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.

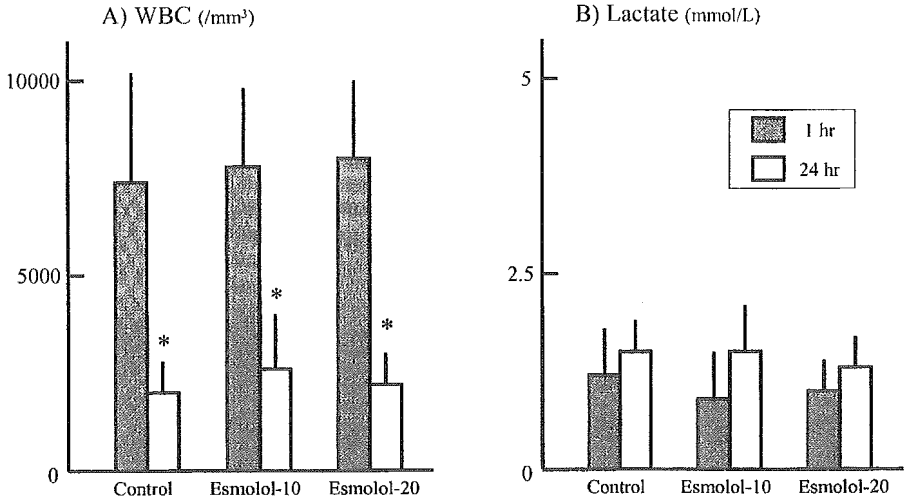

Figure 1. Changes of white blood cells (*WBC*) and arterial lactate at 1- and 24-hr study periods. The WBC counts were significantly reduced at 24 hrs in all groups compared with 1 hr ($p < .05$).

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-

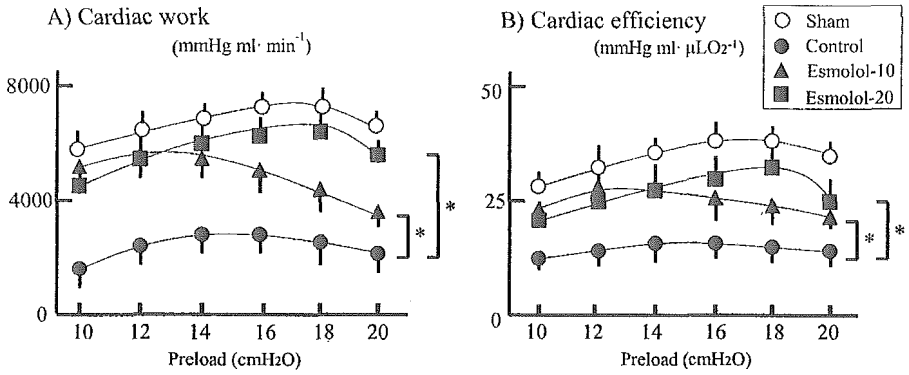

Figure 2. Changes of cardiac work and cardiac efficiency in an isolated working heart between the groups at different preloads. Cardiac efficiency = the ratio of cardiac work, the product of cardiac output × left ventricle peak systolic pressure, to myocardial oxygen consumption. Significant difference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

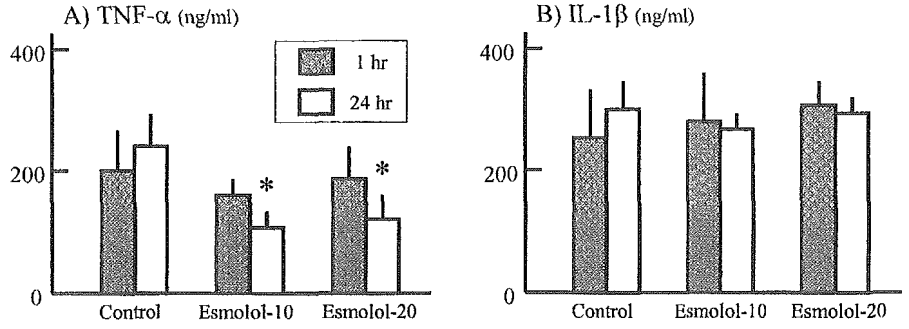

Figure 3. Changes of plasma tumor necrosis factor (*TNF*)-α and interleukin (*IL*)-1β concentrations at 1- and 24-hr study periods. *$p < .05$ vs. the control group.

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

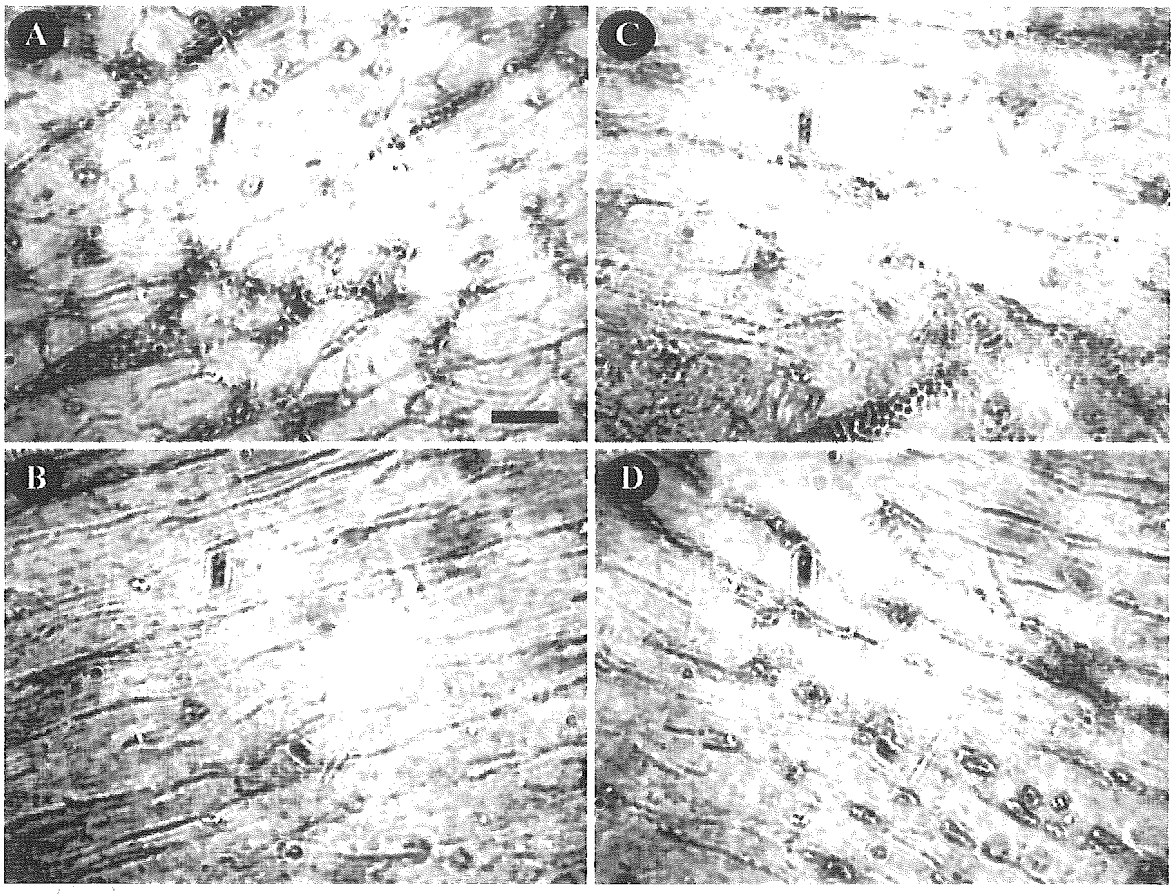

Figure 4. Representative pictures showing immunohistochemistry of β1-adrenergic receptor captured by light microscopy (×100) from rat myocardium. Positive staining for β1-adrenergic receptor is brown. *A*, representative immunostaining of myocardium from a sham rat. *Bar*, 50 μm. *B*, representative 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.

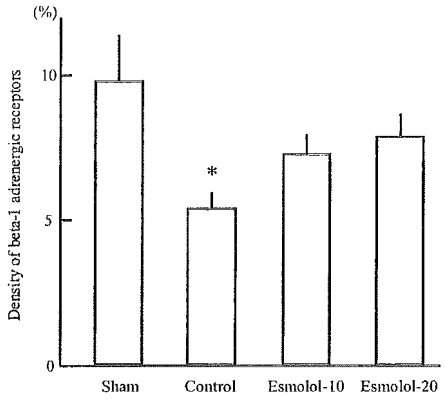

Figure 5. Comparison of β1-adrenergic receptor density in myocardium between sham and septic rats with or without esmolol infusion. *$p < .05$ vs. sham rat.

