Possible clinical implications

Clinically, hepatoenteric ischemia-reperfusion carries a significantly high mortality. Although the beneficial effect of allopurinol or tungsten by suppressing xanthine oxidase has been reported in experimental and clinical situations (4, 37), these measures usually require some pretreatment. On the contrary, this study shows that sivelestat is effective when it is administered just before the establishment of ischemia. This is advantageous in many clinical situations, and further study is warranted to determine whether NE inhibition after reperfusion is effective for attenuating reperfusion injuries.

In conclusion, we found that a specific NE inhibitor suppressed postreperfusion neutrophil priming and subsequent intestinal, hepatic, and renal reperfusion injuries in rabbit models of descending aortic occlusion-reperfusion. It also attenuated pulmonary microvascular leaks, as evidenced by the reduced protein concentration in *post mortem* BALF.

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Infusion of the β-adrenergic blocker esmolol attenuates myocardial dysfunction in septic rats*

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Objective: Since β-blocker therapy is known to be effective in patients with an injured heart, such as infarction, we designed the present study to examine the protective effects of infusion of the β1-selective blocker esmolol on myocardial function in peritonitis-induced septic rats using an isolated working heart preparation.

Design: Randomized animal study.

Setting: University research laboratory.

Subjects: Thirty-one rats treated with cecal ligation and perforation to evoke peritonitis.

Interventions: After cecal ligation and perforation, rats were randomly allocated to the control group (normal saline 2 mL/hr, n = 11), low-dose esmolol group (10 mg/kg/hr, n = 10), or high-dose esmolol group (20 mg/kg/hr, n = 10). After obtaining blood samples for measurement of arterial lactate and tumor necrosis factor-α at 24 hrs, we assessed cardiac output, myocardial oxygen consumption, and cardiac efficiency (cardiac output × peak systolic pressure/myocardial oxygen consumption) at various preloads in an isolated perfused heart preparation.

Measurements and Main Results: Esmolol infusion did not cause an elevation of arterial lactate levels but reduced tumor necrosis factor-α concentrations vs. the control group ($p < .05$). Both cardiac output and cardiac efficiency in the esmolol-treated rats were significantly higher throughout the study periods vs. the control group ($p < .05$).

Conclusions: Esmolol infusion in sepsis improved oxygen utilization of myocardium and preserved myocardial function. (Crit Care Med 2005; 33:2294–2301)

Key Words: β1-adrenergic receptor; hyperdynamic sepsis; tumor necrosis factor-α; working heart preparation

Sepsis is a clinical syndrome that arises from an inappropriate and excessive systemic inflammatory response against infection (1). With adequate fluid resuscitation and pharmacologic interventions, systemic hemodynamics of sepsis is characterized by a hyperdynamic circulatory state, resulting in augmentation of oxygen supply to tissues. The importance of sufficient tissue oxygenation was recently addressed in a large-scale clinical trial in which patients at the early stage of sepsis were treated by aggressive management to optimize hemodynamic function (2). During the progression of sepsis, however, regional tissue dysoxia becomes evident and organ dysfunction including heart dysfunction develops (3). Several mechanisms are considered to be responsible for myocardial dysfunction in sepsis (4, 5). For example, proinflammatory cytokines like tumor necrosis factor (TNF)-α have been shown to play a consequential role in the pathogenesis of myocardial dysfunction in sepsis (6, 7). In addition, the number of β-adrenergic receptors was reduced in critically ill patients involving sepsis (8). Few specific strategies, however, have been demonstrated to restore myocardial dysfunction effectively in sepsis.

There is increasing evidence that β-blocker therapy during perioperative periods improves morbidity and mortality in high-risk patients with ischemic heart disease (9). Another study showed a tenfold decrease in the 30-day perioperative incidence of death from cardiac causes and nonfatal myocardial infarction in β-blocker-treated patients undergoing vascular surgery (10). Such protective effects of β-blocker for ischemic myocardium could be accounted for by an improved balance between oxygen supply and consumption (11), regulation of cytokine release (12, 13), and/or restoration of down-regulated β-receptor (14). A question remains, however, whether β-blocker therapy would improve myocardial dysfunction at a hyperdynamic stage of sepsis where cardiac work is augmented. We therefore designed the present study to examine if infusion of esmolol, a β1-selective adrenergic blocker, suppressed the progression of myocardial dysfunction in a normotensive, hyperdynamic model of septic rats.

METHODS

This study protocol was approved by the animal care and use committee of Keio University School of Medicine.

Animal Preparation. Thirty-one male Wistar rats, weighing 320–360 g, were studied after 3- to 7-day acclimatization periods in our laboratory. With sevoflurane anesthesia in oxygen, the jugular vein and carotid artery were cannulated with a catheter (PE50; Intermedic, Sparks, MD) under sterile conditions. Then a laparotomy was performed and a ligature was placed around the cecum immediately distal to the ileocecal valve. The cecum was then punctured twice with an 18-gauge needle. Fol-

*See also p. 2433.

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lowing this preparatory surgery, normal saline was infused at 2 mL/hr via the catheter for the next 24 hrs. Rats were allowed to move freely in cages and have water and laboratory chow *ad libitum*. This preparation was demonstrated to produce normotensive, hyperdynamic sepsis without apparent tissue hypoxia as previously described (15, 16). In addition, to determine *ex vivo* myocardial function of normal rats in our experimental setting, we studied seven sham rats, which received the same catheterization without cecal ligation and perforation (CLP), under the same study protocol described next.

Study Protocol. After the preparatory surgery, the animals were randomized into three groups: Group C (n = 11) received normal saline, group E-10 (n = 10) received esmolol infusion at 10 mg/kg/hr, and group E-20 (n = 10) received esmolol infusion at 20 mg/kg/hr for the next 24 hrs. Our pilot study using a P-U conductance system (ARIA-1, Millar, Houston, TX) demonstrated that esmolol at the rate of 20 mg/kg/hr reduced cardiac output to an approximately 20% lower level compared with the baseline in normal rats under general anesthesia and 10 mg/kg/hr did not significantly influence cardiac output values. At 1 and 24 hrs after randomization, blood samples were taken via the arterial catheter for measurements of white blood cell count, lactate, and proinflammatory cytokines. Twenty-four hours after randomization, animals were reanesthetized with sevoflurane in oxygen. After the intravenous injection of heparin (3000 IU/kg), the hearts of animals were rapidly excised, mounted on a nonrecirculating Langendorff apparatus to allow further preparation, and perfused with modified Krebs-Henseleit solution at 37°C (composition in mM: NaCl 120, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.25, $NaHCO_3$ 25, and glucose 11) as described previously (17). The perfusion buffer was equilibrated with a 95% oxygen/5% CO_2 gas mixture, resulting in a buffer Po_2 >400 mm Hg.

Spontaneous beating heart was used throughout the isolated-perfusion study periods. Following the placement of an aortic cannula, the left atrium was cannulated through the pulmonary vein to allow filling and contraction of the left atrium. With the left atrial cannula open, the Langendorff column was closed and the aortic outflow line opened so that buffer entering through the left atrium could be pumped out of the left ventricle (i.e., the antegrade working mode). Since perfusion of the left atrium provided sufficient heart rate, electrical pacing was obviated to examine left ventricular function in this experiment. During this working mode, a mean aortic pressure of 70 cm H_2O was maintained by a column height equivalent to 70 cm H_2O. Following the ligation of superior and inferior vena cava as well as pulmonary veins to ensure that the coronary effluent passed through the pulmonary artery, the pulmonary artery was cannulated with a 16-gauge steel cannula, and right ventricular outflow was monitored with transit-time ultrasound flowmeter (T206, Transonic Systems, NY) for the measurement of coronary flow. The probe of this flowmeter was also attached to the aortic flow root. Simultaneously, a catheter-tip pressure transducer (postcraniotomy subdural pressure monitoring kit, 110-4G, Neuro Care Group, Camino, CA) was inserted into left ventricle through the left atrium.

After initial 10 mins of perfusion in the working heart model, baseline measurement at 10 cm H_2O preload, by adjusting the height of the left atrial buffer reservoir above the heart, was performed and repeated at various preloads increasing every 2 cm H_2O up to 20 cm H_2O after 15 mins of stabilization.

Specific Measurements and Calculations. Cardiac output (CO) was defined as the sum of coronary and aortic flows in this experimental setting. Left ventricular pressure was continuously monitored with a transducer (postcraniotomy subdural pressure monitoring kit, 110-4G, Neuro Care Group, Camino, CA, and Life Scope II, Nihon Koden, Tokyo) and recorded in a digital recorder (PC208, Sony, Tokyo). Using computer software (Acqknowledge version 3.0 data acquisition systems, Biopac Systems, Goleta, CA, and Microsoft Excel, Microsoft, Redmond, A), left ventricular peak systolic pressure, left ventricular end-diastolic pressure, maximum rate of left ventricular pressure increase (dP/dt_{max}), minimum rate of left ventricular pressure increase (dP/dt_{min}), and heart rate were calculated, based on the averages of five beats. Left ventricular developed pressure, which was regarded as a marker of contractility of the isolated rat heart, was calculated by subtracting left ventricular end-diastolic pressure from left ventricular peak systolic pressure.

The Po_2 of the perfusion medium obtained at preperfusion (inflow) and right ventricular outflow (outflow) was determined by using a standard blood gas analyzer (ABL 700 series, Radiometer Trading, Copenhagen). Oxygen delivery was calculated as inflow oxygen tension, in millimeters of mercury, multiplied by oxygen solubility (24 μL/mL Krebs-Ringer's solution at 760 mm Hg·oxygen and 37°C) and coronary flow (in milliliters per minute). Myocardial oxygen consumption was calculated as oxygen solubility multiplied by the difference between inflow and outflow oxygen tension times coronary flow. Cardiac efficiency was determined as the ratio of cardiac work, the product of cardiac output (mL/min) × left ventricular peak systolic pressure (mm Hg), to myocardial oxygen consumption, as described previously (18).

