Evaluation of intestinal intramucosal pH, arterial and portal venous blood gas values, and intestinal blood flow during small intestinal ischemia and reperfusion in dogs

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Objectives—To determine whether small intestinal ischemia and reperfusion affects intestinal intramucosal pH (pHi), arterial and portal venous blood gas values, and intestinal blood flow (IBF) and to investigate relationships between regional intestinal tissue oxygenation and systemic variables in dogs.

Animals—15 healthy adult Beagles.

Procedure—Occlusion of superior mesenteric artery (SMA) for 0, 30, or 60 minutes, followed by reperfusion for 180 minutes, was performed; IBF, pHi, arterial and portal venous blood gas values, arterial pressure, and heart rate were measured at various time points; and intestinal mucosal injury was histologically graded.

Results—Occlusion of the SMA induced significant decreases in pHi and IBF. After the release of the occlusion, IBF returned rapidly to baseline values, but improvement in pHi was slow. Arterial and portal venous blood gas analyses were less sensitive than tonometric measurements of pH, and there was no correlation between results of blood gas analyses and tonometric measurements. Histologic score for intestinal mucosal injury increased significantly, depending on duration of ischemia, and there was a correlation between tonometric results and the histologic score.

Conclusions and Clinical Relevance—Results suggest that it is difficult to accurately evaluate local oxygenation disorders by monitoring at the systemic level, whereas clinically pHi is the only reliable indicator of inadequate regional intestinal tissue oxygenation in dogs. (Am J Vet Res 2002;63:804–810)

The intestine has been proposed as a central organ in the pathogenesis of multiple organ dysfunction syndrome in critically ill patients. The intestinal mucosa is affected by hypoxia in the earliest stage of various critical conditions, including shock, trauma, and sepsis. When systemic blood pressure is reduced, splanchnic organ blood vessels immediately contract and redistribute blood to vital organs such as the brain and heart. Furthermore, the intestinal villus has a specific microvascular structure that promotes oxygen shunting via countercurrent exchange. Especially at the tips of the villi, these physiologic and anatomic features contribute to disorders in tissue oxygenation in low-flow states. In addition, results of many studies associate reperfusion of ischemic intestine with remote organ dysfunction via bacterial translocation and the generation of proinflammatory cytokines. Thus, in the management of critically ill patients, early detection and accurate evaluation of intestinal hypoperfusion are important, as is the maintenance of a balance between oxygen delivery and tissue demand in the intestines, because imbalance is a key to the development of multiple organ dysfunction syndrome.

In clinical veterinary practice, monitoring of tissue oxygenation relies largely on systemic variables such as systemic hemodynamics; blood oxygen delivery; consumption, or both; and acid-base balance. However, these types of systemic-level monitoring are useful for comprehensively evaluating respiration, circulation, and metabolism when all tissues in the body are regarded as 1 compartment but not for evaluating local tissue oxygenation. In clinical practice in humans, tonometric measurement of intramucosal pH, which was proposed by Fiddian-Green et al, is highly valued as the only method for noninvasively and specifically measuring intestinal tissue oxygenation. In clinical studies in humans, reduction of intramucosal pH is related to high mortality rate, and treatment using intramucosal pH as an indicator of inadequate tissue oxygenation improves survival rate. These findings suggest the efficacy of managing patients by use of tonometry.

We hypothesized that tonometry is the most appropriate method available to detect intestinal hypoxia during ischemic and reperfusion states. Thus, the purpose of the study reported here was to determine whether small intestinal ischemia-reperfusion affects intestinal intramucosal pH, arterial and portal venous blood gas values, and intestinal blood flow (IBF) and to investigate the relationship between regional intestinal tissue oxygenation and systemic parameters in dogs.

