major cause of death in intensive care units. Arterial hypoxemia is common in sepsis and frequently occurs without a direct inflammatory process present within the lungs. Infusion of endotoxin or tumor necrosis factor mimics the pathophysiologic changes observed in sepsis and has been shown to result in arterial hypoxemia and acute lung injury in experimental animal[1]. Patients with sepsis demonstrate elevated plasma concentrations of endotoxin and other proinflammatory mediators, thus suggesting that systemic mediator release is associated with the indirect organ injury observed in sepsis.

Recently hemofiltration has been investigated as a potential supportive therapy that might be effective in removing a number of circulating proinflamatory molecules in clinical sepsis. Several studies have evaluated the effects of high volume hemofiltration (HVHF) on hemodynamics during septic shock in animal models[2,3]. Therefore, we hypothesized that HVHF would reduce the concentrations of inflammatory mediators in the plasma and correct the instability of hemodynamics and reduce lung inflammations in experimental acute lung injury.

Ultrasonography has been used to evaluate acute lung injury. Ultrasonography provide direct image of the lung region[4]. Area of density has been adapted the indices of ARDS. TEE was

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reported as useful method to observe region of injured lung in clinical study[5,6]. TEE seems an excellent method to evaluate the lung lesion with ultrasonography in large animals. Therefore we used TEE to evaluate the effect of endotoxin on piglet lung and the effect of HVHF on lung lesion.

Methods

This study was approved by our institutional Animal Experiment Committee. Piglet of both sexes weighing 25-30 kg was involved in this study. Animals were anesthetized using ketamine 40 mg/kg intramuscularly and intubated with 7 mm tracheal tube. Anesthesia was maintained with pentobarbital and vecuronium. Ventilation was started at a tidal volume of 10 mlk/kg, respiratory rate between 20 and 25 breath/ min and ZEEP. Polyethylen catheters were placed in the right carotid artery. A 7.5 Fr Swan-Ganz catheter was positioned in the proximal pulmonary artery via the right jugular vein. A 14 Fr double was inserted into the left jugular vein and served as hemofiltration access. Electrical heart activity was derived from needle electrode and body temperature from Swan-Ganz catheter.

The extracorporeal circuit consisted of a roller pump with air detector, bubble trap and pressure limiter, blood pump lines and a hemofilter. Hemofeel® is a polysulphone hollow fiber filter with a cutoff point of less than 50,000 Da. Hemofiltration was performed from the left cervical vein to cervical vein by use of a double lumen catheter. The speed of the roller pump was adjusted to achieve an ultra filtration rate of 150 ml/kg/hr. The ultra-filtered volume was continuously weighed with a balance and replaced before the filter with warmed acetate-buffered electrolyte substitution fluid.

A baseline measurement animal received an intravenous infusion of 20 μg/kg endotoxin over 1 hour. After endotoxin infusion, animals were randomly assigned to HVHF or control groups. The animals were monitored thereafter for 5 hr and killed with a bolus injection of 20 ml KCl. Ringer lactate was started at a rate of 7ml/kg/hr intravenously. Body temperature was kept constant around 37-38 ℃ by cooling or warming the circuit. Measurement of hemodynamics, blood gas, and metabolic variables were obtained every hour. All other measurement were taken at the end of HVHF.

Systolic arterial pressure, systolic pulmonary pressure, central venous pressure were continuously monitored using biomedical amplifiers. The expiratory tidal volume was measured with a flowmeter, and static lung compliance was calculated as (Pplateau-Ppeep) /Vtexp where Pplateau was inspiratory plateau pressure, Ppeep was positive end-expiratory pressure, and Vtexp was expiratory tidal volume. Cardiac output was determined by thermodilution technique. Blood samples were immediately analyzed for arterial and mixed venous oxygen and carbon dioxide tension and saturation.

TEE was performed using an ultrasound system (Hewlett Packard SONOS 1500; Andover, MA) equipped with a 5-MHz 64-element transesophageal multiplane echoprobe and recorded on 0.5-inch vide tape. The lower left lung area was observed through the descending aorta at the low esophageal position. The four chamber view of the heart was observed and the probe was rotated 90° clockwise. Pulmonary blood flow was observed using pulse Doppler method. Pulmonary artery and vein flow were differentiated using color Doppler technique. Maximum blood flow velocity and velocity time integral of the blood vessel were evaluated in both regional pulmonary artery and vein. Resistive index ([peak systolic velocity-end diastolic velocity]/peak systolic velocity) was calculated in regional pulmonary artery. Sensitivity to low velocity flow was maximized by choosing the low velocity scale at 30cm/sec.