Biochemical Analyses. The arterial blood samples at 1 and 24 hrs were used to determine white blood cell counts by an analyzer (Celltac, Nihon Kohden, Tokyo, Japan) and lactate by a standard blood gas analyzer (ABL 700 series, Radiometer Trading, Copenhagen). Residual blood was centrifuged at 2500 rpm for 10 mins at 4°C and then stored at −80°C for measurements of cytokines. TNF-α and interleukin-1β were measured in duplicate by enzyme-linked immunosorbent assays using commercially available antibodies (Immunoassay Kit, Biosource International, CA). Briefly, after incubation in the immobilized antibody and a biotinylated antibody specific for each cytokine, streptavidine-peroxidase is added. This binds to the biotinylated antibody to complete the four-member sandwich. Then, a substrate solution is added, which is acted on by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of each cytokine. The intensity was measured using a plate analyzer (enzyme-linked immunosorbent assay ETY-3A, Toyosokki, Tokyo, Japan).

Immunohistochemical Quantification of Myocardial β1-Receptor. In a separate series of experiments, density of myocardial β1-receptor was determined by immunohistochemistry. In addition to three sham rats, three rats in each group were examined. At 24 hrs after CLP, the rats in three groups were reanesthetized and the hearts were immediately excised and freeze-clamped between blocks of metal cooled in liquid nitrogen and stored at −80°C until the analyses. The β1-adrenergic receptor was analyzed using antihuman β1-adrenergic receptor rabbit polyclonal antibody (PA1-049), which detects β1-adrenergic receptor from mouse and rat tissues. Briefly, sections of hearts were prepared from each of two randomly chosen blocks per tissue per animal and were fixed in dimethyl ketone at 4°C for 20 mins. Then these sections were immersed in 0.3% hydrogen peroxide in methanol for 5 mins to block endogenous peroxidase activity, followed by three rinses in phosphate buffered saline. Sections were incubated with antihuman β1-adrenergic receptor rabbit polyclonal antibody (PA1-049) at a dilution of 1:200 at 4°C overnight. After three rinses in phosphate buffered saline, sections were incubated for 10 mins with secondary antibody: biotin-conjugated mouse and rabbit antigoat immunoglobulin, followed by peroxidase-conjugated streptoavidin. After three rinses in phosphate buffered saline, positive staining was visualized using 3,3'-diaminobenzidine tetrahydrochloride. Slides were then counterstained with hematoxyline. Ten images per each sample were randomly photographed using a microscope attached modular photomicrographic system (Nikon Eclipse TS 100, Nikon, Tokyo). The immunostaining area of the myocardium was measured using a computer software (NIH image 1.63 software, NIH, Bethesda, MD) in a blinded manner, and the ratio of this area to all field of the image was determined as the density of β1-receptors.

Statistical Analysis. Values were described as mean ± SD unless otherwise specified. To compare and contrast the effects of esmolol across the groups during working heart preparation, two-way analysis of variance was employed using the statistical package SPSS/10.0J for Windows (SPSS, Chicago, IL). Where

Table 1. Changes of heart rate and mean arterial pressure after cecal ligation and perforation in all study groups

	Group	0 hr	1 hr	3 hrs	24 hrs
Heart rate, beats/min	Control	372 ± 53	386 ± 42	370 ± 33	437 ± 48[a]
	Esmolol-10	337 ± 16	289 ± 19	316 ± 20	370 ± 23[b]
	Esmolol-20	333 ± 16	290 ± 42	317 ± 20	380 ± 35[b]
	Sham	409 ± 34	383 ± 16	363 ± 24	382 ± 13
Mean arterial pressure, mm Hg	Control	121 ± 12	126 ± 19	128 ± 6	116 ± 14
	Esmolol-10	132 ± 8	124 ± 12	123 ± 10	96 ± 8[a,b]
	Esmolol-20	127 ± 13	119 ± 4	114 ± 5	107 ± 18[a]
	Sham	132 ± 11	129 ± 9	125 ± 8	127 ± 7

[a] $p < .05$ vs. 0 hr; [b] $p < .05$ vs. control group.
Data expressed as mean ± SD.

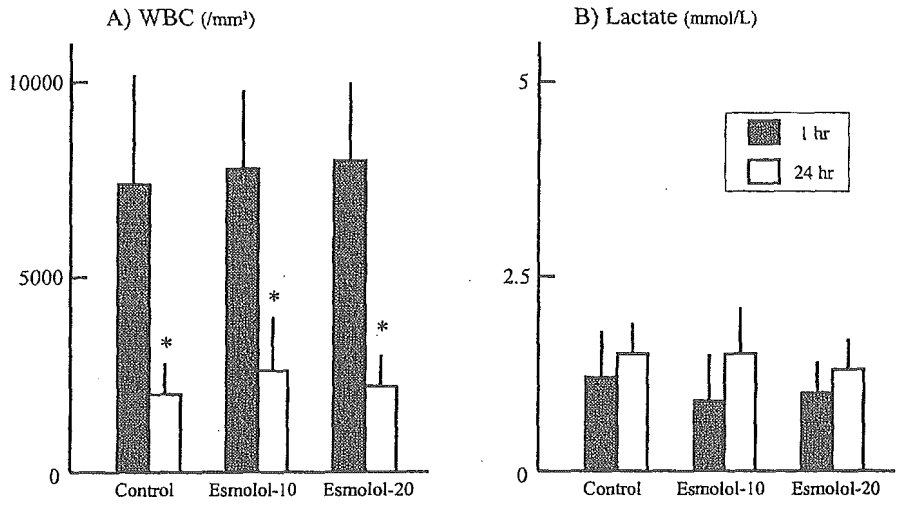

Figure 1. Changes of white blood cells (*WBC*) and arterial lactate at 1- and 24-hr study periods. The WBC counts were significantly reduced at 24 hrs in all groups compared with 1 hr ($p < .05$).

appropriate, directed pairwise comparisons of individual groups were conducted using a Bonferroni-corrected 95% confidence interval. Since the sham rats were not randomized with other animals, statistical analyses vs. the septic rats were not applied. The data of sham rats are presented in the results as the standard of hemodynamic changes and myocardial function in our experimental setting. For analysis of β1-adrenergic receptor density, one-way analysis of variance was employed. Directed comparisons of individual groups were conducted using Scheffé's test. We considered $p < .05$ as significant.

RESULTS

All CLP rats demonstrated a reduction of activity, pilo-excretion, and exudation around eyes and nose at 24 hrs after operation. At postmortem, panperitonitis with moderate volume of ascites was confirmed in all CLP rats.

In Vivo *Hemodynamic Changes in Sham and CLP-Induced Septic Rats.* Table 1 shows the changes of heart rate and mean arterial pressure for 24 hrs in sham and CLP-treated rats with or without esmolol infusion. In the control group, heart rate at 24 hrs increased significantly compared with 0 hr ($p < .05$), whereas mean arterial pressure remained constant during the 24-hr periods. At 24 hrs, heart rates in both esmolol groups, which appeared to be comparable with the sham group, were significantly lower than those in the control group ($p < .05$). Mean arterial pressure at 24 hrs in both esmolol groups was significantly reduced compared with 0 hr ($p < .05$), but only the E-10 group showed a significant difference vs. the control group. In the sham group, both variables remained constant throughout the study periods. As shown in Figure 1, white blood cell counts, which were comparable at 1 hr between the three groups, were significantly reduced to a similar extent at 24 hrs ($p < .05$). Arterial lactate levels were not significantly different and ranged within normal limits at both 1 and 24 hrs in all study groups.

Ex Vivo *Myocardial Function in Sham and CLP-Induced Septic Rats.* Table 2 illustrates the changes of heart rate, stroke volume, and myocardial function at various levels of preload, ranging between 10 and 20 cm H_2O, in a working heart preparation. Heart rate was not significantly different in all three groups, whereas stroke volumes in both esmolol groups were significantly higher almost throughout the study periods vs. the control group. In the sham group, heart rate showed an increasing trend with the elevation of preload, whereas stroke volume remained constant throughout the study periods. CO in both esmolol groups was approximately twice as high as that in the control group ($p < .05$) at all preload levels. In particular, the changes of CO in the E-20 group were almost identical to those of the sham group. The variables to reflect left ventricular contractility or dilation such as left ventricular developed pressure, dP/dt_{max}, and dP/dt_{min} in both esmolol groups were basically maintained higher than those in the control group ($p < .05$), whereas statistical significance was not achieved at some preload periods. There were no significant differences between two esmolol groups in these variables such as left ventricular developed pressure, dP/dt_{max}, and dP/dt_{min}. All these variables in the sham group were slightly higher than those in esmolol-treated groups throughout the study periods. The myocardial oxygen consumption in the E-20 group, which was comparable with the sham group, was significantly higher vs. the control group ($p < .05$), whereas the E-10 group, which ranged between the values of the control and E-20 groups, did not show a significant difference.