Materials and Methods

Anesthesia and surgical preparation—This study was approved by the Bioethics Guidelines of Nippon Veterinary and Animal Science University. Fifteen healthy adult Beagles of both sexes, weighing 9 to 12 kg, were used. Food was withheld from the dogs for 18 hours prior to the experiment, and water was supplied ad libitum. Anesthesia was induced via inhalation of isoflurane in 100% oxygen. After endotracheal intubation, the endotracheal tube was connected to a pressure-limited ventilator, and the dog was mechanically ventilated with fraction of inspired oxygen (FiO2) of 1.0. Spontaneous respiration was...
completely stopped by an IV bolus of pancuronium bromide (0.1 mg/kg), and throughout the experiment additional pancuronium bromide was administered as necessary. Minute ventilation was adjusted to maintain normocarbia (PaCO₂, 40.0 ± 5.0 mm Hg). Anesthesia was maintained by inhalation of isoflurane in 100% oxygen. To minimize the influence of isoflurane on systemic circulation, the minimal concentration of isoflurane was established in accordance with the condition of each dog individually, within the range necessary for sufficient depth of anesthesia. During the experiment, dogs received a continuous IV infusion (10 ml/kg/h) of lactated Ringer's solution as fluid replacement. Body temperature was maintained between 36.5 and 37.5 °C by use of a heating mat.

Both femoral arteries were cannulated with sterile catheters positioned in the abdominal aorta for continuous determination of arterial blood pressure and sampling. The left cephalic vein was cannulated for fluid and drug infusions. A midline laparotomy was performed. The superior mesenteric artery (SMA) was identified and cleaned from the surrounding tissue close to its origin from the aorta. A silastic vessel loop was positioned around the SMA for complete occlusion of the vessel. A splenic vein was cannulated, and a sterile catheter was inserted and positioned with its tip in the portal vein for blood sampling. Subsequently, a small antimesenteric incision was made in the terminal of the descending part of the duodenum, and a tonometric catheter was inserted and placed in the lumen of the proximal portion of the jejunum. The incised wound in the duodenum was closed with a purse-string suture, and the tonometric catheter was fixed. After all measurement systems were in place, the abdominal incised wound was sutured and closed, except for the portion necessary for the experimental procedures. The open area was covered with a sterile, moist gauze pad to prevent evaporation of body fluids. After surgical preparation was completed, 100% oxygen was switched to air for ventilation, and the FiO₂ value was reduced to 0.21. After preparation, a 60-minute period was allowed for all parameters to stabilize before baseline measurements.

Experimental protocol—The 15 dogs were allocated into 3 experimental groups. Group 1 (n = 5) was subjected to 30 minutes of SMA occlusion and 3 hours of reperfusion after occlusion was released. Group 2 (n = 5) was subjected to 60 minutes of SMA occlusion and 3 hours of reperfusion after occlusion was released. Group 3 (n = 5) served as a sham-operated control group. Our aim was to have group 1 with moderate ischemia and group 2 with severe ischemia. In groups 1 and 2, to prepare the ischemic phase, SMA was completely occluded by adding a constant tensile strength to a silastic vessel loop positioned around the SMA for occlusion of the vessel. The reperfusion phase was achieved by opening the occlusion.

Blood sampling and hemodynamic measurements were performed before SMA occlusion (baseline); immediately before occlusion was relieved; and 15, 30, 60, 90, 120, 150, and 180 minutes after occlusion was relieved. Tonometry was performed before SMA occlusion (baseline); immediately before occlusion was relieved; and 30, 60, 90, 120, 150, and 180 minutes after occlusion was relieved.

Hemodynamics—Arterial pressure and heart rate were serially measured and recorded by connecting a catheter inserted into the abdominal aorta with a pressure transducer and a polygraph. Intestinal tissue blood flow was measured by use of a laser blood-flow meter on the antimesenteric serous membrane surface approximately 20 cm distant from the jejunal site where the tonometer was placed; IBF was measured in triplicate, and a mean value was calculated.