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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Epidural Analgesia Prevents Endotoxin-Induced Gut Mucosal Injury in Rabbits

Shizuko Kosugi, MD*, Hiroshi Morisaki, MD*, Tomoyuki Satoh, MD*, Kimiaki Ai, MD*, Michiko Yamamoto, BA*, Junko Soejima, MD†, Ryohei Serita, MD*, Yoshifumi Kotake, MD*, Akitoshi Ishizaka, MD†, and Junzo Takeda, MD*

Departments of *Anesthesiology and †Medicine, Keio University School of Medicine, Tokyo, Japan

In the present study, we evaluated the effect of epidural analgesia on the alterations of gut barrier function elicited by endotoxin in rabbits. After the placement of an epidural catheter, 28 male rabbits were randomized into either 0.5% lidocaine (group E) or saline (group C) group. The solutions (0.4 mL/kg) were epidurally injected, followed by continuous infusion (0.1 $mL \cdot kg^{-1} \cdot h^{-1}$) throughout the study period. Under a continuous infusion of lipopolysaccharide (15 $\mu g \cdot kg^{-1} \cdot h^{-1}$), mean arterial blood pressure, intramucosal pH, and plasma thrombomodulin concentrations were measured. At 4 h, mean arterial blood pressure was lower ($P < 0.05$), intramucosal pH was higher ($P < 0.01$), and the progression of hemodilution more profound ($P < 0.05$) in group E versus group C, whereas plasma thrombomodulin levels were increased to a similar extent between the groups. With less wet-to-dry weight ratio of ileum, histopathological injury scores of gut mucosa were significantly less in group E versus group C ($P < 0.01$). In a separate series of experiments ($n = 10$ each group), mucosal permeability in group E was significantly less compared with group C ($P < 0.05$). Collectively, these studies showed that despite a significant decrease of perfusion pressure and arterial oxygen content, epidural analgesia minimized endotoxin-induced functional and structural injury of gut mucosa possibly through endothelium-independent mechanisms.

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Intestinal mucosa is anatomically vulnerable to any type of oxygen deficit because of its low oxygen tension, right angle branching microvessels, and countercurrent blood supply (1). Through the loss of its barrier function, the gut becomes a significant supplier of microorganisms and toxins to the systemic circulation, evoking the discharge of proinflammatory mediators and the development of multiple organ dysfunction syndrome (2). Whereas the preservation of gut integrity has become a therapeutic goal for critically ill patients, few approaches are clinically relevant in preventing the progression of gut injury (2,3).

Epidural analgesia has been demonstrated, in both clinical and experimental examinations, to attenuate the decrease of intramucosal pH (pHi) in patients undergoing major abdominal surgery (4) and to augment intramucosal microcirculation of the gut in rats (5). In addition, epidural analgesia increased splanchnic venous capacitance by depressing sympathetic nerve activity in a rabbit model (6). We previously demonstrated that thoracic epidural anesthesia and analgesia retarded the progression of intramucosal acidosis and prevented endotoxin influx to the portal vein during acute hypoxia in rabbits (7). However, a question remains whether epidural anesthesia and analgesia are still protective for gut mucosa in clinically relevant disease conditions such as sepsis, where tissue hypoxia is more complicated and substantial. Among several pathophysiological profiles, sepsis is characterized by massive discharges of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) (8), by pathologic releases of nitric oxide (NO) to counteract oxygen radicals (9), and by considerable liberation of thrombomodulin, which acts as a cofactor of thrombin for protein C activation from injured endothelium (10). With a focus on the alterations of these mediators, we designed the present study to examine whether epidural analgesia preserved functional

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Address correspondence and reprint requests to H. Morisaki, MD, Department of Anesthesiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. Address e-mail to anesmrsk@sc.itc.keio.ac.jp.

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and structural integrity of the gut in a rabbit model of endotoxemia.

Methods

This protocol was approved by the Keio University School of Medicine Council on Animal Care in accordance with the guidelines of the National Institutes of Health.

Fifty healthy rabbits (New Zealand White, male; SEASCO, Saitama, Japan), weighing 2.0–2.5 kg (average, 2.3 kg) and fasted for 24 h, underwent instrumentation under general anesthesia. With sevoflurane 3%–4% inhaled in oxygen (3–4 L/min) via a face mask, the rabbits underwent tracheostomy and IV line access on the marginal ear vein. The rabbits were then mechanically ventilated to maintain normocapnia (fraction of inspiratory oxygen, 0.3; inspiratory pressure, 12–15 cm H_2O; and 10–12 breaths/min) using an intensive care unit type ventilator (New Port E-100; New Port Medical Inc, Newport Beach, CA). An epidural catheter was placed via T11-12 interspace, as previously described (11), and an indwelling arterial catheter (22-gauge) was inserted into the right carotid artery. After a midline abdominal incision, a silastic catheter was inserted through the mesenteric vein to the distal portion of the portal vein. A perivascular probe was attached around the portal vein for measurement of portal blood flow (Transit-Time Ultrasound Flowmeter, T206; Transomic System Inc, Ithaca, NY) (12). A sigmoid tonometer catheter (Tonometrics, Worcester, MA) was surgically inserted into the terminal ileum via the ileocecal portion. To obviate the effects of inhaled anesthesia and to mimic the rabbits the condition of sepsis in the intensive care unit, inhaled anesthesia was discontinued, and a mixture of buprenorphine (0.1 mg/mL) and midazolam (2 mg/mL) was continuously infused at a rate of 1 mL/h. Rectal temperature was monitored and maintained at approximately 37°C. Rabbits were observed for 30 min before baseline measurements were made.

Study Protocol 1

After baseline measurements (baseline), 30 rabbits were randomly assigned to a control (group C; $n = 14$) or epidural (group E; $n = 16$) group using computer-generated random numbers. All rabbits in group E received a 0.4-mL/kg bolus injection of 0.5% lidocaine through the epidural catheter, followed by a continuous infusion of 0.1 $mL \cdot kg^{-1} \cdot h^{-1}$, as described previously (13). Group C received the same doses of normal saline alone epidurally. After an equilibration period, the measurements of systemic and splanchnic variables were described in the measurement of pHi, and the specific measurements and calculations were performed (0 h). Thereafter, both groups received continuous infusion of 15 $\mu g \cdot kg^{-1} \cdot h^{-1}$ of lipopolysaccharide (LPS) (*Escherichia coli* serotype 055:55B5; Sigma Chemical Co, St Louis, MO), accompanied by Ringer's acetate solution infusion at a rate of 25 $mL \cdot kg^{-1} \cdot h^{-1}$, throughout the study periods. We chose the identical fluid volume between the groups to obviate a confounding factor for edema formation. In our pilot study using periaortic flow measurements, as previously described (12), the animals demonstrated a hyperdynamic circulatory pattern by manifesting an approximately 25% increase of cardiac output for 5 h, indicating that the animal model could be clinically relevant to septic patients throughout the 4-h study periods. The measurements of systemic and splanchnic variables were repeated at 2- and 4-h time periods. At the completion of the experiment, 0.4 mL/kg of indocyanine green was injected through epidural catheter, and if the cephalic spread of dye did not cover the range between the T4 and L1 level of the spine, the rabbit was excluded from the data collection. After tissue sampling was performed to determine wet-to-dry weight ratio and histological analysis of terminal ileum, the rabbits were killed with an IV pentobarbital overdose.

Gut pHi was monitored using automated air tonometry (Tonocap, Datex Ohmeda, Helsinki, Finland) (14). The measured regional Pco_2 ($Prco_2$), together with simultaneously obtained arterial [HCO_3^-], were applied in the Henderson-Hasselbalch equation for calculation of pHi according to the manufacture's instruction:

$$pHi = 6.1 + \log[HCO_3^-]/0.03 \times PrCO_2$$

[HCO_3^-] being the arterial bicarbonate concentration, 6.1 the dissociation constant of HCO_3^-, and 0.03 the solubility of CO_2 in plasma.

Arterial TNF-α plasma concentrations were measured using enzyme-linked immunosorbent assay that was developed in our laboratory. The assays were performed by using a combination of purified polyclonal goat anti-rabbit TNF antibody as a capture antibody and biotinylated polyclonal goat anti-rabbit TNF antibody for detection (15). Standard material, which was used in the rabbit TNF-conditioned medium (PharMingen, San Diego, CA), and obtained samples were run in duplicate. The detection limit in this assay was 13.7 pg/mL, and linear standard curves were obtained that ranged from 123 to 10,000 pg/mL.

The arterial thrombomodulin plasma level was measured by using enzyme-linked immunosorbent assay, as described elsewhere (16). Briefly, each well of a microtiter plate was coated with 1 μg/mL of goat

anti-rabbit thrombomodulin immunoglobulin G dissolved in 0.1 mol/L of $NaHCO_3$ (pH value of 9.6), and the plate was incubated overnight at 4°C. After blocking with Block Ace (Dainippon Seiyaku, CO, Ltd, Osaka, Japan), rabbit standard thrombomodulin (American Diagnostica Inc, Greenwich, CT) and test specimens were loaded into the wells, and the plate was incubated for 60 min at room temperature. Biotylated anti-thrombomodulin immunoglobulin G was added to the wells, followed by a 30-min incubation. After a 15-min incubation with avidin-peroxidase complex, a substrate solution containing 0.01% H_2O_2 and 0.4 mg/mL of o-phenyl-enediamine was added. The reaction was stopped using 4.5 N of H_2SO_4, and the absorbance at 490 nm was measured using the plate analyzer (ETY-3A, Toyo Sokki, Zama, Japan).

At the completion of experiments in Study Protocol 1, mucosal edema and microstructure of the terminal ileum were examined. Wet weights of five 2-cm parts of ileal tissues were measured and then dried in a vacuum oven (DP22; Yamato Scientific, Tokyo, Japan) at 95°C and −20 cm H_2O for 48 h. The dry weights were determined, and the wet-to-dry weight ratio was calculated (12). Ileal samples for microscopic examination were obtained from the terminal ileum, which was distant from the tonometer catheter placement. Histological sections were evaluated in a blinded manner using light microscopy. Twenty-five random fields from each tissue were examined, and the degree of mucosal damage was graded on a scale of 0–4, with a modification of grading system previously described (17). In this classification, normal villi were graded as 0, mucosal edema limited to the apex of the villous tip and development of the subepithelial space as 1, extension of subepithelial space as 2, localized area of mucosal destruction or extensive submucosal edema was 3, and severe cell disruption was grade 4.