Lung morphometry, at the end of the experiment, the right middle lobe was excised and placed into 10% buffered formaline. Using standard techniques, paraffin and semithin sections were obtained and stained with hematoxylin-eosin stain and toluidin blue, respectively. Lung sections were examined by light microscopy by an experienced pathologist unaware of group or treatment assignment. Severity of alvolar congestion, bleeding atelectasis leukostasis, and perivascular edema was estimated, respectively, using a four grade scoring system, where 0=absence (<25% of maximum pathology), 1=mild (<50%), 2=moderate (50-75%) and 3=severe (>75%).

Blood samples were obtained from the arterial line. The blood was collected in EDTA-tube and then centrifuged and plasma was stored and frozen at -80℃. Sample aliquots of plasma were subjected to specific enzyme-linked immunosorbent assay for high mobility group B1 protein (HMBG1), using an antibody raised against HMGB1, according to the manufacture's instructions (Shino-test corporation, Japan, HMGB1 test kit). Cortisol was measured using a fluorometric method[6]. The recovery rate was 102%, the coefficient of variation was 1.5% and the minimum range of sensitivity was 5 ng. Catecholamines were measured using a gaschromatography-mass spectrometry[7].

All data are presented as mean±SD and analyzed using the paired Student's t-test or one-way analysis of variance. The Dunnet post test was performed if results of one-way analysis of variance were significant. The correlation coefficient between two variables was tested by linear regression analysis. A value of $p<0.05$ was considered to be significant.

Results

Endotoxin challenge induced sustained and progressive derangement of systemic and pulmonary hemodynamics, including tachycardia, systemic hypotension, pulmonary hypertension and low cardiac output. CVP and pulmonary artery wedge pressure were maintained with fluid substitution. Treatment with HVHF did not alter systemic or pulmonary hemodynamics (Table 1).

The PaO_2 decreased significantly 1 hr after completion of endotoxin infusion and maintained low below baseline. Endotoxin induced a widening of the shunt fraction at 1 hr after endotoxin, with further deterioration present the end of the experiment. There was significant difference in PaO_2 and shunt fraction between control and HVHF group (Figure 1,2). These beneficial effects of HVHF on gas were associated with an improvement in lung mechanics.

TEE can observe the vessels around the descending aorta. The density area observed at the left lung begun to appear after 2-3 hours from endotoxin administration and continued to increase up to hours (Figure 3). There was significant relationship between PaO_2 and density area ($p<0.05$). Regional pulmonary artery and vein could be observed 3-4 hour after endotoxin administration (Figure 4). Resistive index, velocity-time integral and maximum flow increased until 5 hours after endotoxin administration. Induction of HVHF reduced the change of these indices of blood flow ($p<0.05$, respectively) (Figure 5). These changes showed the HVHF cause more constricted vascular and reduced shunting blood flow and improve oxygenation.

The concentration of HMGB1 increased at 5 hours after endotoxin administration. The induction of HVHF did not cause changes by HVHF induction. The concentration of cortisol did not changes significantly. The concentration of catecholamines increased significantly especially in norepinephrine. HVHF did not change their concentrations (Table 2).

Endotoxin induced diffuse alveolar damage represented by alveolar bleeding, atelectasis,

Table 1. Changes of parameters during perfusion.

