

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

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**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 ( $0.2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  olprinone; n = 10), or a high-dose group ( $0.6 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  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.<sup>1,2</sup> 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.<sup>3</sup> 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.<sup>4</sup> On the contrary, dobutamine is likely to increase blood flow to the gut concurrently with the improvement of intramucosal pH (pHi) in endotoxin shock.<sup>5</sup> 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.<sup>6</sup> Such therapeutic intervention aimed at preserving gut barrier function could improve the mortality rate in the critically ill.<sup>7</sup>

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.<sup>8</sup> 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.<sup>9-11</sup> 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.<sup>11</sup> 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.<sup>12,13</sup> 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*.<sup>14</sup> 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.

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## 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<sub>2</sub>O 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.<sup>6</sup> 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<sup>-1</sup> · h<sup>-1</sup> 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 μg · kg<sup>-1</sup> · min<sup>-1</sup>, and those in the high-dose group (n = 10) received a continuous infusion of olprinone at a rate of 0.6 μg · kg<sup>-1</sup> · min<sup>-1</sup> throughout the study period. Our pilot study demonstrated that olprinone at a rate of 0.6 μg · kg<sup>-1</sup> · min<sup>-1</sup> increased cardiac output to an approximately 20% higher level compared to baseline in rabbits, whereas 0.2 μg · kg<sup>-1</sup> · min<sup>-1</sup> did not influence

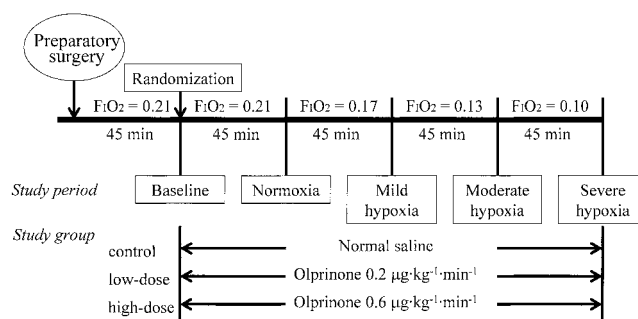


Fig. 1. Schematic drawing of experimental protocol.

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, pH<sub>i</sub>, 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 (FiO<sub>2</sub>) was reduced in three stages (mild hypoxia FiO<sub>2</sub> = 0.17, moderate hypoxia FiO<sub>2</sub> = 0.13, and severe hypoxia FiO<sub>2</sub> = 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.

### Measurement of pH<sub>i</sub>

Gut pH<sub>i</sub> was monitored using a tonometric method described previously.<sup>15,16</sup> Air in the silicone balloon was equilibrated for 10 min, and the Pco<sub>2</sub> 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 Pco<sub>2</sub> (Prco<sub>2</sub>), together with simultaneously obtained arterial [HCO<sub>3</sub><sup>-</sup>], were applied in the Henderson-Hasselbalch equation for calculation of pH<sub>i</sub> according to the manufacturer's instruction:

$$\text{pHi} = 6.1 + \log[\text{HCO}_3^-] \div 0.03 \times \text{Prco}_2$$

where [HCO<sub>3</sub><sup>-</sup>] is the arterial bicarbonate concentration, 6.1 is the dissociation constant of HCO<sub>3</sub><sup>-</sup>, and 0.03 is the solubility of carbon dioxide in plasma. The Pco<sub>2</sub> gap was defined as Prco<sub>2</sub> minus Paco<sub>2</sub> to examine the effects of systemic acidosis on pH<sub>i</sub> as previously reported.<sup>17</sup> Previous studies demonstrated that air tonometry

exhibited good correlation with saline tonometry, with particular advantages with shorter equilibration times.<sup>16</sup>