Arterial and portal pH, Pco_2, Po_2, and lactate concentrations were determined by using a blood gas analyzer (Chiron 860 series, Chiron Diagnostics Corp, East Walpole, MA). Hemoglobin and hemoglobin oxygen saturation were measured using a co-oximeter (OSM3, Radiometer, Copenhagen). Splanchnic oxygen extraction ratio was calculated using standard formulae: splanchnic oxygen extraction ratio (%) = $100 \times (CaO_2 - CpO_2)/CaO_2$. Arterial and portal blood samples were centrifuged, and the plasma was stored at −80°C until analysis. NO release was assessed by the determination of stable NO metabolites (NO_2^- + NO_3^-; NOx) in plasma using spectrophotometric assay (Cayman, Detroit, MI). Plasma lidocaine concentrations at 4 h in both groups were determined by fluorescence polarization immunoassay (TDX system, Abbot, North Chicago, IL). Measurements of all variables were performed in duplicate, and mean values were used for results.

Study Protocol 2

In a separate series of experiments, we determined the changes of gut permeability using fluorescein isothiocyanate-conjugated dextran with a molecular weight of 4000 Da (FD4) (13). After the same preparatory surgery, excluding the placement of the tonometer catheter and perivascular flow probe, 20 rabbits (New Zealand White, male; SEASCO) were randomized into groups C or E (n = 10 each group). The rabbits received the same dose of endotoxin infusion as those in Study Protocol 1. At 4 h, the abdomen was opened for preparation of an *in situ* loop of the gut. Briefly, double ligatures at both ends were made on the 10-cm length of the terminal ileum. Through a cannula placed into this segment of terminal ileum, FD4 (50 mg) was injected. After 30 min, blood samples from both the portal vein and artery were taken and centrifuged, and plasma FD4 concentrations were measured using fluorescence spectrometry (Spectrofluorophotometer:RF-1500; Shimadzu, Kyoto, Japan). Results were corrected for the plasma protein contents measured by the Lowry method.

Data are expressed as mean ± SD unless otherwise specified. Analysis of variance with repeated measures was used to evaluate the differences as shown using SPSS/11.0J for Windows (SPSS Inc, Chicago, IL). Separate analysis was performed if the interaction was statistically significant. When $P < 0.05$, the Scheffe multiple-comparison test was applied to distinguish differences between measurement variables. If the data were not normally distributed, the Friedman test was used to evaluate pair-wise comparisons. The histological scoring data were analyzed by χ^2 test. Differences were considered statistically significant if $P < 0.05$.

Results

Plasma lidocaine concentrations in both groups were all less than the undetectable level (<1.0 μg/mL). Because of misplacement of the epidural catheter, 2 rabbits in group E in Study Protocol 1 were excluded from the data collection.

Compared to the 0-h period, LPS infusion decreased mean arterial blood pressure (MAP) in both study groups ($P < 0.05$). The extent of hypotension in group E was significantly more than group C ($P < 0.05$) (Table 1). Heart rate was significantly depressed to a similar extent in both study groups compared with 0 h ($P < 0.05$). Whereas arterial Po_2 remained constant throughout the study periods in both study groups, arterial oxygen content was significantly depressed to a larger extent in group E compared with group C ($P < 0.05$), mainly because of the progressive reduction of hemoglobin levels. Macroscopic hemolysis and persistent hemorrhage were not observed in either study

Table 1. Effects of Epidural Analgesia on Systemic Circulatory Variables in Endotoxemic Rabbits

	Group	Baseline	0 h	2 h	4 h
Mean arterial blood pressure (mm Hg)	C	92 ± 14	93 ± 14	83 ± 18	81 ± 15
	E	90 ± 14	85 ± 15	59 ± 11*†	59 ± 11*†
Heart rate (bpm)	C	279 ± 25	278 ± 28	257 ± 37†	248 ± 41†
	E	277 ± 34	275 ± 37	245 ± 43†	240 ± 51†
Arterial Po_2 (mm Hg)	C	179 ± 20	182 ± 25	184 ± 17	187 ± 22
	E	181 ± 17	176 ± 28	206 ± 33	187 ± 26
Arterial oxygen content (mL O_2/L)	C	13.9 ± 1.2	13.2 ± 1.2	11.7 ± 1.2†	10.3 ± 1.3†
	E	12.9 ± 1.9	11.6 ± 1.5*	9.6 ± 1.4*†	8.0 ± 0.9*‡
Arterial hemoglobin (g/dL)	C	9.8 ± 0.9	9.3 ± 0.9	8.2 ± 0.9	7.1 ± 1.0‡
	E	9.1 ± 1.3	8.1 ± 1.1	6.6 ± 1.0*†	5.5 ± 0.7*‡
Arterial pH	C	7.39 ± 0.09	7.41 ± 0.06	7.33 ± 0.06	7.32 ± 0.05
	E	7.36 ± 0.05	7.39 ± 0.05	7.41 ± 0.07	7.34 ± 0.05
Arterial lactate (mmol/L)	C	3.6 ± 1.5	3.1 ± 1.5	4.7 ± 2.2	5.9 ± 2.4†
	E	3.5 ± 1.6	3.3 ± 1.7	4.5 ± 2.5	4.8 ± 2.4†

Data are expressed as mean ± SD. The number of rabbits examined is 14 in each group.
* $P < 0.05$ versus Group C; † $P < 0.05$; ‡ $P < 0.01$ versus 0 h.

Table 2. Effects of Epidural Analgesia on Splanchnic Circulation Variables in Endotoxemic Rabbits

	Group	Baseline	0 h	2 h	4 h
Portal blood flow (mL/min)	C	126 ± 32	93 ± 24	116 ± 18	111 ± 29
	E	97 ± 21	82 ± 26	102 ± 33	97 ± 20
Portal oxygen content (mL O_2/L)	C	11.4 ± 1.9	10.6 ± 1.7	10.3 ± 1.5	9.3 ± 2.0
	E	11.0 ± 2.2	9.1 ± 2.2	8.1 ± 1.5*	6.6 ± 1.2*†
Oxygen extraction ratio (%)	C	18 ± 14	20 ± 11	14 ± 10	17 ± 16
	E	16 ± 7	23 ± 12	17 ± 6	22 ± 12
Portal lactate (mmol/L)	C	3.6 ± 1.5	3.1 ± 1.4	4.8 ± 2.2	5.9 ± 2.4†
	E	3.4 ± 1.6	3.3 ± 1.7	4.3 ± 2.2	4.7 ± 2.3†
Portal pH	C	7.39 ± 0.07	7.41 ± 0.06	7.31 ± 0.07†	7.30 ± 0.05†
	E	7.37 ± 0.05	7.39 ± 0.04	7.36 ± 0.09	7.31 ± 0.04†
pHi	C	7.36 ± 0.05	7.36 ± 0.05	7.21 ± 0.09†	7.20 ± 0.07†
	E	7.36 ± 0.06	7.38 ± 0.08	7.36 ± 0.11**	7.33 ± 0.08**

Data are expressed as mean ± SD. The number of rabbits examined is 14 in each group.
Hb = hemoglobin; pHi = intramucosal pH.
* $P < 0.05$; ** $P < 0.01$ versus Group C; † $P < 0.05$; ‡ $P < 0.01$ versus 0 h.

group. Arterial pH values remained unchanged, whereas arterial lactate showed a mild increase at 4 h of LPS infusion in both groups ($P < 0.05$).

Portal blood flow remained constant during the study periods in both study groups (Table 2). Portal pH values decreased, and lactate concentration increased in both groups ($P < 0.05$). Splanchnic oxygen extraction ratio remained constant during LPS infusion despite a slight increase of portal lactate at 4 h ($P < 0.05$). The pHi values of group E were preserved within the normal range (>7.32), whereas those of group C decreased significantly after LPS administration ($P < 0.05$) (Table 2). The wet-to-dry weight ratio of terminal ileum in group E was significantly smaller versus group C (3.40 ± 1.99 versus 6.15 ± 1.27; $P < 0.01$), and the plasma FD4 concentrations in group E were also significantly less (3.26 ± 0.47 versus 4.13 ± 0.98 mg/mg-protein; $P < 0.05$), indicating that the increase of endothelial and intestinal wall permeability induced by LPS infusion were significantly reduced by epidural analgesia.

Figure 1 illustrates representative microscopic pictures of villi of distal ileum in normal rabbits ($n = 3$), group C rabbits, and group E rabbits using light microscopy. The villi of the rabbits in group C showed definitive evidence of mucosal injury, such as disruption of microvilli, lifting of the epithelium from the basal lamina, and submucosal edema (Panel B), whereas those in group E seemed near normal (Panel C). The mucosal injury of distal ileum observed in group C was significantly attenuated in group E (Fig. 2) ($P < 0.01$).