Figure 2 shows the changes of cardiac work and cardiac efficiency at various lev-

Table 2. Changes of heart rate and the variables of myocardial function in working heart model

		Preload					
	Group	10 cm H_2O	12 cm H_2O	14 cm H_2O	16 cm H_2O	18 cm H_2O	20 cm H_2O
Heart rate, beats/min	Control	341 ± 69	351 ± 74	360 ± 72	349 ± 69	349 ± 64	352 ± 68
	Esmolol-10	349 ± 48	354 ± 55	356 ± 49	373 ± 55	373 ± 41	376 ± 44
	Esmolol-20	333 ± 59	341 ± 50	359 ± 54	358 ± 50	360 ± 42	379 ± 36
	Sham	318 ± 27	322 ± 23	331 ± 25	342 ± 28	342 ± 24	343 ± 17
Stroke volume, mL	Control	0.10 ± 0.04	0.11 ± 0.04	0.11 ± 0.04	0.11 ± 0.04	0.10 ± 0.04	0.09 ± 0.04
	Esmolol-10	0.18 ± 0.04[a]	0.19 ± 0.04[a]	0.19 ± 0.05[a]	0.18 ± 0.06[a]	0.16 ± 0.06	0.15 ± 0.06
	Esmolol-20	0.18 ± 0.04[a]	0.19 ± 0.03[a]	0.20 ± 0.03[a]	0.21 ± 0.03[a]	0.20 ± 0.04[a]	0.18 ± 0.03[a]
	Sham	0.21 ± 0.03	0.22 ± 0.03	0.22 ± 0.03	0.22 ± 0.02	0.22 ± 0.03	0.21 ± 0.04
Cardiac output, mL/min	Control	28.4 ± 5.6	33.6 ± 7.1	34.7 ± 7.7	34.1 ± 8.9	32.3 ± 9.3	29.7 ± 8.1
	Esmolol-10	61.9 ± 11.6[a]	66.2 ± 12.5[a]	65.9 ± 13.5[a]	64.8 ± 15.3[a]	59.2 ± 18.4[a]	55.7 ± 18.5[a]
	Esmolol-20	58.6 ± 13.1[a]	66.0 ± 13.8[a]	72.4 ± 13.3[a]	73.9 ± 14.1[a]	75.5 ± 9.2[a]	70.0 ± 14.2[a]
	Sham	66.2 ± 9.6	71.0 ± 8.9	74.1 ± 6.8	76.2 ± 6.9	76.7 ± 10.3	74.5 ± 13.4
Left ventricular developed pressure, mm Hg	Control	57 ± 17	60 ± 19	61 ± 16	64 ± 12	61 ± 12	32 ± 10
	Esmolol-10	78 ± 7[a]	78 ± 9[a]	77 ± 8[a]	73 ± 8[a]	70 ± 7	66 ± 8
	Esmolol-20	79 ± 9[a]	78 ± 8[a]	78 ± 6[a]	76 ± 7[a]	74 ± 7[a]	71 ± 7
	Sham	85 ± 5	87 ± 6	85 ± 7	82 ± 5	82 ± 4	80 ± 3
dP/dt_{max}, mm Hg/sec	Control	1557 ± 541	1685 ± 593	1639 ± 489	1725 ± 399	1564 ± 347	1587 ± 300
	Esmolol-10	2293 ± 265[a]	2317 ± 308[a]	2258 ± 250[a]	2138 ± 181[a]	2007 ± 216	1834 ± 177
	Esmolol-20	2289 ± 307[a]	2261 ± 305[a]	2261 ± 256[a]	2153 ± 288[a]	2149 ± 288[a]	1985 ± 305[a]
	Sham	2540 ± 58	2584 ± 206	2459 ± 255	2283 ± 210	2328 ± 203	2260 ± 138
dP/dt_{min}, mm Hg/sec	Control	−1777 ± 370	−1937 ± 338	−1794 ± 429	−1855 ± 275	−1771 ± 212	−1740 ± 225
	Esmolol-10	−2452 ± 324[a]	−2445 ± 352[a]	−2410 ± 257[a]	−2228 ± 174[a]	−2069 ± 84[a]	−1969 ± 167
	Esmolol-20	−2404 ± 364[a]	−2434 ± 306[a]	−2361 ± 282[a]	−2274 ± 320[a]	−2260 ± 224[a]	−2159 ± 308[a]
	Sham	−2806 ± 191	−2792 ± 294	−2698 ± 237	−2482 ± 217	−2568 ± 253	−2444 ± 119
Myocardial oxygen consumption, μLO_2/min	Control	175 ± 15	168 ± 15	158 ± 15	161 ± 15	154 ± 19	148 ± 17
	Esmolol-10	192 ± 40	201 ± 35	189 ± 36	186 ± 38	180 ± 42	185 ± 50
	Esmolol-20	209 ± 35[a]	211 ± 28[a]	215 ± 28[a]	211 ± 34[a]	206 ± 33[a]	200 ± 41[a]
	Sham	210 ± 33	214 ± 39	217 ± 53	210 ± 53	209 ± 54	213 ± 47

dP/dt_{max}, maximum rate of left ventricular pressure increase; dP/dt_{min}, minimum rate of left ventricular pressure increase.
[a] $p < .05$ vs. control group. Data expressed as mean ± SD.

els of preload, ranging between 10 and 20 cm H_2O, in working heart preparation. In two esmolol-treated groups, both cardiac work and cardiac efficiency were significantly higher than in the control group throughout the study period ($p < .05$), indicating that esmolol infusion preserved cardiac work more efficiently (i.e., more efficient oxygen utilization to a certain extent of cardiac work) in septic rats. In addition, the rightward and upward shift of the peak values in the esmolol-treated rats suggests that the Frank-Starling curve in septic myocardium is improved by esmolol infusion. The values at various preloads in the E-20 group were similar to the sham group.

Esmolol and Proinflammatory Cytokines in Septic Rats. Figure 3 illustrates the changes of TNF-α and interleukin-1β at 1- and 24-hr periods in all CLP groups. In the control group, TNF-α showed an increasing, but not significant, trend at 24 hrs vs. 1 hr, whereas esmolol infusion at either dose caused a significant reduction of TNF-α at 24 hrs compared with the control group ($p < .05$). Interleukin-1β level in plasma did not change significantly in all study groups.

Esmolol and β1-Adrenergic Receptor Density in Septic Myocardium. Figure 4 illustrates the representative microscopic images of positive immunostaining β1-adrenergic receptor in myocardium from sham rat and three groups. Less immunostaining for β1-adrenergic receptor was observed in the control group compared with sham rat as shown in Figure 4*A* and 4*B*. In contrast, with esmolol infusion at either dose, β1-receptor expression on myocardium was much higher than in the control group (Fig. 4*C* and 4*D*). Figure 5 shows the density of β1-adrenergic receptors. The ratio of positive immunostaining area to whole area in the control group was significantly reduced to approximately the 60% level of the sham rat ($p < 0.05$). Those ratios in the esmolol-treated septic rats ranged between the sham and control groups, but no statistical difference was found vs. the sham group.

DISCUSSION

Although augmenting systemic oxygen delivery to match oxygen needs was considered the basic concept for the treatment of sepsis (19), clinical trials demonstrated that hemodynamic therapy aimed at achieving supranormal values of CO did not necessarily improve the outcome in critically ill patients (20, 21). A new guideline clearly documented that a strategy of increasing CO to accomplish a predefined elevated level was not recommended (22). Despite no evidence of a global deficit of oxygen supply (23), septic hearts *per se* are recognized as relatively ischemic due to disturbed microcirculation and mitochondrial dysfunction (24–26). We therefore wondered whether augmentation of CO in hyperdynamic sepsis, requiring further cardiac work, became another circumstance to deteriorate its function and subsequently did not improve the outcome in septic patients. The present study demonstrated that, in a peritonitis-induced septic rat, continuous infusion of esmolol preserved myocardial function without evidence of deteriorating tissue hypoxia. Simultaneously, this study suggested that such cardioprotective effects of esmolol were caused by improved myocardial oxygen utilization, possibly based on the blockade of persistent β-adrenergic stimulation, the reduc-

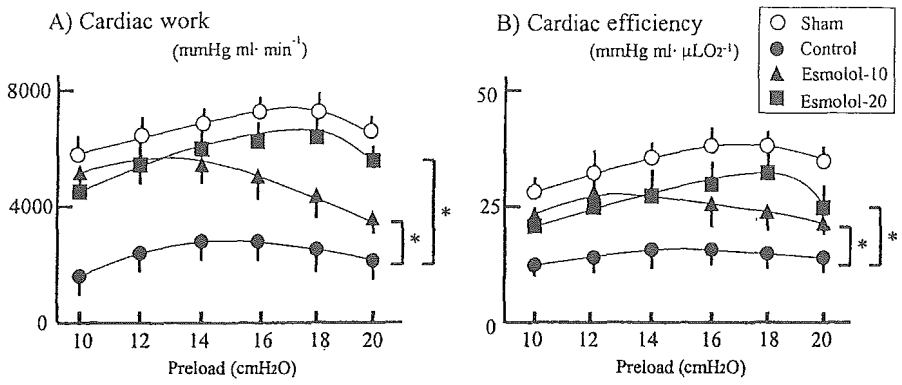

Figure 2. Changes of cardiac work and cardiac efficiency in an isolated working heart between the groups at different preloads. Cardiac efficiency = the ratio of cardiac work, the product of cardiac output × left ventricle peak systolic pressure, to myocardial oxygen consumption. Significant difference was found between the groups through all preloads (*$p < .05$).

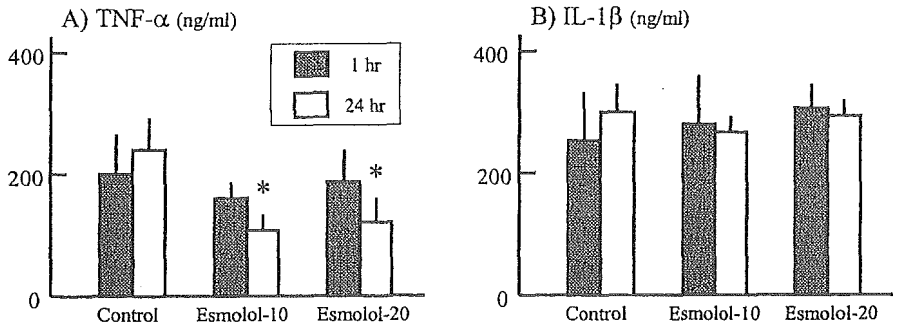

Figure 3. Changes of plasma tumor necrosis factor (*TNF*)-α and interleukin (*IL*)-1β concentrations at 1- and 24-hr study periods. *$p < .05$ vs. the control group.

tion of TNF-α discharges, and the restoration of suppressed β1-adrenergic receptor density in myocardium during the progression of sepsis.