		baseline	ET 0hr	ET 1 hr	2 hr	3 hr	4 hr	5 hr
HR	control	130±24	178±21	180±22	191±28	186±25	187±36	191±35
(l/min)	HVHF	138±24	157±34	169±25	177±39	180±39	192±48	196±47
SBP	control	130±17	112±12	123±17	130±12	133±18	148±19	146±20
(mmHg)	HVHF	136±24	130±27	119±21	133±25	143±21	146±14	144±14
CO	control	2.77±0.81	1.90±0.50	1.77±0.56**	1.65±0.47**	1.82±0.79**	1.77±0.66**	1.92±0.56**
(l/min)	HVHF	3.00±0.97	2.11±0.62	2.09±0.76**	1.80±0.75**	1.82±0.75**	1.84±0.76**	1.86±1.13**
CVP	control	11.8±2.7	11.4±2.7	10.6±2.4	10.6±1.5	10.4±2.4	10.4±1.4	11.1±1.8
(mmHg)	HVHF	10.7±1.9	11.2±1.3	10.4±1.8	9.8±2.0	10.5±1.3	11.0±2.0	10.3±1.3
SPAP	control	38.8±8.7	45.4±7.7	53.4±7.1	54.3±5.6	52.7±7.1	51.7±6.5	53.4±6.8
(mmHg)	HVHF	34.2±4.8	43.0±4.0	51.8±3.0	51.4±2.5	52.0±4.5	51.6±5.0	50.3±3.3
PAWP	control	14.1±3.8	13.0±2.8	12.0±3.1	12.1±2.9	11.5±3.6	14.0±3.9	12.5±2.9
(mmHg)	HVHF	11.0±2.3	11.8±1.8	11.1±2.3	10.7±2.6	10.8±1.8	11.1±2.1	11.4±2.7
Shunt	control	3.31±1.70	9.67±8.44	12.67±9.27*	15.88±8.75*	18.03±9.99*	17.18±10.62**	21.30±8.80**
(%)	HVHF	4.80±1.53	5.14±2.49	5.05±2.45	8.83±7.19	8.91±5.76	7.68±3.24*#	7.38±2.72*#
Compliance	control	15.2±3.4	14.4±3.8	11.1±2.2*	10.9±2.1*	10.9±2.1*	10.9±2.1*	11.3±2.1*
(cc/cmH2O)	HVHF	16.6±6.5	12.8±3.3	12.4±2.9	11.4±2.6	11.9±2.6	11.7±2.3	11.4±2.5*
ETCO2	control	4.45±1.02	4.16±0.56	4.22±0.51	4.27±0.47	4.23±0.49	4.10±0.38	4.26±0.98
(%)	HVHF	4.52±0.84	4.15±0.62	4.31±0.56	4.08±0.79	4.14±0.55	3.8±0.65	3.88±0.25
Flow max	control					34.9±36.7	59.2±32.8	56.8±17.6
(cm/sec)	HVHF						31.2±34.7	45.3±21.7
VTI	control					5.01±7.15	5.73±6.70	9.86±5.86
(cm)	HVHF						1.16±1.50#	1.45±1.66#
BT	control	38.5±1.4	38.3±2.1	38.8±1.9	38.7±1.8	38.7±1.8	38.8±1.8	38.9±1.7
(℃)	HVHF	38.5±1.3	38.5±1.3	38.4±1.3	38.1±1.7	38.1±1.6	38.1±1.4	38.2±1.3

HR: heart rate, SBP: systolic blood pressure, CO: cardiac output, CVP:central venous pressure, SPAP:systolic pulmonary artery pressure, PAWP: Pulmonary artery wedge pressure, $ETCO_2$: endotidal CO2, Flow max: maximum flow velocity, VTI: velocity time integral, BT: body temperature,
* $p<0.05$, ** $p<0.01$ compared with baseline, # $p<0.05$ between groups, ET: endotoxin, ET 1hr: 1hr after completion of ET administration.

leukocyte sequestration, and perivascular edema within lungs at 5 hr after endotoxin challenges.

Discussion

Reports from clinical studies suggested that treatment with hemofiltration when used for non-renal application might be useful in improving respiratory and cardiac function. Honore PM[8] suggested that early initiation of hemofiltration in septic patients may improve hemodynamic and metabolic response. Bagshow ONT[9] reported that a significant improvement in respiratory function occurred in patients with multiple organ failure who had undergone a prolonged period of hemofiltration. However, respiratory and cardiac function effect vice versa, therefore, it remains incompletely understand whether hemofiltration exerts a specific effect on the injured lungs in sepsis. Recently Ullrich R[4] and Su X[5] reported the improvement of oxygenation during HVHF is independent of hemodynamics in animal ARDS studies. In our study we also found the improvement of oxygenation in endotoxin ARDS piglets without significant difference between control and HVHF group in hemodynamics.