Figure 3 illustrates the changes of plasma NOx and TNF-α concentrations at the 2- and 4-h study periods. Both arterial and portal NOx levels were not changed during endotoxin infusion in either study group (Fig. 3A). The level of TNF-α in group C was significantly reduced in group E after 2 h of endotoxin infusion (Fig. 3B). The difference of TNF-α levels found at the 2-h period between the groups disappeared at 4 h. The portal thrombomodulin level was comparable at 0 h and increased significantly to the similar extent in both group C

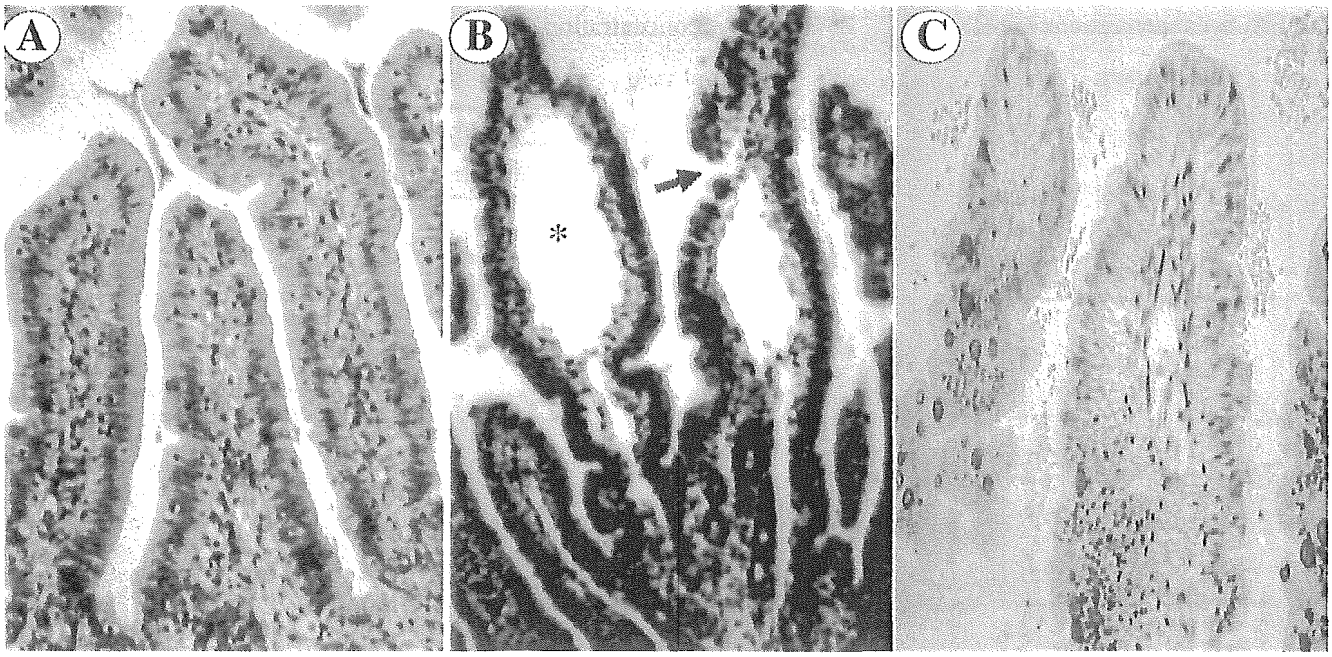

Figure 1. Representative pictures showing villi of distal ileum captured by light microscopy (×100) from rabbits with toluidine blue staining. (A) A representative villi of distal ileum from a normal rabbit. Bar = 50 μm. (B) A representative villi of distal ileum from a rabbit in group C. Note that the epithelial lifting to denuded tips of villi (arrow) and increased cellularity of lamina propria (asterisk) are evident in the ileum. (C) A representative villi of distal ileum from a rabbit in group E. Note near norma-appearing structure of ileal mucosa.

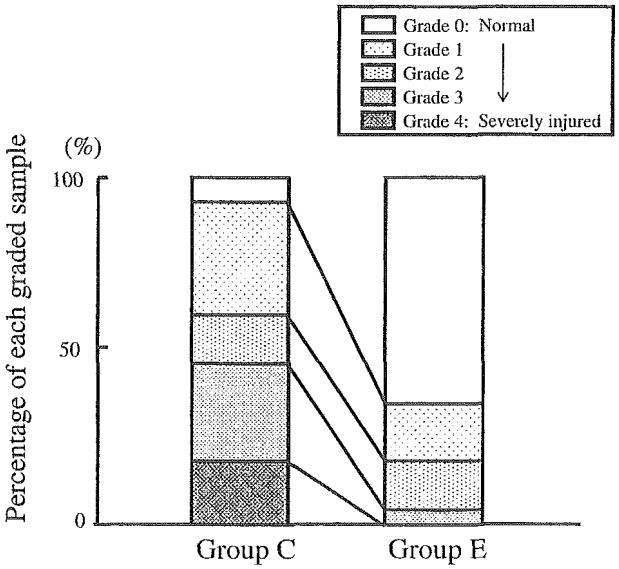

Figure 2. Effects of epidural analgesia on the severity of mucosal injury during endotoxemia. The degree of mucosal injury was graded on a scale of 0–4, with 0 considered normal and 4 representing severe cell disruption. Data are expressed as percentage of 25 fields for each rabbit (n = 14 each group). χ^2 test showed a significant difference between the groups ($P < 0.01$).

and E at 4 h after LPS infusion (213 ± 46 ng/mL to 1037 ± 991 ng/mL versus 225 ± 28 ng/mL to 1395 ± 878 ng/mL, respectively; $P < 0.01$), indicating that endotoxin-induced endothelial injury was not modulated by epidural analgesia.

Discussion

The current study indicates that the application of epidural analgesia with lidocaine in endotoxemic hosts attenuates the progression of intramucosal acidosis, the increase of intestinal permeability, and the structural alterations of intestinal villi, possibly through the restoration of microcirculation. In addition, the protective effects are independent from modulation of endotoxin-induced endothelial cell activation to liberate thrombomodulin. These beneficial effects were noted, even though the epidural block was accompanied by a significant decrease of perfusion pressure and arterial oxygen content, which could be potent confounding factors to deteriorate gut mucosal oxygenation.

Our previous study using an acute hypoxia model showed the similar progression of moderate hemodilution (arterial hemoglobin, 7.7 ± 0.8 g/dL) under approximately one-fifth fluid volume resuscitation compared with this endotoxemia model (7). Because persistent bleeding was not observed after the preparatory surgery, aggressive fluid resuscitation and frequent blood sampling were the primary contributors to this progressive hemodilution in both groups. Thus, the augmentation of hemodilution only observed in group E could be attributed to the presence of epidural analgesia *per se*. A previous study demonstrated that thoracic epidural anesthesia with extra intravascular fluid administration induced significant hemodilution but not with basic fluid infusion in healthy pigs

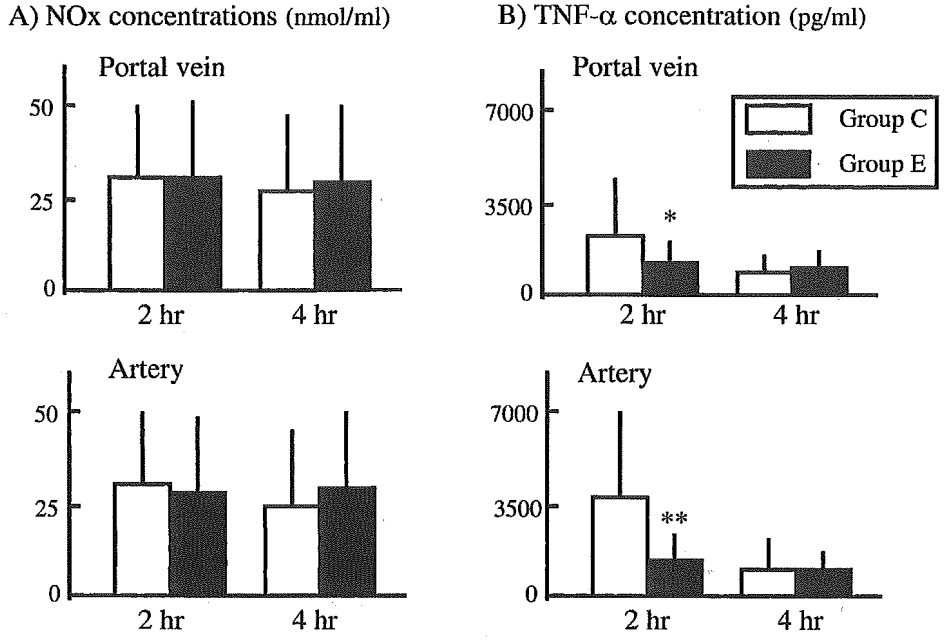

Figure 3. Changes of plasma NOx (A) and tumor necrosis factor (TNF)-α (B) concentrations during endotoxin infusion. Data are expressed as mean ± SD (n = 14 in each group). *P < 0.05; **P < 0.01 versus group C. No significant differences were found in NOx data between the groups.

(18). Interestingly, such additional fluid volume did not restore epidural anesthesia-induced hypotension, which was consistent with our findings. Another study showed that intravascular fluid administration with crystalloid solution resulted in more pronounced hemodilution in patients who developed hypotension during epidural anesthesia (19). Although the precise mechanisms remain unclear (19), either hypovolemia or hypotension caused by epidural anesthesia is able to recruit a considerable quantity of fluid from skin and skeletal muscle (20,21) in combination with its restoring effects of increased vascular permeability during endotoxemia. Some may argue that moderate hemodilution *per se* rather than epidural lidocaine protects mucosal microcirculation of the gut. In a porcine model, gut mucosal oxygen supply was well maintained to systemic hematocrit values of approximately 10%, whereas serosal tissue oxygen supply decreased (22). Conversely, another study reported that acute severe hemodilution from 20% to 14% of hematocrit, close to the level observed in group E, exhausted the compensatory mechanisms of splanchnic circulation (23). In normal rats, the critical hematocrit for intestinal tissue oxygenation was approximately 16% (24). Therefore, the hemoglobin concentration observed in group E seems close to the level of critical hematocrit, not providing further beneficial aspects of hemodilution on the microcirculation.