#### *Specific Measurements and Calculations*

Arterial and portal pH,  $P_{CO_2}$ ,  $P_{O_2}$ , 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  $100 \times \text{portal flow index}/\text{cardiac index}$ . 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  $-20^\circ\text{C}$  until analysis. Using these samples, the portal endotoxin level was measured using a synthetic chromogenic substrate method (Seikagaku-kogyo, 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  $95^\circ\text{C}$  and  $-20\text{ cm H}_2\text{O}$  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.<sup>18</sup> After processing, semithin (2–4  $\mu\text{m}$ ) sections were cut with a diamond knife and stained with 1% toluidine blue. With a modification of Chiu's grading system,<sup>19</sup> 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  $\pm$  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  $P_{aO_2}$  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**

	Baseline	Normoxia	Hypoxia		
			Mild	Moderate	Severe
Arterial O <sub>2</sub> 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 <sub>2</sub> content (ml · O <sub>2</sub> /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 <sup>-1</sup> · kg <sup>-1</sup> )					
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 (Fio<sub>2</sub>) 0.21; mild hypoxia = Fio<sub>2</sub> 0.17; moderate hypoxia = Fio<sub>2</sub> 0.13; severe hypoxia = Fio<sub>2</sub> 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<sub>2</sub>) in the low- and high-dose groups was significantly augmented compared with the control group, whereas spO<sub>2</sub>ER was not different between the all study groups. During acute hypoxia, spDO<sub>2</sub> 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<sub>2</sub>ER 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<sub>2</sub> and spVO<sub>2</sub> 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 pH<sub>i</sub> and portal lactate levels during the study period. The value of pH<sub>i</sub> in the control group was progressively depressed at two hypoxic stages, whereas pH<sub>i</sub> 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 Pco<sub>2</sub> gap, defined as the difference between measured regional Pco<sub>2</sub> (PrCO<sub>2</sub>) and Paco<sub>2</sub>, to examine the effects of systemic acidosis on pH<sub>i</sub> over the hypoxic stages. We

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

	Baseline	Normoxia	Hypoxia		
			Mild	Moderate	Severe
Portal flow index ( $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ )					
Control	27.3 $\pm$ 8.6	23.1 $\pm$ 9.0	14.5 $\pm$ 6.1†	9.0 $\pm$ 3.4†	—
Low-dose	26.7 $\pm$ 6.0	28.2 $\pm$ 8.3‡	25.6 $\pm$ 8.6‡	20.1 $\pm$ 6.8*‡	15.5 $\pm$ 3.9*
High-dose	23.6 $\pm$ 6.6	27.9 $\pm$ 29.3‡	26.1 $\pm$ 7.5‡	24.9 $\pm$ 9.2§	25.0 $\pm$ 8.9
Fraction of portal flow (%)					
Control	32 $\pm$ 8	28 $\pm$ 9	31 $\pm$ 11	23 $\pm$ 7†	—
Low-dose	30 $\pm$ 6	32 $\pm$ 9	33 $\pm$ 11	32 $\pm$ 10‡	29 $\pm$ 5
High-dose	25 $\pm$ 6	27 $\pm$ 8	30 $\pm$ 11	32 $\pm$ 9‡	36 $\pm$ 10‡
Splanchnic oxygen delivery ( $\text{ml} \cdot \text{O}_2/\text{min}$ )					
Control	8.6 $\pm$ 2.8	6.8 $\pm$ 2.0	3.4 $\pm$ 0.9*	1.6 $\pm$ 0.5*	—
Low-dose	8.7 $\pm$ 1.9	8.4 $\pm$ 2.5‡	6.1 $\pm$ 1.5*‡	4.1 $\pm$ 1.0*†	2.1 $\pm$ 0.6*†
High-dose	7.3 $\pm$ 1.8	8.1 $\pm$ 2.6‡	6.5 $\pm$ 2.6*‡	4.1 $\pm$ 1.1*†	3.5 $\pm$ 1.1*†
Splanchnic oxygen consumption ( $\text{ml} \cdot \text{O}_2/\text{min}$ )					
Control	3.9 $\pm$ 1.5	3.4 $\pm$ 1.0	2.1 $\pm$ 1.2*	1.2 $\pm$ 0.4‡	—
Low-dose	2.9 $\pm$ 1.0	4.0 $\pm$ 1.3	3.1 $\pm$ 1.1*	2.1 $\pm$ 1.0*‡	1.4 $\pm$ 0.7†
High-dose	2.8 $\pm$ 0.8	3.4 $\pm$ 1.2	3.1 $\pm$ 1.4	2.3 $\pm$ 0.7*‡	2.0 $\pm$ 0.7†
Splanchnic oxygen extraction ratio (%)					
Control	47 $\pm$ 13	51 $\pm$ 9	59 $\pm$ 24	73 $\pm$ 8†	—
Low-dose	34 $\pm$ 12	48 $\pm$ 10	52 $\pm$ 18	61 $\pm$ 9*‡	63 $\pm$ 14*
High-dose	39 $\pm$ 10	42 $\pm$ 11	48 $\pm$ 16	55 $\pm$ 10*§	60 $\pm$ 15*