"Renal dose" hamofiltration rate has successfully been used to treat acute renal failure for years. This does suffices for renal replacement therapy and can remove inflammatory mediators; however, it does not alter plasma levels of these mediators,

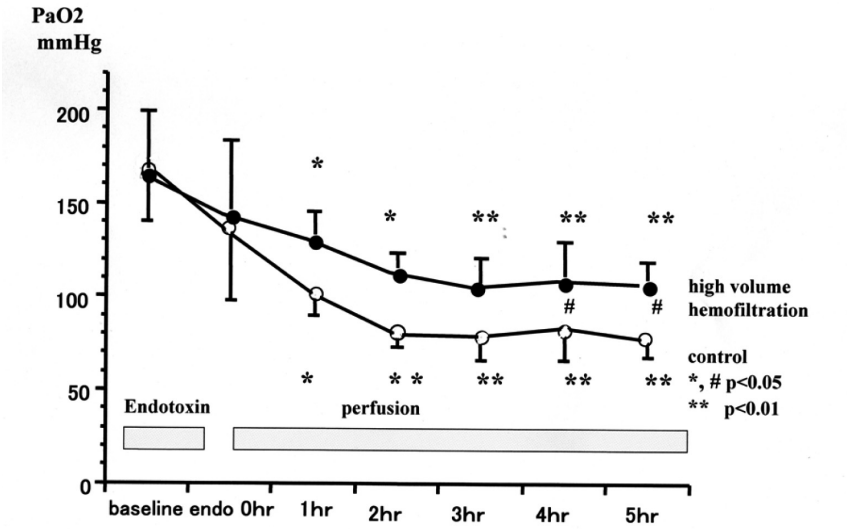

Figure 1 Effects of high volume hemofiltration on PaO_2.

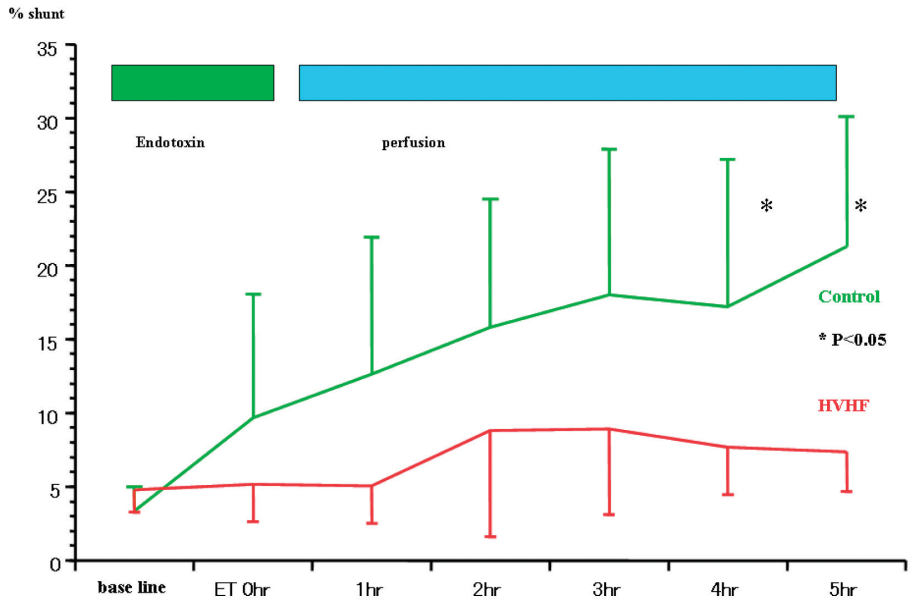

Figure 2 Changes of % shunt during hemofiltration.

suggesting that its ability to clear inflammatory mediators is suboptimal. Hence, the indication for its use in septic patients was abandoned, beyond its function to provide renal support in the presence of renal dysfunction. Ronco C and coworker[10] demonstrated survival benefits by increasing the haemofiltration does beyond the conventional renal does in patients with sepsis. Additionally benefits have been demonstrated in several animal models of sepsis[11]. High-volume haemofiltration, was thus conceived and applied in human sepsis. Findings of improvements in haemodynamics with decreased vasopressor requirements and trends toward improved survival are evidence that HVHF may be efficacious[12].