In this model, systemic inflammatory responses to infection are obvious, reflected by a marked reduction of white blood cell counts and a significant elevation of heart rate at 24 hrs. With adequate fluid resuscitation, it has been appreciated as most clinically relevant to a hyperdynamic sepsis, concomitantly obviating the confounding factors like hypotension or significant tissue hypoxia (15, 16). Under these conditions, however, cardiac performance in septic rats was significantly depressed in the *ex vivo* study, demonstrating that CO in the control group was reduced to approximately 50% level vs. the sham rats when the preload and afterload were kept identical (Table 2). On the contrary, all variables of myocardial function such as left ventricular developed pressure, dP/dt, and CO were significantly improved by continuous infusion of esmolol (Table 2), accompanied by augmented cardiac work and cardiac efficiency (Fig. 2), indicating that esmolol infusion during the development of sepsis improved myocardial oxygen utilization and contractility. Interestingly, heart rate remained constant and stroke volume was kept higher in the esmolol-infused rats. Accordingly, β-blocker not only may augment myocardial contractility but also may increase end-diastolic volume of septic heart by improving its diastolic function. Previous clinical study demonstrated that the survivors from septic shock had an acutely dilated left ventricle with an increased end-diastolic volume for the first few days, whereas nonsurvivors showed normal values throughout the course of their illness until death (27, 28). Infusion of β-blocker could induce such alterations of septic myocardium to enlarge its end-diastolic volume, subsequently protecting the host.

Application of β-blocker has been demonstrated to improve acute and long-term prognosis in ischemic heart disease and to reduce perioperative incidence of death from cardiac causes in high-risk patients (9, 10). Although the present study did not clarify whether such beneficial effects of β-blocker improved the outcome in septic hosts, β-blocker infusion restored the function of septic myocardium up to the level of sham rats. Several possibilities to account for this promising property of β-blocker in septic myocardium have been proposed. First, significant reduction of heart rate induces the improvement of oxygen balance and the prolongation of diastolic phase, subsequently providing an anti-ischemic effect on the myocardium (11). Such effects of β-blocker could contribute to protection of septic hearts. Second, prolonged and intensive β-adrenergic receptors stimulation leads to desensitization and down-regulation of β-adrenergic receptors, which seriously impairs cardiac function (11). Since sepsis is accompanied by a massive discharge of endogenous catecholamines (29), infusion of β-blocker during the development of sepsis may be able to block this pathway of desensitization. Third, attenuation of adrenergic nervous system by β-adrenergic blockade contributes to the suppression of TNF-α and the preservation of cardiac function (6, 30), because TNF-α and interleukin-1β have been shown to synergistically depress myocardial contractility by disrupting the transmembrane β-adrenergic signal transduction (12, 13). Owing to a limited amount of blood sampling in rat, we did not measure these cytokines at the other time period when their discharges might be maximized during 24 hrs after CLP. These effects of β-adrenergic blocker to suppress the discharges of cytokines like TNF-α could be the major mechanism for preservation of myocardial function in sepsis. In addition, recent study supported that TNF-α secreted from myocytes, which produced no measurable rise in plasma, developed myocardial dysfunction after ischemia-reperfusion, shock, or endotoxemia (30–33). The myocardium produces as much TNF-α per gram of tissue in response to endotoxin as either the liver or spleen, both of which include large macrophage populations, the major source of TNF-α (33). Although we did not assess the expression of TNF messenger RNA on the myocardium or the effects of TNF-α converting enzyme inhibitor (34), in the present study, β-blocker infusion might minimize these responses and preserve greater myocardial function as observed in patients with heart failure (35, 36). Finally, although the measurement of receptor density in this study did not allow us to evaluate the accurate number or

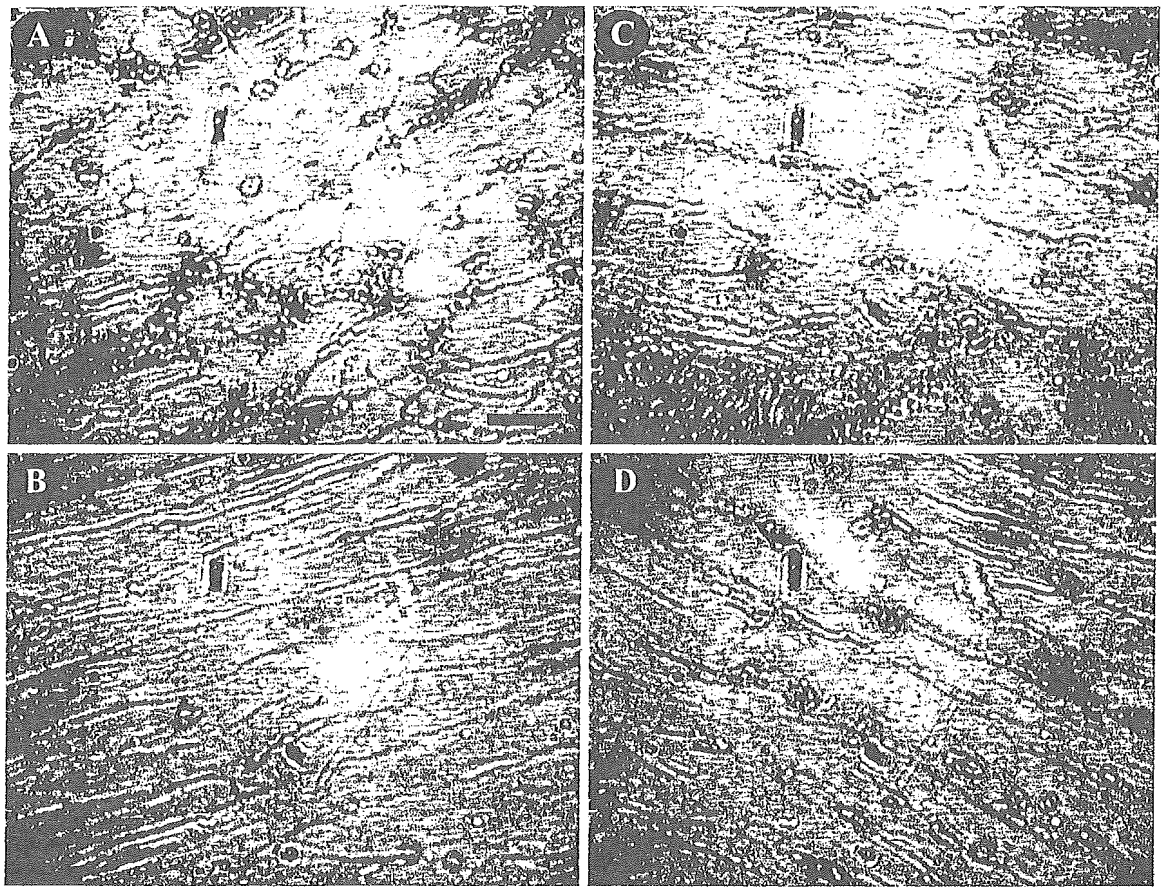

Figure 4. Representative pictures showing immunohistochemistry of β1-adrenergic receptor captured by light microscopy (×100) from rat myocardium. Positive staining for β1-adrenergic receptor is brown. *A*, representative immunostaining of myocardium from a sham rat. *Bar*, 50 μm. *B*, representative immunostaining of myocardium from a rat in the control group, a septic rat without esmolol infusion. Note that the density of β1-receptor is apparently uneven vs. the sham. *C* and *D*, representative immunostaining of myocardium from rat in esmolol-10 and -20 groups, respectively.

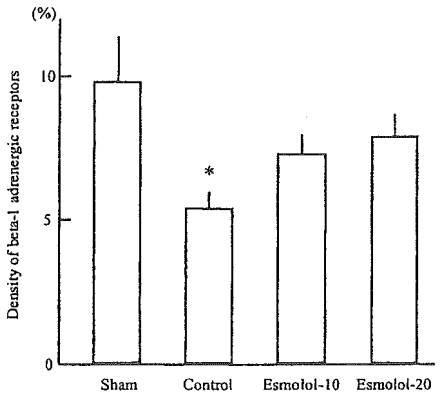

Figure 5. Comparison of β1-adrenergic receptor density in myocardium between sham and septic rats with or without esmolol infusion. *$p < .05$ vs. sham rat.

function of β1-adrenergic receptor, the restoration of receptor density might protect myocardial function. In patients with septic shock, an approximately 40% reduction of the β-adrenergic receptor density was demonstrated (8), indicating that the potency of β-adrenergic stimulation in septic myocardium was reduced at the level of receptors. In the present study, we found comparable results of β-receptor down-regulation in septic myocardium but failed to show the significant restoration of receptor density with esmolol infusion vs. the control group. Although others demonstrated that β-adrenergic receptors in myocardium were initially externalized during hyperdynamic phase but were internalized during the late stage of sepsis (37), further study is needed to elucidate the changes of β-adrenergic receptor density in septic myocardium.