To explain the initial host response against endotoxin exposure, it is postulated that prototypic cytokines like TNF-α are discharged first from macrophages and monocytes, subsequently initiating a second level of inflammatory cascades such as interleukin-6 and damaging gut mucosa by modulating splanchnic oxygen transport and intramucosal microcirculation (25). The TNF-α levels in endotoxemia are characterized by a rapid increase within 30 min, followed by a one-hour peak and a decrease during the next two hours (26). Considering these kinetics, epidural analgesia may have been able to modulate the process to discharge TNF-α at the early stage of endotoxemia. Simultaneously, the decrease of plasma TNF-α levels at a later stage, accompanied by a loss of significant difference between the groups, seems to be rational, although the serum TNF-α profile in human and animal sepsis models is an issue of much debate regarding its time course and magnitude (8,9,26–28). However, a local increase of such cytokines and oxygen radicals could, in turn, upregulate inducible NO synthase expression, leading to a prolonged increase of NOx levels in tissue and plasma. A previous study showed that endogenous production of NO played an important role in the modulation of gut permeability in rats (29). Although we were unable to show any significant alterations of the NOx levels in this model (Fig. 3), it was consistent with a previous animal study that demonstrated a species-specific modulation of NO pathway after endotoxin injection in rabbits (9). Previous studies showing a significant increase of NOx in endotoxemia were applied to a shock model with a single injection of large-dose endotoxin (9,30). However, in the present study, we intended to mimic a more clinically relevant model of sepsis by using small-dose endotoxin infusion, presenting a normotensive hyperdynamic circulatory state. Another important finding of this study was to show a marked increase of plasma thrombomodulin in both study groups. Thrombomodulin, which plays a crucial role in hemostasis by binding thrombin and subsequently converting protein C to its active form, has been recognized as a sensitive marker of endothelial injury

(10). In the present study, such endothelial injury was not modulated by epidural analgesia. However, intestinal edema through an increased vascular permeability was significantly reduced by application of epidural analgesia. These conflicting results suggest that (a) upregulation of NO in this model might not be involved so significantly as is detected, (b) protein leakage through separation of endothelial tight junctions, rather than endothelial cell activation liberating thrombomodulin, is a major element to develop tissue edema, and (c) plasma thrombomodulin does not always mirror the whole profile of endothelial cell injury.

The clinical implications of the present study should be interpreted with caution because endotoxemia or severe sepsis is likely accompanied by the risk of infection such as meningitis or coagulation abnormalities such as low platelet count and coagulation factors that limit the indication of epidural analgesia. Furthermore, application of epidural anesthesia and analgesia may worsen the stability of systemic hemodynamics in critically ill patients who are treated with aggressive fluid resuscitation and vasoactive drugs. Indeed, MAP in group E was significantly depressed versus MAP in group C. Even with such a confounding factor, epidural analgesia shows a protective property on gut mucosa. Although it remains to be determined whether epidural analgesia can restore gut barrier dysfunction when it has already been established, epidural analgesia may be indicated for patients with functional obstruction of gastrointestinal tract or major vascular surgery who may develop increased mucosal permeability and subsequent bacterial translocation (31,32). In addition, potent analgesic effects of epidural blockade in this acutely instrumented model might provide more optimal analgesia and subsequently blunt stress responses, resulting in the reduction of discharges of vasoconstrictive mediators and the preservation of gut mucosal microcirculation. Finally, small plasma concentrations of lidocaine, absorbed from the epidural space in group E, could have modified the results, although they were all less than the detectable limit of the assay we applied. A previous study showed that lidocaine (plasma concentration, 1.4–2.5 μg/mL) reduced the extravasation of albumin in the lungs of endotoxemic rabbits (33). Another study reported that IV pretreatment with the same dose of lidocaine in rats attenuated endotoxin-induced increases in leukocyte adhesion and transvascular leakage against albumin (34). However, there is a possibility that small-dose lidocaine infusion, inducing plasma lidocaine at less than the detectable range, via the parenteral route might have produced the similar results.

In conclusion, the present study demonstrated that epidural analgesia prevented endotoxin-induced functional and structural alterations of gut mucosa in rabbits without modulating injured endothelial cells. Although further investigation is warranted, this study suggests that epidural anesthesia may be given to critically ill patients who are at risk of gut barrier dysfunction as a therapeutic option to preserve functional integrity.

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Olprinone, a Phosphodiesterase III Inhibitor, Reduces Gut Mucosal Injury and Portal Endotoxin Level during Acute Hypoxia in Rabbits

Tomoyuki Satoh, M.D.,* Hiroshi Morisaki, M.D.,† Kimiaki Ai, M.D.,* Shizuko Kosugi, M.D.,* Michiko Yamamoto, B.A.,‡ Ryohei Serita, M.D.,* Yoshifumi Kotake, M.D.,* Junzo Takeda, M.D.§

Background: **Preservation of gut integrity has become a therapeutic goal to obviate bacterial translocation in the critically ill. The authors examined whether olprinone, a phosphodiesterase III inhibitor, protected functional and structural integrity of gut mucosa against acute progressive hypoxia.**

Methods: **Thirty-two animals were randomly allocated to a control group (n = 12), a low-dose group (0.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ olprinone; n = 10), or a high-dose group (0.6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ olprinone; n = 10) after preparatory surgery. Ascending aortic and portal blood flow, intramural pH of the ileum, and portal endotoxin levels were measured at normoxia and through three stages of progressive hypoxia (fraction of inspired oxygen = 0.17, 0.13, and 0.10).**

Results: **At normoxia, ascending aortic flow in the high-dose group was approximately 20% higher than in the control and low-dose groups. During progressive hypoxia, both ascending aortic and portal flow in the control group were depressed, whereas olprinone infusion attenuated such alterations and redistributed blood to the splanchnic area in a dose-dependent manner. On the contrary, the reduction of intramural pH of the ileum and the elevation of portal endotoxin levels observed in the control group were significantly minimized in both the low- and high-dose groups to a similar extent during acute hypoxia. Histopathologic alterations of gut mucosa observed in the control group were minimized by olprinone infusion dose-independently, accompanied by reduction of mortality rate of the animals.**

Conclusions: **Olprinone slows progression of intestinal mucosal acidosis and gut barrier dysfunction, concurrently with preservation of microscopic structures, through both flow-dependent and -independent mechanisms under acute hypoxia. Such properties of olprinone may serve to protect the host under insult.**

RECENT studies show that the mucosal barrier function of the gut, blocking the entry of microorganisms and/or toxins into systemic circulation, plays a consequential role in the discharge of proinflammatory mediators and the development of multiple organ dysfunction.[1,2] Although augmentation of oxygen supply to the gut, anatomically susceptible to oxygen deficit, is a way to maintain its barrier function, pharmacologic interventions do not necessarily improve the performance of oxygen metabolism at intramucosal microcirculation.[3] For example, low-dose dopamine infusion, traditionally considered a procedure to increase splanchnic blood flow, does not improve mucosal blood flow, but redistributes flow away from the mucosa in a porcine model of hemorrhagic shock.[4] On the contrary, dobutamine is likely to increase blood flow to the gut concurrently with the improvement of intramucosal pH (pHi) in endotoxin shock.[5] Recently, we demonstrated that continuous epidural anesthesia with lidocaine slowed the progression of intramucosal acidosis of the gut, subsequently preventing the translocation of endotoxin into the portal vein in an acute hypoxic model.[6] Such therapeutic intervention aimed at preserving gut barrier function could improve the mortality rate in the critically ill.[7]

Olprinone (E-1020; 1,2-dihydro-6-methyl-2-oxo-5-[imidazo(1,2-a) pyridin-6-yl]-3-pyridine carbonitrile hydrochloride monohydrate), clinically used as an inotropic and vasodilator in patients with congestive heart failure, is a newly synthesized imidazopyridine-derivative phosphodiesterase III inhibitor.[8] Among this type of drugs, olprinone is characterized by promising profiles to preserve the function of extracardiac organs such as the diaphragm, bronchus, or gut.[9-11] A previous study demonstrated that olprinone augmented hepatosplanchnic blood flow more than cardiac output in patients undergoing cardiac surgery, suggesting that olprinone was likely to preserve gut integrity under conditions of splanchnic hypoperfusion due to redistribution of blood flow between organs.[11] Furthermore, there is an increasing amount of evidence that phosphodiesterase III inhibitors such as pentoxifylline *per se* work as antiinflammatory agents in the setting of oxidative or inflammatory insults.[12,13] A previous study showed that amrinone, another phosphodiesterase III inhibitor, at clinically relevant concentrations, inhibited cytokine-induced increases in adhesion molecule concentrations of human umbilical vein endothelial cells *in vitro*.[14] Collectively, olprinone may protect the barrier function of the gut from insults not only through its primary action to augment splanchnic flow but also by flow-independent pathways. Therefore, we tested the hypothesis that olprinone infusion redistributed blood flow to splanchnic area under acute hypoxia and subsequently preserved functional and structural integrity of the gut in a rabbit model.