Data are expressed as mean  $\pm$  SD. Baseline = at 45 min after preparatory surgery; normoxia = fraction of inspired oxygen ( $\text{FiO}_2$ ) 0.21; mild hypoxia =  $\text{FiO}_2$  0.17; moderate hypoxia =  $\text{FiO}_2$  0.13; severe hypoxia =  $\text{FiO}_2$  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.

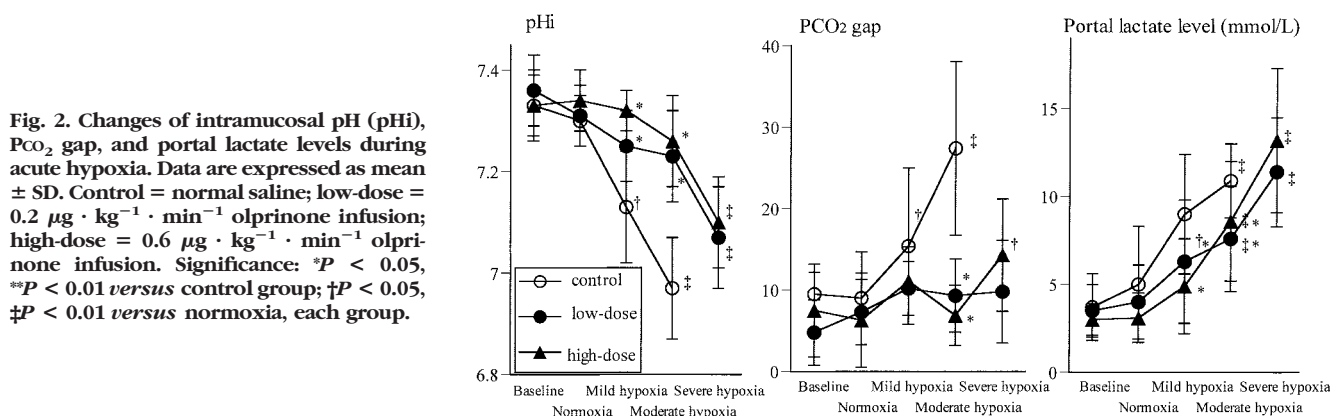
then found that the  $\text{Pco}_2$  gap in the control group was significantly greater at moderate hypoxia than in the other two groups, indicating that the pH<sub>i</sub> behavior in this experiment was not caused by profound systemic acidosis but mainly by intestinal ischemia *per se*. Although both  $\text{spDO}_2$  and  $\text{spVO}_2$  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 endo-

toxin 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 ( $3.76 \pm 1.26$  vs.  $2.99 \pm 1.67$  and  $1.71 \pm 1.15$ , respectively;  $P < 0.05$ ), indicating that the ileum in the control group was edematous



**Fig. 2.** Changes of intramucosal pH (pH<sub>i</sub>),  $\text{PCO}_2$  gap, and portal lactate levels during acute hypoxia. Data are expressed as mean  $\pm$  SD. Control = normal saline; low-dose =  $0.2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  olprinone infusion; high-dose =  $0.6 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  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**

Endotoxin (pg/ml)	Baseline	Normoxia	Hypoxia		
			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 ( $\text{FiO}_2$ ) 0.21; mild hypoxia =  $\text{FiO}_2$  0.17; moderate hypoxia =  $\text{FiO}_2$  0.13; severe hypoxia =  $\text{FiO}_2$  0.10.