Tonometry—In the tonometer’s silicone balloon, 2.5 ml of saline (0.9% NaCl) solution was placed and allowed to equilibrate for 30 minutes. Thereafter, 1 ml of saline solution was aspirated and discharged; this volume represented the dead space of the tonometer. The remaining 1.5 ml was then immediately aspirated, and PCO₂ of the aliquot was determined in a blood gas analyzer. The correction factor (1.29) for the 30-minute equilibration period listed by the manufacturer was used to adjust the measured PCO₂ in the aliquot of saline solution. This value, intramucosal PCO₂, together with the simultaneously obtained arterial HCO₃⁻ concentration, was used in the Henderson-Hasselbalch equation (pH = 6.1 + log([HCO₃⁻]/[PCO₂] X 0.03)) for calculation of intramucosal pH. In addition, to minimize the influence of respiration on intramucosal PCO₂, we calculated the intramucosal-arterial PCO₂ gradient. It has been reported that the intramucosal-arterial PCO₂ gradient is the most advantageous tonometric marker of intestinal tissue oxygenation.

Oxygen metabolism—Arterial and portal venous blood pH, PO₂, and PCO₂ were measured, and HCO₃⁻ and O₂ saturation were calculated by use of a blood gas analyzer. The portal venous-arterial PCO₂ gradient was then calculated.

Histologic evaluation—All dogs were euthanized by administration of an overdose of pentobarbital sodium after all measurements were completed. For histopathologic evaluation, whole-thickness specimens were obtained from the midjejunum. Sections were processed routinely and stained with H&E. Severity of mucosal injury was graded from 0 to 5 according to a scale described by Chiu et al. (Table 1).

Table 1—Histologic grading system for mucosal injury in the intestines of dogs

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
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<tbody>
<tr>
<td>0</td>
<td>Normal mucosal villi.</td>
</tr>
<tr>
<td>1</td>
<td>Development of subepithelial space, usually at apex of villus; often with capillary congestion.</td>
</tr>
<tr>
<td>2</td>
<td>Extension of subepithelial space with moderate lifting of epithelial layer from lamina propria.</td>
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<tr>
<td>3</td>
<td>Massive epithelial lifting down sides of villi. A few villus tips may be denuded.</td>
</tr>
<tr>
<td>4</td>
<td>Denuded villi with exposure of lamina propria and dilated capillaries. Increased cellularity of lamina propria may be evident.</td>
</tr>
<tr>
<td>5</td>
<td>Disintegration of lamina propria; hemorrhage and ulceration.</td>
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</tbody>
</table>

Figure 1—Mean ± SD intestinal tissue blood flow expressed as a percentage of baseline blood-flow values in dogs in which the superior mesenteric artery was occluded for 30 minutes (group 1) or 60 minutes (group 2) and in sham-operated control dogs (group 3). EOI = End of ischemic period. Values (minutes) on x-axis indicate duration of reperfusion (R) after occlusion of the artery was reversed. *Significant (P < 0.05) difference from baseline value within group.
hypothesis was rejected, a multiple comparison test was performed by use of the Dunn test. To detect significant differences between measurement points within a group, the Friedman test was used; when a null hypothesis was rejected, a multiple comparison test was performed by use of the Fisher exact test. Correlation analysis between parameters was conducted by use of linear regression analysis. For all comparisons, a value of $P < 0.05$ was regarded as significant.

**Results**

**Intestinal tissue blood flow**—In groups 1 and 2, IBF was significantly ($P < 0.01$) decreased (7.6 ± 1.5% and 8.1 ± 2.3% of baseline, respectively) at the end of the ischemic period and returned rapidly to baseline values after release of the occlusion (Fig 1). In group 3, there were no significant changes throughout the experiment.

**Tonometry**—In groups 1 and 2, the intramucosal-arterial PCO₂ gradient significantly ($P < 0.01$) increased from –0.8 ± 1.9 to 68.6 ± 31.3 mm Hg and from –3.6 ± 7.9 to 88.4 ± 27.1 mm Hg, respectively (Fig 2). Compared with baseline values, intramucosal pH was significantly ($P < 0.01$) decreased from 7.35 ± 0.03 to 6.91 ± 0.13 and 7.41 ± 0.12 to 6.85 ± 0.09, respectively, at the end of the ischemic period (Fig 3). These values improved gradually after release of the occlusion but not to baseline values. In group 3, there were no significant changes throughout the experiment.