* Instructor, † Assistant Professor, ‡ Research Associate, § Professor, Department of Anesthesiology.

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Address reprint requests to Dr. Morisaki: Department of Anesthesiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan. Address electronic mail to: anesmrsk@sc.itc.keio.ac.jp.

Materials and Methods

This protocol was approved by the Keio University Council on Animal Care (Tokyo, Japan) in accordance with the guidelines of the National Institutes of Health.

Preparatory Surgery

Thirty-two healthy rabbits (Japanese white, male; SEASCO, Saitama, Japan) that weighed 2.0–2.6 kg (average, 2.3 kg) and fasted for 24 h were used. With 3–4% sevoflurane inhalation in oxygen (3–4 l/min) *via* face mask, the rabbits underwent tracheostomy and intravenous line access on the marginal ear vein. The rabbits were then mechanically ventilated to maintain normocapnia (peak inspiratory pressure 12–15 cm H_2O and 10–12/min) using an intensive care unit-type ventilator (New Port E-100; New Port Medical Instruction Inc., CA). The right carotid artery was cannulated to monitor mean arterial pressure. Thereafter, all animals received sternotomy to attach a perivascular probe of the Transit-Time Ultrasound Flowmeter (T206; Transonic Systems Inc., Ithaca, NY) around the ascending aorta for measurement of cardiac output. Following the midline abdominal incision, a silastic catheter was inserted through the mesenteric vein to the distal portion of the portal vein for subsequent blood sampling. Another perivascular probe was attached around the portal vein for measurement of portal blood flow as described previously.[6] Then, a sigmoid tonometer catheter (Tonometrics, Worcester, MA) was surgically inserted intraluminally into the terminal ileum. After closure of the sternotomy and laparotomy, inhalational anesthesia was discontinued, and a continuous 1-ml/h infusion mixture of buprenorphine (0.1 mg/ml), midazolam (2 mg/ml) and vecuronium (0.05 mg/ml) was given throughout the study period to suppress vigorous spontaneous inspiratory efforts during hypoxia as described in the Study Protocol. Ringer's acetate solution (10–12 ml/kg) was infused for 30 min and continuously administered at the speed of 4 ml · kg^{-1} · h^{-1} throughout the study period. Animals were observed for 45 min before baseline measurements.

Study Protocol

After baseline measurements (baseline), 32 rabbits were randomly allocated into three groups using computer-generated random numbers. Twelve animals (control group) received normal saline infusion, whereas the animals in the low-dose group (n = 10) received a continuous infusion of olprinone at a rate of 0.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$, and those in the high-dose group (n = 10) received a continuous infusion of olprinone at a rate of 0.6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ throughout the study period. Our pilot study demonstrated that olprinone at a rate of 0.6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ increased cardiac output to an approximately 20% higher level compared to baseline in rabbits, whereas 0.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ did not influence cardiac output values. Therefore, in the current study, we applied these doses to the high-dose and low-dose groups, respectively. After a 45-min equilibration period, baseline portal flow, pHi, and other analyses described in the Specific Measurements and Calculations section were performed (normoxia). By mixing air with 100% nitrogen through the oxygen blender of the ventilator, the fraction of inspired oxygen (F_{IO_2}) was reduced in three stages (mild hypoxia F_{IO_2} = 0.17, moderate hypoxia F_{IO_2} = 0.13, and severe hypoxia F_{IO_2} = 0.10) under monitoring with an anesthetic gas analyzer (Ohmeda 5250RGM; BOC Health Care, Louisville, CO). The measurements were then repeated after a 45-min equilibration period for each hypoxic stage (fig. 1). After the experiments were executed, rabbits were sacrificed with an intravenous pentobarbital overdose.

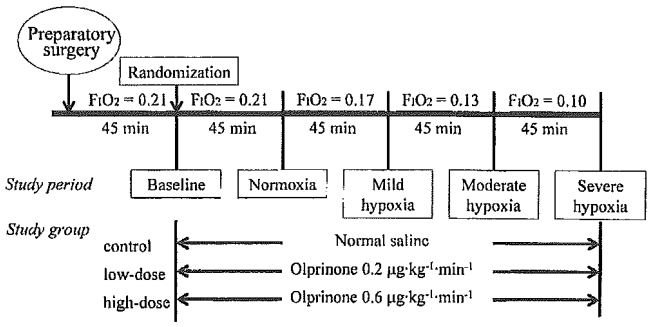

Fig. 1. Schematic drawing of experimental protocol.

Measurement of pHi

Gut pHi was monitored using a tonometric method described previously.[15,16] Air in the silicone balloon was equilibrated for 10 min, and the P_{CO_2} in the air was measured by capnometry using automated air tonometry (Tonocap; Datex Ohmeda, Helsinki, Finland). This device automatically fills the tonometer catheter balloon with 5 ml room air, which is kept in the balloon for a preselected equilibration period to allow carbon dioxide diffusion from the intestinal lumen. The sample then is emptied back into an infrared measuring chamber for analysis. During steady state conditions, there is a correction factor of approximately 2% to allow for catheter dead space. The measured regional P_{CO_2} (Pr_{CO_2}), together with simultaneously obtained arterial [HCO_3^-], were applied in the Henderson-Hasselbalch equation for calculation of pHi according to the manufacturer's instruction:

$$pHi = 6.1 + \log[HCO_3^-] \div 0.03 \times Pr_{CO_2}$$

where [HCO_3^-] is the arterial bicarbonate concentration, 6.1 is the dissociation constant of HCO_3^-, and 0.03 is the solubility of carbon dioxide in plasma. The P_{CO_2} gap was defined as Pr_{CO_2} minus Pa_{CO_2} to examine the effects of systemic acidosis on pHi as previously reported.[17] Previous studies demonstrated that air tonometry

exhibited good correlation with saline tonometry, with particular advantages with shorter equilibration times.[16]

Specific Measurements and Calculations

Arterial and portal pH, Pco_2, Po_2, and lactate concentrations were determined by using a blood gas analyzer (Chiron 860 series; Chiron Diagnostics Corp., East Walpole, MA). Hemoglobin and hemoglobin oxygen saturation were measured using a cooximeter (OSM3; Radiometer, Copenhagen) to calculate oxygen content. Ascending aortic and portal blood flow were indexed by body weight as cardiac index and portal flow index, respectively. The fraction of portal flow was defined as 100 × portal flow index/cardiac index. Splanchnic oxygen transport ($spDO_2$), consumption ($spVO_2$), and extraction ratio (spO_2ER) were calculated using standard formulae. Portal blood samples were centrifuged, and plasma was stored at −20°C until analysis. Using these samples, the portal endotoxin level was measured using a synthetic chromogenic substrate method (Seikagakukogyo, Tokyo). Measurements of all parameters were performed in duplicate, and mean values were used for analysis.

Wet-to-dry Weight Ratio and Histologic Analysis

In a separate set of experiments, mucosal edema and microstructure of the terminal ileum were examined. Because no animal in the control group survived during severe hypoxia, tissue samples were collected from two animals in each group at the moderate hypoxia period. After the wet weights of five 2-cm parts of ileal tissues were measured, the tissues were completely dried in a vacuum oven (DP22; Yamato Scientific, Tokyo, Japan) at 95°C and −20 cm H_2O for 48 h to remove any gravimetrically detectable water. The dry weights of the ileum were determined, and the wet-to-dry ratio was calculated. Samples for microscopic examination were fixed by luminal perfusion and processed as described elsewhere.[18] After processing, semithin (2–4 μm) sections were cut with a diamond knife and stained with 1% toluidine blue. With a modification of Chiu's grading system,[19] the degree of mucosal damage was graded in a blinded fashion by an independent observer on a scale of 0–5, with 0 considered normal and 5 representing severe cell disruption. Thirteen to 15 random villi were assessed per animal.

Statistical Analysis

Data are expressed as mean ± SD unless otherwise specified. To compare and contrast the effects of olprinone infusion before the induction of hypoxic stress, two-way analysis of variance was applied to the data of baseline and normoxia, supported by SPSS/9.0J for Windows (SPSS Inc., Chicago, IL). After induction of progressive hypoxia, results between the normoxia period and the three stages of hypoxia periods were analyzed using a repeated-measures analysis of variance approach. If the interaction was statistically significant, the Scheffé multiple comparison test was applied to distinguish differences between measured variables. The Kaplan-Meier test was applied to examine the differences in mortality rate between the groups. The wet-to-dry ratio and histologic scoring data were analyzed by the Friedman test and chi-square test, respectively. Differences were considered statistically significant if P was less than 0.05.

Results

All 12 animals in the control group were unable to complete all stages of hypoxia, and half of them died at moderate hypoxia, whereas 4 of 10 in both the low- and high-dose groups died at severe hypoxia. The mortality rate was significantly different between the control group and the two olprinone groups ($P < 0.01$), suggesting that olprinone infusion at either low or high dose protected the animals from acute hypoxic stress.