It is possible to observe some lung lesion

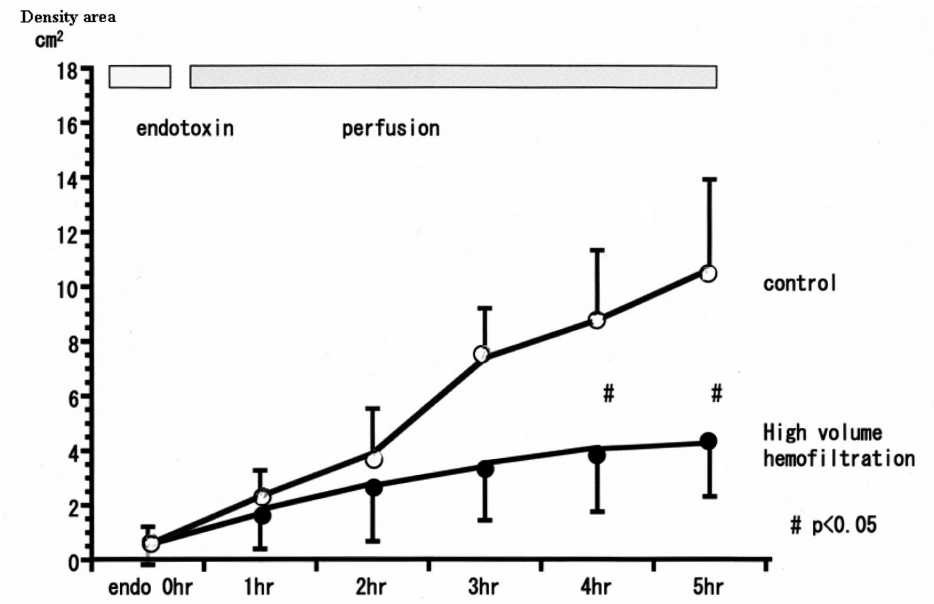

Figure 3 effects of high volume hemofiltration on density area.

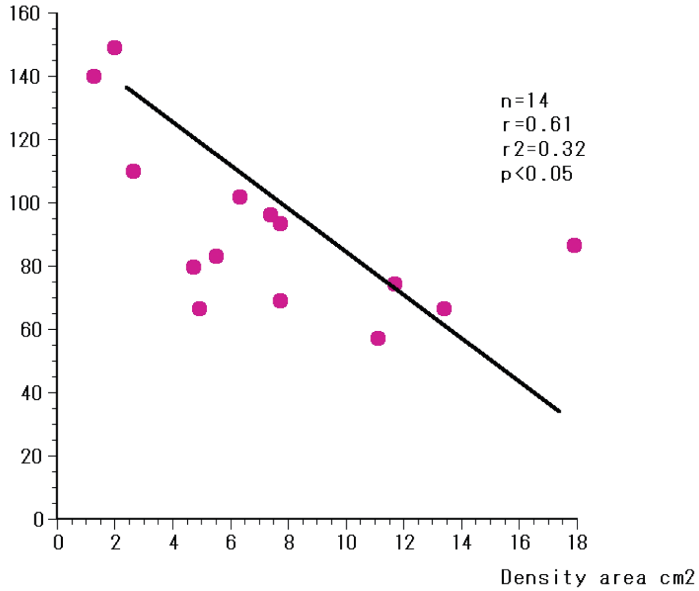

Figure 4 Relation between density area and PaO_2.

using transthoracic ultrasonography such as lung consolidation, pleural effusion, tumor and alveolar-interstitial syndrome[4,13,14]. Even observation of blood flow in pulmonary vessels was reported[15]. However, it is impossible to use transthoracic procedure to observe lung lesion in large animals. TEE has been originally used to observe the heart and the great vessels. However, the lungs located akin to the esophagus and the descending aorta can be used as windows to observe the dependent area in the left lung. Dorsal part of the lungs is the major lesion in acute lung injury patients. The observation of pleural effusion and atelectasis of the lungs has been reported[16,17]. We