**Oxygen metabolism**—In group 2, arterial and portal venous pH was significantly ($P < 0.05$) reduced after reperfusion, compared with the baseline value (Fig 3). In groups 1 and 3, there were no significant changes in arterial and portal venous pH throughout the experiment.

In group 2, portal venous PCO₂ was significantly ($P < 0.05$) higher than that in group 3 after 30 minutes of reperfusion (group 2, 44.8 ± 2.9 mm Hg; group 3, 39.0 ± 3.3 mm Hg). There were no significant changes in arterial blood PCO₂ in any of the 3 groups throughout the experiment. Likewise, there were no significant changes in portal venous PCO₂ in groups 1 and 3 throughout the experiment.

In all measurement point values of group 3 and baseline values of groups 1 and 2, there was a correlation between intramucosal pH and portal venous pH.
(r = 0.696; P < 0.001) and between intramucosal-arterial P\textsubscript{CO\textsubscript{2}} gradient and portal venous-arterial P\textsubscript{CO\textsubscript{2}} gradient (r = 0.507; P < 0.001). At the end of the ischemic phase or during the reperfusion phase in groups 1 and 2, there was no correlation between intramucosal pH and portal venous pH or between intramucosal-arterial P\textsubscript{CO\textsubscript{2}} gradient and portal venous-arterial P\textsubscript{CO\textsubscript{2}} gradient. There were no significant changes in arterial and portal venous PO\textsubscript{2} and O\textsubscript{2} saturation in any of the groups.

Hemodynamics—In groups 1 and 2, the release of SMA occlusion rapidly reduced mean arterial pressure from 105 ± 15 to 93 ± 13 mm Hg and from 109 ± 17 to 95 ± 22 mm Hg, respectively, although the difference did not reach significance. During the reperfusion phase, mean arterial pressure gradually increased to the baseline value in group 1, whereas there was no increase in group 2. There were no significant changes in heart rate in any of the groups.

Histologic evaluation—At the end of the experiment, the histologic grading score for intestinal mucosal injury was significantly (group 1 vs group 2, P < 0.01; group 1 vs group 3, P < 0.01; group 2 vs group 3, P < 0.01) high with the duration of ischemia (group 1, 3.3 ± 0.5; group 2, 4.8 ± 0.4; group 3, 0.2 ± 0.4), compared among the 3 experimental groups. There was a correlation between intramucosal-arterial P\textsubscript{CO\textsubscript{2}} and grading score (r = 0.751; P < 0.01; Fig 4). In group 3, histologic grade was typically 0 (Fig 5). In group 1, extensive epithelial lifting down the sides of villi and exfoliation of some epithelial cells at the tips of villi were evident (grade 3). In group 2, massive exfoliation of epithelial cells, disintegration of lamina propria, and increased cellularity of lamina propria were evident (grade 5). Furthermore, all histologic lesions were localized in the mucosal area; no histologic lesions were detected in the smooth muscle area.

Discussion

In this study, we detected some characteristics of regional intestinal tissue oxygenation in ischemia-reperfusion states in dogs. The main results were that the oxygenation parameters, such as intramucosal pH, IBF, and arterial and portal venous blood gas values, were not correlated with each other and that intestinal tissue acidosis persisted after reperfusion.

Tonometry is a noninvasive method for measuring intramucosal pH and was proposed by Fiddian-Green et al. Tonometry depends on the following hypothesis: CO\textsubscript{2} freely passes through a tonometer silicone balloon, the intestinal lumen, intestinal fluid, and superficial mucosal layer; P\textsubscript{CO\textsubscript{2}} in the physiologic saline solution in the silicone balloon corresponds to intestinal intramucosal P\textsubscript{CO\textsubscript{2}}; and the bicarbonate concentration of the superficial mucosal layer is similar to the arterial bicarbonate concentration. A correlation has been revealed between tonometric findings and results of direct measurement of intramucosal pH with a needle pH electrode or a glass microprobe.