Systemic Effects of Progressive Hypoxia and Olprinone Infusion

Table 1 illustrates the changes of systemic hemodynamic and oxygen metabolism during acute hypoxia. Both Pao_2 and arterial oxygen content declined linearly to a similar extent during the study periods in all three groups. Olprinone at low- or high-dose infusion did not significantly decrease mean arterial pressure during normoxia. Mean arterial pressure was gradually depressed over the hypoxia study periods in all three groups ($P < 0.05$). During acute progression of hypoxia, however, mean arterial pressure in the high-dose group was reduced to a lesser extent than in the control group. The cardiac index (CI) in the high-dose group at normoxia was significantly elevated to approximately 20% from the baseline value, whereas those in the low-dose and control groups remained constant. During acute hypoxia stages, CI in all groups was linearly reduced, but CI in the low- and high-dose groups was depressed to a lesser extent than that in control group. Both arterial pH and hemoglobin concentrations were not different between the groups at baseline and normoxia, whereas arterial pH declined during acute hypoxia in all groups. At severe hypoxia, hemoglobin values in both the low- and high-dose groups were significantly depressed compared to normoxia, mainly due to hemodilution associated with frequent blood sampling: Macroscopic hemolysis was not observed during hypoxia stages. Arterial pH in the high-dose group was higher than in other groups at moderate hypoxia, but no difference was found at severe hypoxia between the low- and high-dose groups. Simultaneously, arterial lactate levels were not statistically different at the baseline and normoxia stages but progressively increased during hypoxia in all study groups.

Table 1. Effects of Three Levels of Progressive Hypoxia and Olprinone Infusion on Systemic Circulatory Variables

			Hypoxia		
	Baseline	Normoxia	Mild	Moderate	Severe
Arterial O_2 tension (mmHg)					
Control	70.7 ± 11.6	70.7 ± 11.9	61.4 ± 7.4*	55.0 ± 13.3*	–
Low-dose	73.4 ± 8.9	76.0 ± 13.4	56.1 ± 10.5*	47.1 ± 7.8*	44.4 ± 10.6†
High-dose	76.0 ± 10.4	70.5 ± 8.8	58.9 ± 8.4*	45.9 ± 6.2*	43.5 ± 8.5†
Arterial O_2 content (ml · O_2/l)					
Control	13.7 ± 1.9	12.7 ± 2.9	11.9 ± 2.5*	9.0 ± 2.4†	–
Low-dose	14.1 ± 1.9	12.9 ± 2.5	10.8 ± 2.5*	9.0 ± 1.4†	5.9 ± 1.6†
High-dose	13.8 ± 2.8	12.8 ± 2.2	10.9 ± 2.4*	8.3 ± 1.9†	6.5 ± 2.4†
Mean arterial pressure (mmHg)					
Control	87.2 ± 11.2	84.9 ± 12.5	72.7 ± 20.1*	70.3 ± 28.4*	–
Low-dose	92.0 ± 8.6	89.1 ± 11.5	78.5 ± 16.6*	68.2 ± 19.5*	64.2 ± 15.9†
High-dose	97.5 ± 10.5	93.4 ± 9.0	85.3 ± 7.0‡	77.3 ± 13.9*‡	62.9 ± 10.8†
Cardiac index (ml · min^{-1} · kg^{-1})					
Control	85.1 ± 15.7	83.1 ± 17.4	50.5 ± 22.4†	40.6 ± 17.5†	–
Low-dose	90.2 ± 15.5	90.4 ± 11.9	77.3 ± 9.8‡	62.8 ± 12.7*‡	55.6 ± 16.3†
High-dose	93.3 ± 12.3	110.7 ± 17.9§‖	89.1 ± 17.5‡‖	80.8 ± 18.5*‡‖	71.8 ± 19.5†‖
Arterial pH					
Control	7.36 ± 0.04	7.31 ± 0.08	7.31 ± 0.08	7.24 ± 0.10*	–
Low-dose	7.34 ± 0.08	7.34 ± 0.08	7.31 ± 0.09	7.25 ± 0.13*	7.11 ± 0.10†
High-dose	7.38 ± 0.05	7.38 ± 0.05	7.36 ± 0.04	7.34 ± 0.08‡	7.15 ± 0.07†
Arterial hemoglobin (g/dl)					
Control	10.7 ± 1.5	9.9 ± 2.3	10.0 ± 1.7	9.3 ± 0.7	–
Low-dose	10.9 ± 1.8	10.2 ± 1.9	9.8 ± 1.9	9.8 ± 1.5	7.7 ± 0.8*
High-dose	10.7 ± 1.7	9.7 ± 1.5	9.1 ± 1.5	8.3 ± 1.7	7.9 ± 1.7*
Arterial lactate (mM)					
Control	3.5 ± 1.8	4.8 ± 3.3	8.4 ± 4.8†	10.9 ± 2.6†	–
Low-dose	3.2 ± 1.5	3.8 ± 2.2	6.0 ± 3.5†	7.3 ± 3.4†‡	10.6 ± 3.2†
High-dose	2.6 ± 1.0	2.9 ± 1.4	4.7 ± 2.7†‡	8.2 ± 3.6†‡	12.5 ± 3.8†

Data are expressed as mean ± SD. Baseline = at 45 min after preparatory surgery; normoxia = fraction of inspired oxygen (F_{IO_2}) 0.21; mild hypoxia = F_{IO_2} 0.17; moderate hypoxia = F_{IO_2} 0.13; severe hypoxia = F_{IO_2} 0.10.

* $P < 0.05$. † $P < 0.01$ *vs.* normoxia (reports the hypoxia stage effect). ‡ $P < 0.05$. § $P < 0.01$ *vs.* control group. ‖ $P < 0.05$ *vs.* low-dose group.

Lactate levels in the low- and high-dose groups were significantly less than in the control group at moderate hypoxia. No differences in arterial lactate were found between the olprinone groups.

Splanchnic Effects of Progressive Hypoxia and Olprinone Infusion

Table 2 shows the changes of several parameters in splanchnic blood flow and oxygen metabolism during acute progression of hypoxia. At normoxia, the portal flow index but not fraction of portal flow was significantly elevated in the low- and high-dose groups compared to the control group. With the progression of acute hypoxia, both the fraction of portal flow and the portal flow index in the control group showed a marked reduction compared to normoxia. On the contrary, olprinone infusion modified the responses of portal flow to progressive hypoxia: In the high-dose group, the portal flow index was well preserved throughout all the hypoxic stages, and the fraction of portal flow was even augmented at the severe hypoxia stage. In the low-dose group, the portal flow index was linearly reduced from normoxia in parallel with the reduction in CI, whereas the fraction of portal flow remained constant.

At normoxia, oxygen delivery to the splanchnic area ($spDO_2$) in the low- and high-dose groups was significantly augmented compared with the control group, whereas spO_2ER was not different between the all study groups. During acute hypoxia, $spDO_2$ in all groups was reduced linearly, but to a greater extent in the control group than in the low- and high-dose groups ($P < 0.05$). Simultaneously, spO_2ER showed a marked increase, but that in control group was significantly higher than in other groups at the moderate hypoxia stage. In addition, both $spDO_2$ and $spVO_2$ in the control group were significantly lower than in the high-dose group at moderate hypoxia.

Gut Mucosal Effects of Progressive Hypoxia and Olprinone Infusion

Figure 2 illustrates the changes of pHi and portal lactate levels during the study period. The value of pHi in the control group was progressively depressed at two hypoxic stages, whereas pHi in the low- and high-dose groups remained unchanged at the moderate hypoxia stage compared with normoxia and significantly dropped at severe hypoxia. We further examined the P_{CO_2} gap, defined as the difference between measured regional P_{CO_2} ($PrCO_2$) and $Paco_2$, to examine the effects of systemic acidosis on pHi over the hypoxic stages. We

Table 2. Effects of Three Levels of Progressive Hypoxia and Olprinone Infusion on Portal Blood Flow and Oxygen Metabolisms

			Hypoxia		
	Baseline	Normoxia	Mild	Moderate	Severe
Portal flow index (ml · min^{-1} · kg^{-1})					
Control	27.3 ± 8.6	23.1 ± 9.0	14.5 ± 6.1†	9.0 ± 3.4†	–
Low-dose	26.7 ± 6.0	28.2 ± 8.3‡	25.6 ± 8.6‡	20.1 ± 6.8*‡	15.5 ± 3.9*
High-dose	23.6 ± 6.6	27.9 ± 29.3‡	26.1 ± 7.5‡	24.9 ± 9.2§	25.0 ± 8.9‖
Fraction of portal flow (%)					
Control	32 ± 8	28 ± 9	31 ± 11	23 ± 7†	–
Low-dose	30 ± 6	32 ± 9	33 ± 11	32 ± 10‡	29 ± 5
High-dose	25 ± 6	27 ± 8	30 ± 11	32 ± 9‡	36 ± 10‡‖
Splanchnic oxygen delivery (ml · O_2/min)					
Control	8.6 ± 2.8	6.8 ± 2.0	3.4 ± 0.9*	1.6 ± 0.5*	–
Low-dose	8.7 ± 1.9	8.4 ± 2.5‡	6.1 ± 1.5*‡	4.1 ± 1.0*†	2.1 ± 0.6*†
High-dose	7.3 ± 1.8	8.1 ± 2.6‡	6.5 ± 2.6*‡	4.1 ± 1.1*†	3.5 ± 1.1*†‖
Splanchnic oxygen consumption (ml · O_2/min)					
Control	3.9 ± 1.5	3.4 ± 1.0	2.1 ± 1.2*	1.2 ± 0.4‡	–
Low-dose	2.9 ± 1.0	4.0 ± 1.3	3.1 ± 1.1*	2.1 ± 1.0*‡	1.4 ± 0.7†
High-dose	2.8 ± 0.8	3.4 ± 1.2	3.1 ± 1.4	2.3 ± 0.7*‡	2.0 ± 0.7†‖
Splanchnic oxygen extraction ratio (%)					
Control	47 ± 13	51 ± 9	59 ± 24	73 ± 8†	–
Low-dose	34 ± 12	48 ± 10	52 ± 18	61 ± 9*‡	63 ± 14*
High-dose	39 ± 10	42 ± 11	48 ± 16	55 ± 10*§	60 ± 15*

Data are expressed as mean ± SD. Baseline = at 45 min after preparatory surgery; normoxia = fraction of inspired oxygen (F_{IO_2}) 0.21; mild hypoxia = F_{IO_2} 0.17; moderate hypoxia = F_{IO_2} 0.13; severe hypoxia = F_{IO_2} 0.10.