There are several issues to interpret the data herein. Compared with *in vivo* analyses, an isolated working heart preparation eliminates the impacts of circulating endogenous humoral mediators and inflammatory cells. If these components, which are able to modulate sepsis-related myocardial dysfunction, persist during the evaluation periods, the protective effects of esmolol might not become apparent. To this point, however, it should be noted that esmolol *per se* was excluded from the perfusion medium. Additionally, the dose of esmolol infusion was determined in healthy, but not septic, rats under general anesthesia in our pilot study. In other words, infusion of esmolol at 20 mg/kg/hr, which could reduce CO to an approximately 80% level in healthy rats, might possess more profound cardiodepressive effects on septic hearts *in vivo*. Although the dose of esmolol should be reduced in a disease condition like sepsis, the half dose in this study showed a similar protective property on myocardial function during the development of sepsis. Some may argue that arterial lactate is a reliable marker to reflect the progression of anaerobic tissue metabolism in sepsis (38). Several other mechanisms such as dysfunction of pyruvate dehydrogenase, delayed lactate clearance, or epinephrine-stimulated aerobic glycolysis could contribute to an eleva-

The present study shows that infusion of esmolol, a β1-selective blocker, provides cardioprotective effects in septic hearts by improving myocardial oxygen utilization.

tion of arterial lactate in critically ill (39). Although it is true that the absence of lactate elevation may not directly correspond to the absence of tissue hypoxia, the 24-hr esmolol infusion to septic animals in this study did not cause a significant increase of lactate production, which was associated with high mortality rate in critically ill patients (40, 41). Finally, apart from the benefits to myocardial function, the results do not allow us to make any further conclusions regarding the roles of β-blocker in sepsis. However, the primary goal was to examine whether esmolol infusion in septic animals was beneficial to myocardial function, and this goal was accomplished.

CONCLUSIONS

The present study shows that infusion of esmolol, a β1-selective blocker, provides cardioprotective effects in septic hearts by improving myocardial oxygen utilization. As for the clinical application of β-blockers, further investigation is warranted to evaluate their effects on other organs as well as mortality in sepsis.

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Off-Pump Coronary Artery Bypass Attenuates Transient Hepatocellular Damage After Myocardial Revascularization

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Midori Ishibashi, MT,† and Kiyoaki Watanabe, MD†

Objective: Cardiopulmonary bypass (CPB) affects hepatocellular integrity and occasionally results in liver dysfunction after cardiac surgery. Performing coronary artery bypass graft surgery without CPB may help to reduce the risk of this complication and better preserve perioperative liver function. This study compared perioperative hepatocellular damage in patients undergoing on-pump and off-pump bypass surgery.

Design: Prospective study.

Setting: University hospital.

Participants: Patients scheduled for elective on-pump (n = 21) and off-pump (n = 17) coronary artery bypass surgery.

Measurements and Main Results: Liver function was assessed by serum levels of alcohol dehydrogenase (AD) and α-glutathione S-transferase (α-GST), which serve as more sensitive indices of hepatocellular injury than do conventional transaminases. Arterial blood was sampled at 6 stages: after induction of anesthesia (baseline); at the end of CPB in the on-pump group or on completion of the last distal anastomosis in the off-pump group; at the end of surgery; and 6 hours, 12 hours, and 24 hours after the end of anesthesia. The off-pump patients showed significantly lower increases in serum AD and α-GST levels than did the on-pump group. AD and α-GST values increased in the on-pump patients after the initiation of CPB and peaked at the end of surgery, with a return to baseline at 12 hours and 24 hours after the end of anesthesia. No clinically relevant liver dysfunction was observed in either group.

Conclusions: CPB induced transient subclinical hepatocellular damage, whereas off-pump revascularization attenuated this damage.

KEY WORDS: coronary artery bypass surgery, off-pump, cardiopulmonary bypass, liver function

LIVER DYSFUNCTION AFTER cardiac surgery results in increased mortality and morbidity and prolonged hospital stays. The pathogenesis of this complication involves multiple factors, including exposure to hepatotoxic agents and decreased perfusion of the liver secondary to perioperative hypotension or low cardiac output. Cardiopulmonary bypass (CPB) is an additional risk factor specific to cardiac surgery. The injurious action of nonphysiologic hemodynamics during CPB on liver function is caused by several mechanisms, including the effects of nonpulsatile perfusion, a low-flow state, increased levels of circulating catecholamines, and formation of free radicals.[1] The risks associated with CPB can be eliminated by a new approach using off-pump coronary artery bypass graft surgery, which is performed on the beating heart.[2,3] Standard laboratory indicators of liver function such as transaminases or bilirubin have limited use in the detection of minor degrees of liver damage. The hepatic isoenzymes of alcohol dehydrogenase (AD)[4,5] and α-glutathione S-transferase (α-GST)[6,7] are highly specific markers of alterations in hepatocellular function, and their serum levels correlate better with histologic changes than do the transaminases commonly used as monitoring parameters. This study was conducted to evaluate hepatocellular impairment by measuring serum AD activity and α-GST concentrations in patients undergoing on-pump and off-pump coronary artery bypass grafting.

METHODS

Twenty-one patients who underwent elective on-pump and 17 scheduled to undergo off-pump coronary artery bypass grafting were enrolled in the study. Patients known to have a history of hepatic or renal disease were excluded from the study. After receiving approval from the institutional committee of human research, informed consent was obtained from all patients. Coronary artery bypass surgery was performed by the same surgical team. The selection of the operative procedure was made based on the surgeon's recommendation and the patient's choice.

Patients were premedicated with oral diazepam (5 mg) and intramuscular meperidine (0.5-1.0 mg/kg). Anesthesia was induced using midazolam (2-4 mg), fentanyl (5-10 μg/kg), and vecuronium (0.15 mg/kg) and maintained with fentanyl (30-40 μg/kg), midazolam (0.2-0.3 mg/kg), sevoflurane, and vecuronium. Intraoperative monitoring parameters included 5-lead electrocardiography, arterial pressure, central venous pressure, pulmonary artery pressures, pulse oximetry, capnography, urine output, and nasopharyngeal and bladder temperatures. Transesophageal echocardiography was also monitored to assess ventricular and valvular function.

Off-pump coronary artery bypass was performed using an Octopus suction stabilization device (Medtronic Inc, Minneapolis, MN) through a median sternotomy. Deep pericardial sutures were placed to lift the myocardial apex and facilitate exposure to the posterior and lateral aspects of the myocardium. If cardiac output and blood pressure dropped during the heart displacement, patients first were placed in a slight Trendelenburg position and then received fluids and vasoconstrictor such as phenylephrine. In most patients, small doses of inotropic drugs (dopamine and/or dobutamine) were also given to achieve acceptable hemodynamics (cardiac index [CI]>2.5 L/min/m^2 and systolic arterial pressure >90 mmHg). Infusion doses of dopamine and dobutamine were titrated according to the CI measurements. When CI was satisfactory (>2.5 L/min/m^2) but a low blood pressure persisted, norepinephrine was infused to maintain systolic arterial pressure >90 mmHg. Mixed venous oxygen saturation was monitored for detecting abrupt changes in cardiac performance caused by surgical manipulation of the heart. Allogeneic red blood cells were transfused to achieve a hematocrit greater than 27%. None of the patients received aprotinin.

In the on-pump group, CPB was performed using a centrifugal pump (HPM-15; Nikkiso Co, Tokyo, Japan), a membrane oxygenator (HPO-20H-C; Senko Medical Inc, Tokyo, Japan) and an arterial catheter filter (Auto Vent-SV; Pall Biomedical Products Co, Glen Cove, NY). Non-

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Table 1. Demographic and Intraoperative Data

Characteristic	On-Pump Group (n = 21)	Off-Pump Group (n = 17)	p Value
Age (y)	67 ± 7	69 ± 6	0.373
Gender (female/male)	3/18	3/14	0.778
Height (cm)	164 ± 7	164 ± 7	0.843
Weight (kg)	63 ± 8	64 ± 10	0.793
Hypertension (n)	19	13	0.239
Diabetes mellitus (n)	11	10	0.691
Hypercholesterolemia (n)	7	6	0.899
NYHA class			0.927
I (n)	5	5	
II (n)	12	9	
III (n)	4	3	
Left ventricular ejection fraction (%)	63 ± 11	65 ± 13	0.494
Old myocardial infarction (n)	12	10	0.917
No. of diseased vessels	2.7 ± 0.6	2.2 ± 0.5	0.005
No. of grafts	3.7 ± 1.0	2.4 ± 0.6	<0.001
Cardiopulmonary bypass time (min)	171 ± 39	–	
Operation time (min)	414 ± 67	340 ± 71	0.002
Anesthetic time (min)	534 ± 72	457 ± 88	0.006
Infusion dose of dopamine (μg/kg/min)	3.4 ± 0.7	3.4 ± 1.2	0.920
Infusion dose of dobutamine (μg/kg/min)	3.3 ± 0.7	3.4 ± 0.8	0.707
Use of phenylephrine (n)	6	15	<0.001
Use of norepinephrine (n)	2	9	0.005

NOTE. Values are mean ± SD.
Abbreviation: NYHA, New York Heart Association.

pulsatile flow was maintained at a pump flow rate of between 2.2 and 2.6 L/min/m^2. Phenylephrine or sodium nitroprusside was administered as needed to maintain mean systemic perfusion pressure between 50 and 80 mmHg. Moderate hemodilution (hematocrit 18%-24%) and moderate hypothermia (bladder temperature 28°C) were maintained during aortic cross-clamping. Myocardial preservation was performed by antegrade and retrograde cold potassium blood cardioplegia. Heparin was administered to maintain an activated coagulation time longer than 480 seconds. After the termination of CPB, a minimal dose of dopamine and/or dobutamine was used routinely because of the hemodynamic instability and then the infusion was titrated to maintain the CI >2.5 L/min/m^2. Norepinephrine was infused if systolic arterial pressure decreased below 90 mmHg and vascular resistance was lower than 800 dyne/sec/cm^{-5}. After CPB, red blood cells were transfused to maintain a hematocrit greater than 24%, preferentially by reinfusion of salvaged autologous blood.