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

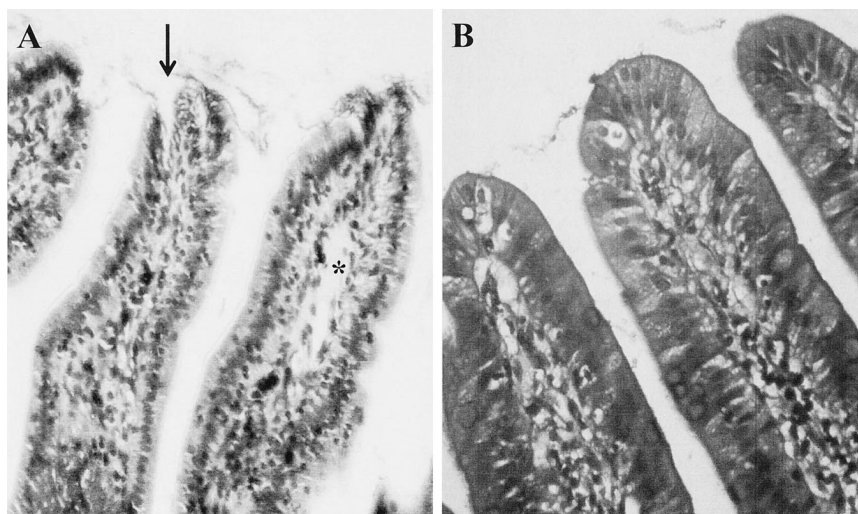
compared to the two olprinone groups. Figure 3 illustrates representative pictures of villi of the distal ileum in the control and high-dose olprinone groups by light microscopy. The villi of the animals subjected to acute progressive hypoxia in the control group showed definitive evidence of mucosal 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, irrespective 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 pH<sub>i</sub> is considered a valuable marker to mirror the outcome of critically ill patients,<sup>1,15</sup> olprinone

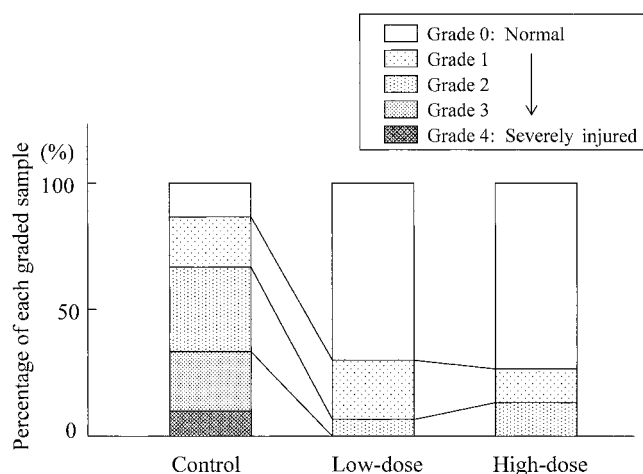
infusion may be able to protect those who experience such life-threatening injuries.

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.<sup>20</sup> 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.<sup>21,22</sup> 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 pH<sub>i</sub> 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.**





**Fig. 4. Severity of mucosal injury during acute hypoxia.** The degree of mucosal injury was graded on a scale of 0–5, with 0 considered normal and 5 representing severe cell disruption. Data are expressed as percentage of 27–28 fields. Control = normal saline; low-dose =  $0.2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  olprinone infusion; high-dose =  $0.6 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  olprinone infusion. Chi-square test showed significant difference between control and two olprinone groups.

of activated leukocytes is considered a cause of exaggerated ischemia under inflammatory conditions.<sup>2,23</sup> Previous studies showed that hypoxia increased “vascular permeability” through the reduction of cyclic 3',5' adenosine monophosphate (cAMP) levels in endothelial cells.<sup>24</sup> If such mechanism also works in “mucosal permeability” of the gut, olprinone, a phosphodiesterase III inhibitor, may antagonize hypoxia-induced alterations of cAMP contents through its original action to elevate intracellular cAMP, subsequently preserving cellular barrier function.<sup>20,25</sup> Further investigation is now under way to address whether involvements of inflammatory cells and intramucosal cAMP levels could be modulated by olprinone infusion in this model.