In our study, IBF was measured by use of a laser blood-flow meter, which facilitates direct stable real-time measurement of blood flow at the small intestinal tissue level. The laser blood-flow meter used in this study measures blood flow to within a half diameter of 1 mm from a probe; thus, tissue blood flow measured on the intestinal serous membrane surface reflects mainly perfusion in the muscle layer.

In our study, IBF was significantly decreased, and intestinal mucosal acidosis and CO\textsubscript{2} accumulation developed after complete SMA occlusion. Increases in intestinal intramucosal CO\textsubscript{2} are associated with 2 mechanisms. First, excessive CO\textsubscript{2} accumulation results from buffering H\textsuperscript{+} produced by unresorbed hydrolysis of ATP and lactic acid derived from anaerobic glycolysis in tissues under hypoxia. Second, CO\textsubscript{2}
can increase because of the reduction of CO₂ washout related to low perfusion.

Furthermore, our study revealed that intestinal tissue blood flow and muscle layer perfusion rapidly improved after reperfusion, but improvement in intestinal mucosal acidosis and CO₂ accumulation was slow. This finding suggests that disorders in coupling between oxygen demand and blood flow in the local intestinal mucosal microcirculation unit persisted in the reperfusion phase. In several studies, it has been reported that the persistence of mucosal acidosis in the reperfusion phase following complete occlusion of the SMA was related to increases in oxygen demand associated with both the return of oxygen debt and increases in the generation of reactive oxygen metabolites or the persistent partial lack of microcirculation (no-reflow phenomenon) associated with injury during ischemia. The mechanism of the no-reflow phenomenon is multifactorial and includes compression of the capillary bed by cellular swelling during ischemia, vasoconstriction of microcirculation, and capillary plugging. The disturbance in local balance between the production of vasoconstrictive factors (such as thromboxane A₂ and endothelin) and relaxing factors (such as prostacyclin and nitrous oxide) from the endothelium may be of pathophysiologic importance in the mechanism of the no-reflow phenomenon.

Interestingly, in this experiment, tissue acidosis related to SMA occlusion in group 2 slightly improved in the initial stage of reperfusion but deteriorated again without changes in intestinal tissue blood flow or muscle layer perfusion after 90 minutes of reperfusion. This indicated reperfusion injury at the local oxygenation. It is speculated that various factors, such as reactive oxygen metabolites, arachidonic acid metabolites, platelet activating factor, and proinflammatory cytokines are involved in reperfusion injury. Cellular ATP depletion during ischemia depresses the action of the ATP-dependent calcium sequestering system, which results in intracellular calcium overload. Increased intracellular calcium may activate phospholipase A₂, generating arachidonic acid metabolites and platelet activating factor, and may convert xanthine dehydrogenase to xanthine oxidase, producing reactive oxygen metabolites when oxygen is reintroduced at reperfusion. In the intestines, xanthine oxidase is found exclusively in the mucosal layer; concentration increases from the base to the tip of the villus. This may explain our histologic finding that after an episode of intestinal ischemia-reperfusion, mucosal injury was more severe at the tops of the villi than at their bases or at the submucosal layer. Failure of the mucosal barrier caused by the intestinal ischemia-reperfusion may allow invasion of bacteria and their products, which results in the production of proinflammatory cytokines by intestinal macrophages, gut-associated lymphatic tissue, or intestinal epithelial cells. In addition, intestinal ischemia-reperfusion itself activates a cascade of stress-sensitive protein kinases that converge on specific transcriptional factors such as nuclear factor-κB to regulate expression of proinflammatory genes that encode proinflammatory cytokines, chemokines, and adhesion molecules. Thus, proinflammatory cytokines involved in local inflammatory response following intestinal ischemia-reperfusion also may have contributed to reperfusion injury in our study.