* $P < 0.05$, † $P < 0.01$ *vs.* normoxia in each group. ‡ $P < 0.05$. § $P < 0.01$ *vs.* control group at each experimental stage. ‖ $P < 0.05$ *vs.* low-dose group.

then found that the P_{CO_2} gap in the control group was significantly greater at moderate hypoxia than in the other two groups, indicating that the pHi behavior in this experiment was not caused by profound systemic acidosis but mainly by intestinal ischemia *per se*. Although both $spDO_2$ and $spVO_2$ were modulated dose-dependently, it should be noted that the progression of intramucosal acidosis was attenuated to a similar extent irrespective of olprinone dose. Furthermore, the increase in portal lactate levels was significantly attenuated in both the low- and high-dose groups compared with the control group at mild and moderate hypoxia ($P < 0.05$).

To evaluate the changes of gut mucosal permeability against endotoxin, we measured portal concentrations of endotoxin at each experimental stage. Portal endotoxin levels in the control group showed a marked elevation at mild and moderate hypoxia stages, whereas those in the low- and high-dose groups remained unchanged until severe hypoxia (table 3), indicating that translocation of endotoxin through the gut mucosal layer was significantly attenuated in the low- and high-dose groups under the progression of acute hypoxia.

Structural Alterations of Gut Mucosa during Hypoxia and Olprinone Infusion

The wet-to-dry weight ratio of the terminal ileum in the control group was significantly greater than in the low- or high-dose olprinone group (3.76 ± 1.26 *vs.* 2.99 ± 1.67 and 1.71 ± 1.15, respectively; $P < 0.05$), indicating that the ileum in the control group was edematous

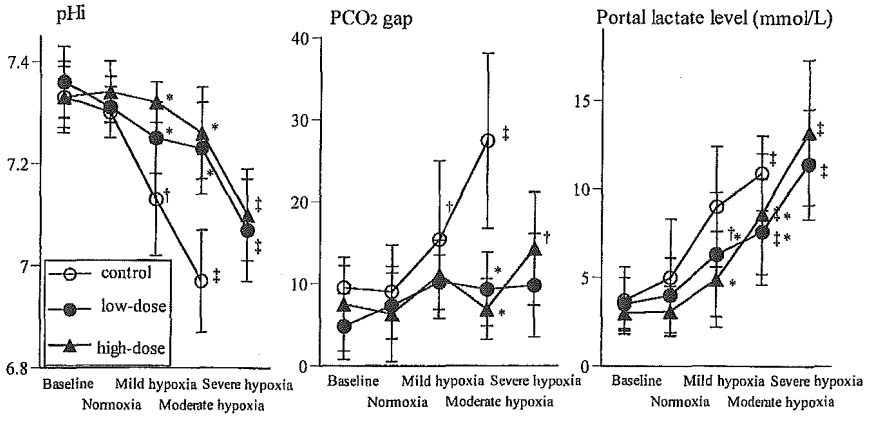

Fig. 2. Changes of intramucosal pH (pHi), P_{CO_2} gap, and portal lactate levels during acute hypoxia. Data are expressed as mean ± SD. Control = normal saline; low-dose = 0.2 μg · kg^{-1} · min^{-1} olprinone infusion; high-dose = 0.6 μg · kg^{-1} · min^{-1} olprinone infusion. Significance: *$P < 0.05$, **$P < 0.01$ *versus* control group; †$P < 0.05$, ‡$P < 0.01$ *versus* normoxia, each group.

Table 3. Effects of Three Levels of Progressive Hypoxia and Olprinone Infusion on Portal Endotoxin Concentration

			Hypoxia		
Endotoxin (pg/ml)	Baseline	Normoxia	Mild	Moderate	Severe
Control	38 ± 13	67 ± 30	236 ± 121†	455 ± 259†	–
Low-dose	45 ± 34	69 ± 51	89 ± 62‡	118 ± 79‡	175 ± 125*
High-dose	63 ± 19	70 ± 29	95 ± 51‡	137 ± 95‡	186 ± 160*

Data are expressed as mean ± SD. Baseline = at 45 min after preparatory surgery; normoxia = fraction of inspired oxygen (Fio_2) 0.21; mild hypoxia = Fio_2 0.17; moderate hypoxia = Fio_2 0.13; severe hypoxia = Fio_2 0.10.

* $P < 0.05$, † $P < 0.01$ *vs.* normoxia in each group. ‡ $P < 0.05$ *vs.* control group at each hypoxia stage.

compared to the two olprinone groups. Figure 3 illustrates representative pictures of villi of the distal ileum in the control and high-dose olprinone groups by light microscopy. The villi of the animals subjected to acute progressive hypoxia in the control group showed definitive evidence of mucosal injury, such as disruption of microvilli and submucosal edema (*panel A*), whereas those in the high-dose group appeared normal (*panel B*). Simultaneously, the data of injury scores demonstrated that mucosal injury of distal ileum was significantly attenuated by olprinone infusion as shown in figure 4 ($P < 0.01$).

Discussion

The current study indicates that olprinone infusion redistributes blood flow to the splanchnic area under acute hypoxia in a dose-dependent manner and that it slows the progression of both functional and structural alterations accompanied by intramucosal acidosis, irrespectively of its flow-augmenting properties. More importantly, considering the lower mortality rate in the olprinone-treated groups, olprinone infusion *per se* not only preserved gut integrity of the barrier function but also might provide some protective properties to the host. Because pHi is considered a valuable marker to mirror the outcome of critically ill patients,[1,15] olprinone infusion may be able to protect those who experience such life-threatening injuries.

Although the current study does not elucidate the precise mechanisms, the dose-dependent augmentation of blood flow and oxygen delivery to the gut appears to be a key profile for preservation of intramucosal circulation during olprinone infusion. Indeed, a previous study showed that this agent relaxed the mesenteric small artery of rabbits dose-dependently *via* its direct action on vascular smooth muscle.[20] Under insult, splanchnic microvascular resistance is elevated because of increased arteriole and/or precapillary sphincter tones, resulting in the redistribution of blood flow away from the gut mucosa.[21,22] Olprinone could attenuate such responses against hypoxia not only at the level of mesenteric circulation but also in intramucosal microcirculation. Furthermore, it should be noted that low-dose olprinone infusion showed the comparable reduction of pHi values, elevation of portal endotoxin concentrations, and severity of structural alterations in gut mucosa compared to the high-dose group, despite significantly less oxygen delivery to splanchnic circulation during hypoxia. Collectively, in addition to the primary action of olprinone to augment oxygen delivery, other mechanisms, such as suppression of inflammatory cells, are likely to account for protecting the intramucosal integrity of the gut. For example, microvascular entrapment

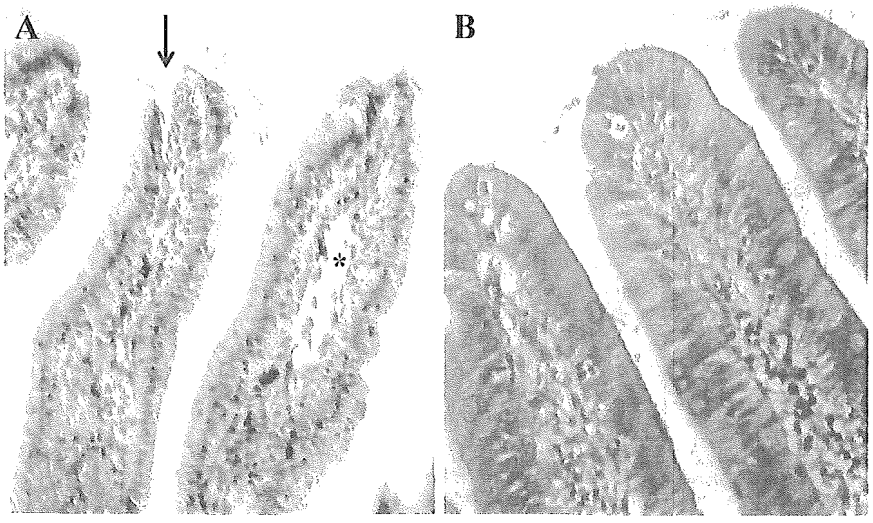

Fig. 3. Representative pictures showing villi of distal ileum captured by light microscopy (×100) from rabbits. (*A*) Representative villi of distal ileum from a rabbit in the control group at moderate hypoxia. Note that the patent breaks (*arrow*) and submucosal edema (*asterisk*) are evident in the ileum. (*B*) Representative villi of distal ileum from a rabbit in the high-dose group at moderate hypoxia. Note near-normal–appearing structure of ileal mucosa.