Arterial blood was sampled and measurements of aspartate transaminase (AST), alanine transaminase (ALT), AD, and α-GST were made at the following 6 stages: after induction of anesthesia (baseline); at the end of CPB in the on-pump group and completion of the last distal anastomosis in the off-pump group; at the end of surgery; and 6 hours, 12 hours, and 24 hours after the end of anesthesia. Serum AST and ALT activities were analyzed using standard clinical laboratory techniques. AD serum activity was measured spectrophotometrically using Kato et al's method,[8] and the serum concentration of α-GST was determined using a Hepakit-Alpha α-GST enzyme-linked immunosorbent assay test kit (Biotrin International, Dublin, Ireland). Reference ranges for AD and α-GST were less than 4.0 IU/L and less than 8.0 μg/L, respectively.[8,9]

A power analysis was performed after a pilot study with a sample size of 10 cases (5 on-pump and 5 off-pump). Based on the pilot data and a desired increase in variables of AD and α-GST by 50% of the each reference range (2.0 IU/L and 4.0 μg/L for AD and α-GST, respectively), a power of 0.8 at $\alpha = 0.05$ resulted in 16 patients for each group. In this study, a total of 38 patients were included (21 on-pump and 17 off-pump). Results are expressed as the mean ± standard deviation. Statistical analysis was performed using 2-way repeated analysis of variance, Student t test, Mann-Whitney *U* test, or chi-square test. A p value < 0.05 was considered significant.

RESULTS

Table 1 shows patient demographic and intraoperative data. There were no significant differences between the 2 groups in terms of age, gender distribution, height, weight, or in the number of patients with hypertension or diabetes mellitus. The number of patients with hypercholesterolemia receiving statin treatment did not differ between the groups. Preoperative evaluations of cardiac function as assessed by New York Heart Association class and left ventricular ejection fraction also did not differ between the groups. Patients in the on-pump group had a higher incidence of coronary artery pathology than did those in the off-pump group. The number of bypass grafts was 3.7 ± 1.0 in the on-pump group and 2.4 ± 0.6 in the off-pump group ($p < 0.001$). Surgical and anesthetic times were significantly longer in the on-pump group. Dopamine and dobutamine were administered in 10 and 18 patients of the on-pump group and 8 and 12 patients of the off-pump groups, respectively. The infusion doses of dopamine and dobutamine did not differ between the groups. The off-pump patients received more phenylephrine and norepinephrine compared with the on-pump patients. No patient in either group received a phosphodiesterase III inhibitor or needed an intra-aortic balloon pump. None of the patients experienced notable complications in either the surgical or postoperative periods.

Changes in serum activity of AST, ALT, and AD and concentration of α-GST are shown in Figure 1. Serum AST and ALT activities remained stable throughout the entire observa-

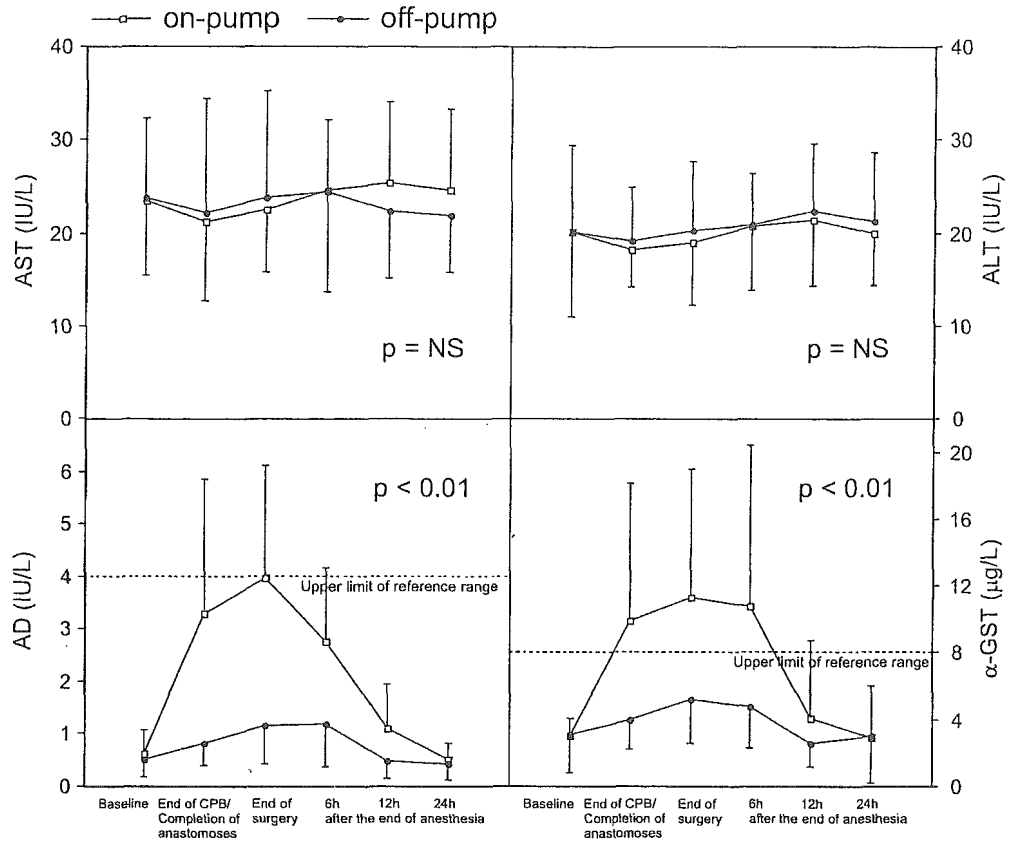

Fig 1. Changes in serum activity of AST, ALT, and AD and concentration of α-GST in the on-pump and off-pump groups (mean ± SD). CPB, cardiopulmonary bypass.

tion period, and measurements did not differ between groups. Serum AD activity was similar and within the reference range at baseline in both groups. Intraoperative AD activity increased in the on-pump group, showing a peak at the end of surgery, followed by a gradual return to the baseline value at 24 hours after the end of anesthesia. In contrast, AD activity remained unchanged in the off-pump patients. Ten patients in the on-pump group had peak AD values of >4.0 IU/L, whereas AD values in all off-pump patients remained within the reference range. There were no differences between the 2 groups in serum α-GST concentrations at baseline. The concentration of α-GST increased significantly in the on-pump patients after the start of CPB and peaked at the end of surgery, with a return to baseline at 12 hours after the end of anesthesia; but no change was observed in the off-pump patients. Fourteen patients in the on-pump group had peak α-GST concentrations >8.0 μg/L, whereas α-GST values in all but 1 off-pump patient were within the reference range.

DISCUSSION

This study shows that CPB induces transient alterations in hepatocellular integrity in cardiac surgical patients, as evidenced by increased levels of serum AD and α-GST in the on-pump patients. These increases were confined to the intraoperative and immediate postoperative periods and had returned to baseline levels on the first postoperative day. In contrast, these parameters remained stable in the off-pump patients. Baseline measurements of serum AD and α-GST were similar in both groups, indicating that the observed parameter changes were confined to the intraoperative period and might have been because of the detrimental effects of CPB on liver function.

Serum transaminase (AST and ALT) activities showed no significant changes in either group at any time in the study period. One disadvantage of transaminases when used as indices of hepatocellular damage is related to their distributions within the liver. The periportal hepatocytes contain the largest concentrations of transaminases, whereas the centrilobular hepatocytes, which are relatively deficient in transaminases, are more susceptible to damage from hypoxia and exogenous toxins. AD and α-GST are primarily located in the centrilobular hepatocytes and are readily and rapidly released into the circulation after hepatic insult. The short plasma half-lives of AD and α-GST also allow the early detection and resolution of hepatic damage.[4-7] None of the patients in this study showed overt clinically relevant liver dysfunction; nevertheless, CPB might be associated with transient deleterious effects on hep-

atocellular function. Off-pump bypass surgery, on the other hand, preserved perioperative hepatocellular integrity.

CPB-associated liver dysfunction is attributed to multiple factors, including nonpulsatile perfusion, a low-flow state during CPB, and increased levels of circulating catecholamines and cytokines. Disturbances in the hepatic circulation during CPB are believed to be responsible for hepatocellular injury. Hemodilution has been reported to improve hepatic arterial and portal flow.[10] However, hemodilution may result in a net decrease in hepatic oxygen delivery because of the reduction in oxygen content. The changes in AD and α-GST values during CPB might have been partly induced by hemodilution. Hypothermia (bladder temperature 28°C) has little effect on hepatic arterial flow but may cause an increase in portal flow.[11] The authors believe that it is unlikely that hypothermia had much effect on liver function.

Pump flow rate and type of perfusion (pulsatile or nonpulsatile) can affect hepatic blood flow. Mathie et al[12] showed that hepatic blood flow is maintained better with high pump flows (2.4 L/min/m^2) rather than with low flows (1.2 L/min/m^2). Total hepatic blood flow is better preserved during low-flow bypass (1.2 L/min/m^2) by pulsatile than by nonpulsatile perfusion; however, no significant difference was noted between pulsatile and nonpulsatile perfusion at the higher bypass flow rate of 2.4 L/min/m^2.[12] The authors' use of nonpulsatile perfusion might help to explain the changes in AD and α-GST levels, although the effect may be minimized because of relatively high pump flow rates (2.2-2.6 L/min/m^2).

Portal venous blood flow is regulated by the action of α-agonist-responsive resistance vessels within the prehepatic splanchnic vascular bed.[13] In the present study, the off-pump patients received more vasopressors than on-pump patients, although the use of inotropic drugs did not differ between the groups. Reves et al[14] investigated the circulating catecholamine levels during cardiac surgery and showed increases in blood endogenous epinephrine and norepinephrine released at the initiation of CPB. These endogenous catecholamines in on-pump patients may have the potential for adverse effects including the reduction of liver perfusion and impaired liver function.