Because of its microcirculatory properties and redistribution of blood flow under insult, the gut mucosal layer is considered vulnerable to any type of reduction in oxygen delivery, such as ischemic, anemic, or hypoxic hypoxia.<sup>26</sup> Among them, hemorrhagic shock causing both ischemic and anemic hypoxia has been well investigated to elucidate the pathophysiology of bacterial translocation.<sup>18,27</sup> In this experiment, we applied hypoxic hypoxia to examine alterations of gut mucosal integrity under noninflammatory impacts and found that structural changes in gut mucosa caused by acute progressive hypoxia were ameliorated with olprinone infusion. Although the current study was not aimed at clarifying the presence of bacteria in mesenteric lymph nodes or portal blood by cultures, such findings as the alterations in pHi, portal endotoxin level, and mucosal structures indicated a possibility that olprinone infusion minimized the risk of bacterial translocation.

There are several limitations to the interpretation of

the data reported. First, it could be argued that our study protocol was able to mirror the clinical situation. To mimic the clinical situation, we first determined the dose of olprinone infusion to augment CI to approximately 20% in rabbits. Then, we confirmed that one third of such an olprinone dose did not increase CI at normoxia. Even at a low-dose infusion, however, olprinone infusion attenuated hypoxia-induced myocardial suppression and subsequent hypoperfusion of the splanchnic area observed in the control group, indicating that characteristic profiles of olprinone, *i.e.*, the redistribution of blood flow to splanchnic circulation, was preserved without apparent augmentation of systemic blood flow. On the other hand, acute progressive hypoxia in this study might be too severe to mimic the clinical situation, according to high lethality in the control group. Second, some may argue that other pharmacologic interventions to augment CI show protective roles for splanchnic organs and an outcome that are similar to that of olprinone. In this experimental setting, we found no changes in pHi and mucosal structures between the low- and high-dose olprinone groups, although both  $\text{spDO}_2$  and  $\text{spVO}_2$  in the high-dose group were significantly greater than in the low-dose group at severe hypoxia. Previous studies demonstrated that dopamine infusion caused an imbalance between oxygen demand and supply in the splanchnic region because of flow redistribution away from the gut mucosa.<sup>3,4</sup> Also, we previously showed that epidural anesthesia protected gut integrity and minimized translocation of endotoxin under similar experimental conditions compared with dobutamine infusion alone,<sup>6</sup> indicating that augmentation of CI is not a key component for protection of intramucosal integrity of the gut under our protocol. Rather, olprinone possibly augments mucosal microcirculation by modulating distribution of blood flow within the gut. Finally, we applied air tonometry to obviate the confounding factors of saline tonometry, such as long equilibration period, temperature correction, and bias produced by the type of blood gas analyzers.<sup>16,28</sup> Although the values of pHi remain controversial,<sup>29</sup> tissue hypoxia of the gut in the current study design was caused by arterial hypoxemia, indicating that  $\text{PrCO}_2$  should be similar to  $\text{Paco}_2$  because of prompt removal of anabolically generated carbon dioxide from the tissues under high-flow dysoxic conditions.<sup>17</sup>

In conclusion, olprinone infusion preserves redistribution of blood flow to the splanchnic area and slows the progression of gut mucosal injury during acute hypoxia through both flow-dependent and -independent mechanisms. This property of olprinone may serve to prevent translocation of endotoxin, indicating a possibility that olprinone protects the host under insult.

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