Recently, it has been reported that leukocyte-endothelium interaction directly contributes to tissue injury in the intestines after ischemia-reperfusion. Oxygen-free radicals or inflammatory mediators generated after ischemia-reperfusion induce the expression of adhesion molecules on leukocytes (eg, CD11b/CD18) and on the endothelium (eg, intercellular adhesion molecule-1, E-selectin, and P-selectin), leading to leukocyte adherence to the endothelium, which results in microcirculatory disturbance and tissue injury. In 1 study that used a cat intestinal ischemia-reperfusion model, it was reported that intestinal accumulation of neutrophils started after 60 minutes of reperfusion. Thus, our finding may have been due to leukocyte-accumulation-related increases in oxygen consumption and microcirculatory disorders. Furthermore, this hypothesis may be supported by the histologic findings in group 2, that is, infiltration of inflammatory cells such as lymphocytes, monocytes, and neutrophils in the intestinal mucosa.

Clinically, blood gas is generally measured as an index of tissue oxygen metabolism. In low perfusion or low oxygenation states, decrease in arterial blood pH is caused mainly by accumulation of lactate that is derived from anaerobic glycolysis. Furthermore, the low-flow states cause venous blood respiratory acidosis and the reduction of oxygen saturation; several studies have revealed the efficacy of blood gas analysis by the use of mixed venous blood as an index of general perfusion. Hepatic venous blood as an index of hepatic perfusion, and venous blood at the jugular bulb as an index of cerebral perfusion. In our study, we analyzed arterial and venous blood gas in the portal vein (a blood drainage route from the intestines) and compared results with the tonometric findings. We confirmed that disorders in oxygen metabolism in localized intestinal regions did not correlate well with results of analysis of portal venous blood and arterial blood. This may have been due to dilution by blood flow from the splanchnic region, other than the small intestine, to the portal vein and to reduction of the washout of anaerobic metabolites from the intestinal tissue in low-flow states.

In addition, in this study, tonometry detected the minimum pH during the ischemic phase, whereas arterial and portal venous blood gas analysis detected minimum pH in the reperfusion phase; this difference represents a time lag. There was no correlation between results of portal venous and arterial blood gas analysis and results of tonometry at the end of the ischemic phase and during the reperfusion phase. The time lag observed in our study may have occurred via the following mechanism: in the ischemic phase, a closed system was formed, communications with outer areas were interrupted, metabolites accumulated, and subsequent reperfusion switched the closed system to an open system, causing the outflow of anaerobic metabolites into the systemic circulation. This scenario suggests that reperfusion-related improvement in local perfusion causes systemic acidosis.
sis when severe ischemia and reperfusion are repeated, which is a paradox.

At the end of this experiment, the grading score for mucosal injury was significantly high with the duration of the ischemia, compared among the 3 experimental groups. Furthermore, there was a correlation between the grading score for mucosal injury and the intramuscular-arterial PCO2 gradient. This finding is in accordance with the findings of Iwanami et al who, in a dog intestinal ischemia-reperfusion model, found a correlation between histologic findings and intramuscular pH and that tonometric monitoring was useful for evaluating graft viability after small intestinal transplantation. Thus, tonometry may be useful for evaluating reperfusion injury after ischemia at the histologic level.

In our canine model of small intestinal ischemia-reperfusion, we found that it was difficult to accurately evaluate local oxygenation disorders by monitoring systemic parameters, which is commonly performed in clinical practice. Because our intestinal ischemia-reperfusion model differed markedly from shock that causes low systemic perfusion, our findings cannot be applied to all clinical cases. However, at the least, our data suggest that management of circulation that solely targets improvement in mesenteric blood flow is insufficient to improve intestinal tissue oxygenation after ischemia and that it is difficult to maintain local intestinal oxygenation appropriately by use of blood gas analysis.

References