CPB also causes the development of inflammation, resulting in alterations in complement, coagulation, and fibrinolytic systems. Proinflammatory cytokines play a key role in the inflammatory cascade after CPB and may induce liver dysfunction. Wan et al[15] compared the production of cytokines in patients undergoing myocardial revascularization with and without CPB. Avoiding the use of CPB may preserve hepatocellular integrity and might have contributed to unchanged serum AD and α-GST levels in the off-pump patients.

The deleterious effects of CPB might have caused some degree of hepatocellular impairment by themselves. However, in this context, they can be thought of as merely setting the stage for additional insults, such as low cardiac output or hypotension, to elicit overt CPB-associated clinically relevant liver dysfunction. Nomoto et al[16] investigated alterations in hepatic mitochondrial function during and after CPB by measuring arterial ketone body ratios. They concluded that CPB has a detrimental effect on liver function and that this effect generally lasts until the first postoperative morning. In the present study, AD and α-GST values returned to baseline by 12 hours after the end of anesthesia, showing that the recovery of hepatocellular function after CPB was prompt.

The limitation of this study was that it was observational and not a randomized controlled trial. However, the groups consisted of consecutive patients, and there were no differences between the groups in demographic data such as age, gender, hypertension, diabetes mellitus, hypercholesterolemia, New York Heart Association functional class, left ventricular ejection fraction, and history of myocardial infarction. In addition, the baseline measurements for liver function were similar in both groups. The differences in cohorts for numbers of diseased vessels and distal grafts and operation and anesthetic times detected in this study might reflect a surgical selection bias. This bias was thought to be because of the selection of the operative procedure for coronary revascularization, which was based on the surgeon's discretion according to optimal patient care. A prospective, randomized trial involving more patients is mandatory to provide incremental evidence comparing the 2 approaches. In conclusion, the present results suggest that on-pump CABG surgery induces reversible subclinical impairment of hepatocellular integrity, whereas off-pump revascularization attenuates this damage.

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トピックス

人工酸素運搬体の開発—現状と将来展望—

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医学書院

は信じられないほどである．同一遺伝子でも表現型(Phenotype)が違うのである．その表現型は環境によって左右されるのである．臨床検査データは一つの表現型であり，環境によって変化した結果も含んだデータである．遺伝子多型などの遺伝子情報と標準化された臨床検査データを蓄積し解析することで，個人が置かれた環境等の要因も考慮することができ，初めて個人の体質に合った医療(テーラーメード医療)が可能になると考えている．このような状況になると医療は劇的に変化するのではないだろうか．

今回JCCLSにできた臨床検査標準化基本検討委員会は，ここに述べたような手段で臨床検査ひいては医療の標準化の礎になることを目指している活動である．

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人工酸素運搬体の開発
—現状と将来展望—

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■歴史的背景

1900年にLandsteinerが血液型を発見，血液型をあわせ，交差試験を行うことにより，輸血の副作用は著しく減少した．20世紀，輸血は出血に対するなくてはならない治療法としてその安全性が追求され続け，その結果多くの血液型の理解が進み，輸血感染症が発見され，スクリーニング方法も確立した．また，移植片対宿主疾患(graft versus host disease, GVHD)などの免疫学的異常反応の解析と治療，輸血後急性肺傷害(transfusion related acute lung injury, TRALI)など，多くの問題に対して研究が行われ，その治療法が開発されて，輸血治療は安全・確実なものとなってきた．しかし，未知のウイルス感染症やプリオン病の問題など輸血治療の安全性にかかわる新たな問題が最近クローズアップされてきている．

いつでも，どこでも安全に投与できる人工血液の開発を目指し，厚生省(当時)は1997年に人工赤血球，人工血小板，人工抗体の創製と開発について科学研究班を組織し，精力的な研究が開始され，現在に至っている．

■人工赤血球とは

輸血に用いる保存血は冷所で保存し，保存期間が21日間と短いこと，輸血を行う直前に交差試験を必要とすることなどの煩雑な点がある．交差試験が必要なく，長期保存のできる人工赤血球の開発は第二次世界大戦以前より始まっていた．戦後，パーフルオロ化合物の合成技術，高分子合成・分離技術，蛋白質精製技術などの成熟とあいまって1960年ごろより人工赤血球として人工酸素運搬体の開発が行われるようになった．日本でも人工赤血球の開発は早くから始められ，旧ミドリ十字社がパーフルオロ化合物の乳剤であるフルオゾールを開発し，一定の成績を収めてFDA(Food and Drug Administration，米国食品医薬品局)より承認を受けたが，特殊な適応症のみの承認であったために広く使用されるには至らなかった．一方，ヘモグロビン(hemoglobin，Hb)を精製・修飾して酸素運搬体として利用するタイプの人工赤血球は北米で多くの企業が開発を行ってきた．Baxter社が開発したDCL-Hbは第3相試験まで研究が進んだが開発が中断された．一方，Biopure社の開発している重合ウシヘモグロビンを用いた人工赤血球は，現在北米を中心に第3相試験を行っており，南アフリカでは臨床応用がなされている．

以上のように開発が開始されて久しいが，投与後の血管抵抗の上昇や，組織機能，酸素運搬などの点で解決しなければならない問題点が多く，真の意味での臨床に用いられている物質はない．

■人工赤血球開発の現状

われわれは厚生労働省科学研究の一環として1985年より早稲田大学理工学部と共同で，人工酸素運搬体の開発と評価を行ってきた．現在二種類の人工酸素運搬体について研究を進めている．一つ目は期限切れの輸血用血液よりヘモグロビンを分離精製し，ウイルス除去，不活化を行ってからリン脂質小胞体(リポソーム)内に封入し，粒径を250 nmに制御したヘモグロビン内包型リポソームであるヘモグロビン小胞体(Hb小胞体)と，二つ目がアルブミンに人工合成のヘムを包接させたアルブミンヘムとである(図)．これらの開発現状について紹介し，臨床検査法の開発についても言及する．

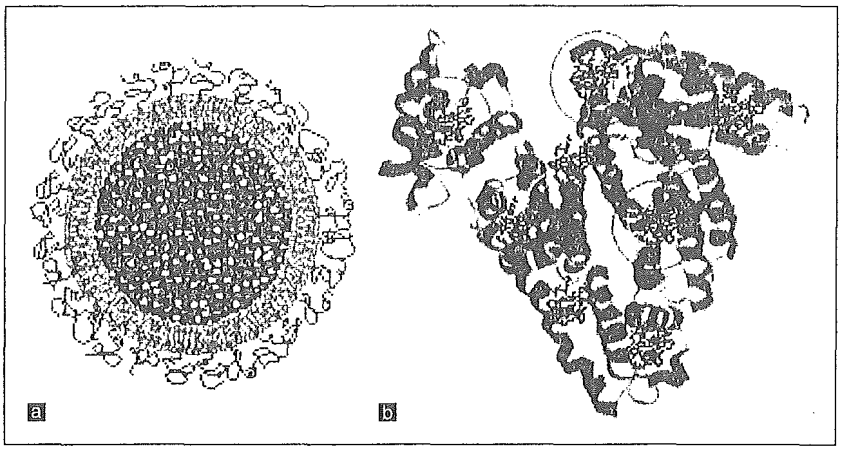

図　ヘモグロビン小胞体(a)とアルブミンヘム(b)の模式図
a：精製したヘモグロビンをリン脂質二重膜に封入している．直径 250 nm．b：人工合成のヘムをアルブミン分子に包接させた．

1．ヘモグロビン小胞体(Hb 小胞体)

Hb 小胞体は精製ヘモグロビンをヒトの赤血球のように脂質二重膜で被覆して粒径を制御し，酸素運搬能を持たせた物質である[1]．通常リポソームは自己粒子間の凝集や内皮細胞への付着，血小板の活性化などが問題となる．互いの凝集を防止し，血管内皮との相互作用を防止するためにポリエチレングリコール(polyethylene glycol，PEG)で膜表面を修飾し，血小板の活性化を抑制する負電荷脂質を用いてリポソームを形成している．また，長期保存を可能とするためには小胞体内のヘモグロビンが酸化されない環境を保持することが重要で，現在では窒素雰囲気下で Hb 小胞体を脱酸素化したデオキシ体として保存することによって1年以上の長期保存を可能としている[2]．

生体内での酸素運搬能，循環保持能力については，交換輸注試験[3]や出血性ショックの蘇生試験を中心に検討が行われている．ラット，およびウサギ，ビーグル犬において種々の検討が行われ，十分な酸素運搬能を有し，ショック蘇生に有効であることが解明されている．生体内での半減期は 35 時間(ラット)近くあることが報告された[4]．

投与後の生体に与える変化であるが，成長阻害もなく，実験動物の体重の増加も順調であった．血中より消失した Hb 小胞体がどのように代謝されるかを病理組織を用いて検討したところ，3時間後より脾臓の赤脾髄に集積し始め，7日後をピークとして脾臓が腫大，重量も最大となり，その後正常域に復することが明らかとなってきた．腫大は赤脾髄のマクロファージが Hb 小胞体を貪食することによって起こり，脾臓内のマクロファージはいったん，貪食胞が飽和に達したと思えるほど Hb 小胞体を貪食した後，経時的に正常像に回復し，7日後には正常の組織構築を呈することを Sakai らが報告している[5]．このような脾臓の変化はラットの保存血液を用いて行った同様の試験でも認められるので，Hb 小胞体に特有の現象ではないことも明らかとなった．