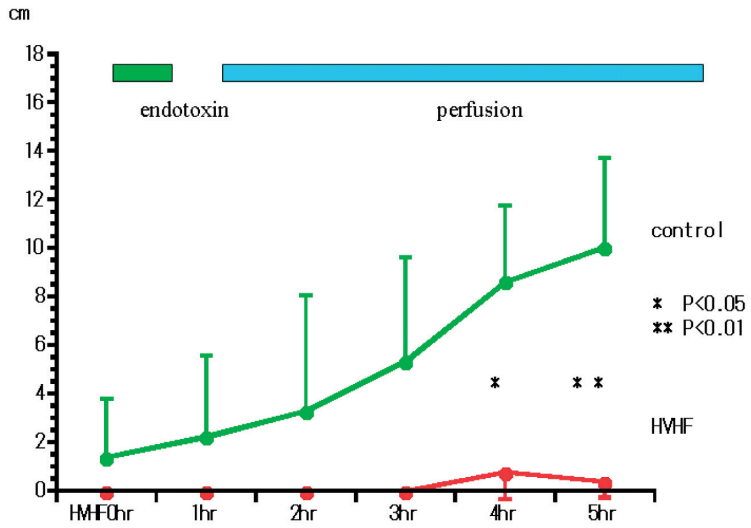

Figure 5 Changes of time-velocity integral during hemofiltration.

Table 2. Changes in the plasma concentration of mediators.

		baseline	ET 0hr	ET 5hr
cortisol	control	13.5±4.3	15.4±7.6	21.9±24.9
(μg/dl)	HVHF	18.4±5.6	19.8±5.0	18.0±5.2
epinephrine	control	110.6±93.1	226.5±259.0	436.8±467.5
(pg/ml)	HVHF	258.7±209.7	304.2±154.8	311.8±366.9
norepinephrine	control	147.5±57.8	258.7±117.2	805.8±548.3*
(pg/ml)	HVHF	228.4±147.1	429.7±475.2	1013.7±1038.4*
dopamine	control	95.6±58.0	92.2±41.4	173.0±106.3
(pg/ml)	HVHF	59.0±30.8	71.7±23.9	86.6±52.0
HMGB1	control	5.86±3.39	7.62±5.76	18.81±18.8*
(ng/ml)	HVHF	3.31±2.06	8.08±7.99	13.77±9.50*

Mean±SD, * p<0.05 vs baseline value, HMGB1: high mobility group box 1 protein, HVHF: high volume hemofiltration group, ET: endotoxin, ET 1hr: 1hr after completion of endotoxin administration.

reported that it was possible to observe density area in the dependent lung region in acute lung injury patients[5,18]. The disadvantage of TEE was the limitation of the observation filed. In this TEE technique the right lung was not observable. However, we reported that it is possible to assume the right lung lesion from the observed left lung lesion[5].

The pulmonary arteries and veins we observed seemed to belong to 3-4 generation by TEE image analysis. Resistive index has been used as the indices of distal impedance of a vessel. Yuan A et al[19] used resistive index to estimate constriction of segmental pulmonary artery. They reported that reactive vasoconstriction was most marked in obstructive pneumonia. Lenz F et al[20] found that the such index is a useful parameter in fetus's pulmonary vein. We also found significant relation between resistive index and oxygenation. We used time-velocity integral as the indices of regional pulmonary artery and venous flow. Time-velocity integral has been used to estimate

blood flow in pulmonary vein in fetuses[21]. Boyd SY et al[22] used time-velocity integral of anastomosed pulmonary vein to measure blood flow with TEE in single-lung transplantation patients. We found significant relation between oxygenation and time-velocity integral both in regional pulmonary artery and vein.

Cortisol and catecholamines are known as stress hormones and play important roles in the hemodynamics and metabolism of critically ill patients. Cortisol has a positive inotropic effect and maintains the vascular tone[23]. The molecular weight of cortisol is only 360, however the part of unbounded cortisol is small[24]. Around 0.2mg of cortisol is excreted from filter during normal hemofiltration. Catecholamine levels increase manifold above the baseline in critically ill patients. Hemodynamics during hemofiltration are maintained partly by increased catecholamines. Bellomo R et al reported the daily loss of catecholamines was minimal during hemofiltration[25]. In our study no difference in concentration of cortisol and catecholamines were seen between control and HVHF.

HMGB1 acts as a late mediator of sepsis and is identified as a new potential target for immunotherapy in sepsis. It plays an important role in developing acute lung injury in sepsis in clinical situation[26]. Mice showed increased HMGB1 concentration at 18 hr after endotoxin administration[27]. However, in our experiment piglet showed elevation of plasma HGMB1 even 6 hrs after endotoxin injection. Molecular of HMGB1 is 27KD and the effects of HVHF on HMGB1 were not studied precisely. We could not find difference of HMGB1 concentration between control group and HVHF group. No clear correlation between levels of HMGB1 and severity of infection was found in clinical study[28].

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