通常の薬品を開発する際には，LD_{50} から，最大投与量を決定し，通常使用量を決定してゆく．しかし人工酸素運搬体に関しては，最大投与量をどのように決定すべきなのかについて明らかなコンセンサスは得られておらず，今後検討すべき課題であると考えられる．

長期生存に与える影響，免疫系に与える影響についても研究が進んでおり反復投与でも成長，血液生化学的検査などでの明らかな異常は認められていない[6]．

リポソーム製剤を血管内に投与する場合，投与後に採血した血液内，特に血漿層にリポソームが分散し，血液・生化学検査が正しく評価できるかについて検討する必要がある．特に比色や比濁法で定量する臨床検査法では，Hb 小胞体の干渉作用のため検査結果に影響が出ることが予想された．この点に関しては採血後に血清を超遠心分離操作にかけて Hb 小胞体を沈殿除去することにより結果に影響を及ぼさないことが確認されている[7]．

2．アルブミンヘム

アルブミンは血中で最も多い蛋白質であり，膠質浸透圧，粘度を維持し，体内のホメオスタシスを保持する重要な蛋白質である．アルブミンヘムはこの蛋白質に人工合成のヘムを包接という方法で導入し，酸素運搬を可能とした物質である．体内で酸素運搬能を有

し，出血性ショックの治療薬として有効である可能性が示唆されており[8,9]，現在研究が続行中である．血中にアルブミンヘムが投与された場合の臨床検査法についても，今後開発を進めてゆく必要がある．

まとめ

人工赤血球は通常の薬剤と異なり，血中で酸素を受け渡しすることで機能を発揮し，血中にある程度の期間とどまることが要求されている．出血に対する治療薬としての用途のほか，虚血領域への酸素運搬を治療法としたOxygen Therapeutics(酸素治療)についても研究が進んでいる[10]．臨床応用が始まれば，臨床検査現場での対応も必要となるため，機器の開発，応用に関しても研究の展開が必要と考えられている．

輸血は20世紀の医療を大きく変えた治療法の1つであり，今日の日本での輸血は限りなく安全になっている．しかし，貯蔵，使用時の注意点を考えると人工血液があることによって輸血を補完できるシステムを構築できる可能性がある．この点で人工赤血球の開発は21世紀の医療の進歩に貢献できると考えられる[11]．

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新規腫瘍マーカーとしての尿中ジアセチルスペルミン

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はじめに

尿中ジアセチルスペルミンはポリアミンの尿中代謝産物の一つである．ポリアミンは活発に増殖する組織に多量に含まれており，細胞増殖に重要な役割を果たしていると考えられるが，アセチル化され，尿へ排泄されるが，その大部分はモノアセチル体である．ジアセチルスペルミンはポリアミンの1種であるスペルミンがジアセチル化されたもので，尿中ポリアミンの1%以下にすぎない．しかし，最近，癌患者に特異的に尿中排泄が増加することが知られるようになってきた．検体が尿であるため，採血の苦痛や針刺し事故の危険がなく，癌マーカーとしての機序より考えると臓器特異性がないことが推察され，癌検診に有用な“汎用性癌マーカー”として期待されている．

本稿では尿中ジアセチルスペルミンの研究の現況について概説した．

1．尿中ポリアミン

複数のアミノ基を持つアルキルアミンをポリアミンと総称する．ヒトの体内には4種類のポリアミンと，

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Superficial contact cryoablation attenuates experimentally created lung air leakage ☆

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Abstract

Previously, we, and others found that cryoablation on normal lung produced localized pulmonary hemorrhage and edema, causing obliteration of air space. Therefore, we hypothesized that lung air leakage may be diminished by this procedure. In the present study, we examined if cryoablation can attenuate experimentally created lung air leakage. Male domestic pigs ($n=4$) underwent a thoracotomy. The lung was resected approximately 5 mm in diameter and 1 mm in depth to create air leakage lesions. An argon gas cryoprobe with a copper plate attached to its tip was used to cryoablate the lesions superficially. After cryoablation, the positive airway pressure that produced macroscopic bubbles from each lesion site was compared between cryoablated and untreated lesions. Also, cryoablation of the lung surface was carried out in male Donryu rats ($n=20$) which were sequentially sacrificed to observe the histological changes over a time course. In the pigs, the air leakage pressure was significantly increased with cryoablation (40 cmH_2O <) compared to no treatment (19 ± 5 cmH_2O) ($p=0.021$, Mann–Whitney U test). Histologically, cryoablation produced acute pulmonary hemorrhage and edema. In the rats, the region with extensive hemorrhage progressed to fibrosis in 1 month, and the areas with edema recovered. This study provides supportive evidence that cryoablation has the potential to stop air leakage from surface pulmonary injury. This procedure may provide a useful adjunct to surgical resection for spontaneous pneumothorax, and the control of air leakage from dissected raw lung surfaces during lung resection.

Keywords: Cryoablation; Pneumothorax; Pulmonary air leakage; Pulmonary edema

Spontaneous pneumothorax in otherwise healthy individuals is nearly always due to the rupture of a subpleural bleb or bulla. At present, the mainstay of surgical therapy is considered to be resection of bullae along with a portion of underlying normal lung plus pleural ablation or pleurectomy. With the use of automatic suturing devices, often excess normal lung is resected, particularly when performed thoracoscopically as the suturing device insertion path is restricted. Moreover, broad based bullae are difficult to resect using auto-suturing devices without

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inclusion of a substantial portion of normal lung parenchyma, and often require the use of many cartridges. Also, it is not infrequent that we encounter cases with multiple bullae or blebs, too many to resect.

Previously, we, and others have examined the effects of cryoablation on normal lung [8,7,3]. The primary finding was localized extensive pulmonary hemorrhage and edema. From these findings, and a clinical report suggesting reduced air leakage by cryoablation following lung biopsies [1], we hypothesized that lung air leakage may be diminished by this procedure because the associated lung air space could be obliterated. In the present study, we examined if superficial contact cryoablation can attenuate experimentally created lung air leakage.

Materials and methods

Male domestic pigs (weight ~40 kg, $n=4$) were used for this study. Under general anesthesia, the animals were mechanically ventilated at a respiratory rate of 15–20 per min, tidal volume 10 ml/kg. The airway pressure was sustained at 10–15 cmH_2O. The animals were placed in a left decubitus position and right thoracotomy was performed. A 3 mm diameter cryoprobe (CRYOcare Cryosurgical Unit, Endocare, Irvine, CA) was used for the study. The cryoprobe uses high-pressure argon and helium gas for freezing and thawing, respectively, based on the Joule–Thomson principle. A 30 mm diameter copper plate, 0.2 mm thick, was placed at the tip of the probe (Fig. 1) to cryoablate the lung superficially through the pleural surface. Cryoablation was performed as 1 cycle of 5 min freeze followed by 5 min thaw. To create air leakage lesions, approximately 5 mm diameter of the lung, together with the pleura, was resected using forceps and scissors. The depth was adjusted to be approximately 1 mm with the lung inflated. Air leakage was confirmed at ventilating pressure of 10–15 cmH_2O. Lesions with macroscopic bleeding were not used. Four lesions were created per animal, 2 each in the upper and lower lobe so as to be well away from each other. Two of these lesions were cryoablated, one each in the upper and lower lobe, respectively. The plates and probes were removed together as soon as they acquired mobility after thawing. Other 2 lesions were left untreated.

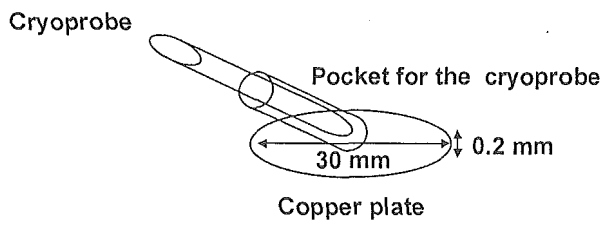

Fig. 1. Schematic representation of the copper plate attached to the cryoprobe.

Approximately 5 min after the thawing, when localized pulmonary hemorrhage becomes macroscopically apparent, air leakage test was done by progressively increasing positive airway pressure. The positive airway pressure that produced macroscopic bubbles from each lesion site was recorded as air leakage pressure. These parameters were compared between cryoablation (cryoablation group, $n=8$) and no treatment (control group, $n=8$). At the end of the experiment, the animals were sacrificed. The lung was removed and fixed in 10% formalin for H and E staining.

Long-term experiments were carried out in male Donryu rats (~250 g, $n=20$) primarily to observe the histological changes in the lung after superficial cryoablation over a time course. The animals were anesthetized with intramuscular injection of a cocktail of 90 mg ketamine hydrochloride (Parke-Davis, Morris Plains, NJ) and 9 mg xylazine (Fermentia, Kansas City, MO) per kg body weight. Tracheostomy was performed and a ventilation tube was placed in the trachea. The animals were placed in a right decubitus position. Under mechanical ventilation, the left chest wall was exposed, and a mini-thoracotomy was performed. The surface of the left lung was injured using an 18 gauge needle (approximately 1 mm in diameter and depth), and presence of air leakage was confirmed macroscopically by dripping saline. To cryoablate the lung surface in rats, an ophthalmic cryosurgical device was used (Cryomaster, Keeler, Windsor, UK). This device utilizes carbon dioxide gas, and the probe has a rounded tip, approximately 2 mm in diameter. The tip was gently placed on the injured lung surface. Cryoablation was performed as 1 cycle of 5 min freeze followed by 5 min thaw as in the pigs. The wound was closed and the animals were allowed to survive. For histological examinations, the animals were sequentially sacrificed at 7, 48 h, 1 week, 1 month, and 2 months, 4 animals per time point. Control experiments were not done in the rats, because in our preliminary experiment the rats showed signs of significant respiratory distress when the lung surface wound was left untreated.