Evaluation of hepatosplanchnic circulation and intestinal oxygenation in dogs with a condition that mimicked septic shock induced by continuous infusion of a low dose of lipopolysaccharide

Yoko Sakaue, DVM; Yoshinori Nezu, DVM, PhD; Shinobu Komori, DVM, PhD; Yasushi Hara, DVM, PhD; Masahiro Tagawa, DVM, PhD; Ryo Ogawa, MD, PhD

Objective—To determine whether continuous infusion of a low dose of lipopolysaccharide (LPS) to induce a condition mimicking septic shock in dogs would affect systemic and hepatosplanchnic circulation and oxygenation.

Animals—12 healthy adult Beagles.

Procedure—Dogs received a low dose of LPS (Escherichia coli O55:B5) by continuous IV infusion at a rate of 1 µg/kg/h for 8 hours. Systemic hemodynamics; systemic oxygenation; blood flow in the cranial mesenteric artery, common hepatic artery, and portal vein; intestinal and hepatic tissue blood flow; mesenteric oxygenation; and intramucosal PCO2 were evaluated before and at selected time points after onset of the LPS infusion.

Results—After onset of the LPS infusion, cardiac index increased and mean arterial pressure (MAP) and systemic vascular resistance decreased, which is characteristic of the hyperdynamic state in septic patients. Hepatosplanchnic blood flow increased during the hyperdynamic state. Intestinal PCO2 was increased even when blood flows increased. During the latter half of the experimental period, MAP was maintained but hepatosplanchnic blood flows decreased and intestinal PCO2 increased further.

Conclusions and Clinical Relevance—Analysis of the results suggested that hepatosplanchnic blood flow enters the hyperdynamic state during the early stages of sepsis and that intestinal tissue oxygenation is threatened even when hepatosplanchnic blood flow is increased or maintained. Hence, improvement of hepatosplanchnic circulation and intestinal tissue oxygenation is important in clinical evidence of a septic condition. (Am J Vet Res 2004;65:1347-1354)

Sepsis is a major cause of death in critically ill patients. It can be induced by administration of lipopolysaccharide (LPS) or other bacterial products and leads to a systemic inflammatory response characterized by the release of inflammatory mediators, including tumor necrosis factor (TNF)-α and interleukin (IL)-6. In these conditions, patients are at risk of developing dysfunction in multiple organs.

Lipopolysaccharide stimulates endothelial cells or monocytes, which leads to the production of inflammatory mediators such as cytokines and vasoactive factors such as prostacyclin and nitric oxide. As a result, local inflammatory mediators dysregulate microcirculation. In particular, the gastrointestinal tract has the body’s largest immune system and can produce cytokines.

In addition, oxygen metabolism is usually increased during septic conditions; thus, oxygen delivery (DO2) may not meet oxygen requirements. The intestinal villus has a specific microvascular structure that promotes oxygen shunting via countercurrent exchange. At the tips of the villi, these physiologic and anatomic features contribute to disorders in tissue oxygenation when oxygen consumption (VO2) increases.

The intestinal lumen contains bacteria. When the intestinal barrier mechanism is impaired, bacterial translocation develops and leads to a systemic cytokine response and dysfunction in multiple organs. The liver, which is the primary organ affected by bacterial translocation, plays an important role in cytokine production and barrier mechanisms. Liver dysfunction during sepsis has been related to a poor outcome. Therefore, the maintenance of hepatosplanchnic perfusion and oxygenation, along with the prevention of hepatosplanchnic dysfunction, are important for the prevention and control of dysfunction in multiple organs attributable to sepsis.

To clarify this problem, studies have been performed in various animals, although the effects of the early stage of sepsis on hepatosplanchnic circulation have not been clarified. Many experiments have been performed in which bolus infusions of lethal doses of LPS have been administered IV, but use of that technique is problematic in that it is not matched to a clinically septic condition. In contrast, induction of a septic condition by use of continuous infusion of a low dose of LPS more closely approximates actual clinical conditions and is recognized as a suitable technique for the study of sepsis.

We hypothesized that hepatosplanchnic circulation and oxygenation become disordered during an early stage of septic conditions. Thus, the purpose of
the study reported here was to determine whether con-

Materials and Methods

Animals—Twelve healthy adult Beagles (males and females) were used in the study. Dogs weighed 8.5 to 12.5 kg. Food was withheld from the dogs for 18 hours prior to the study, but water was available ad libitum. The study was approved by the Committee on Bioethics of Nippon Veterinary and Animal Science University.

Anesthesia and surgical preparation—Anesthesia was induced and maintained by administration of pentobarbital sodium\(^*\) (30 mg/kg, IV, as a bolus, followed by constant infusion at the rate of 2 mg/kg/h, IV). Small supplementary doses were administered when necessary. An endotracheal tube was inserted, and the endotracheal tube was then con-

onset of the LPS infusion, the continuous IV infusion of saline solution was increased to a rate of 20 mL/kg/h; this rate for administration of saline solution was maintained throughout the experimental period. In a preliminary study, we determined that this rate did not reduce preload. Blood samples were collected and hemodynamic mea-

Humodynamic measurements—Mean arterial pressure (MAP), central venous pressure (CVP), mean pulmonary arterial pressure (MPAP), pulmonary capillary wedge pressure (PCWP), and heart rate were serially measured and recorded by connecting one of the aforementioned catheters to a pressure transducer\(^*\) and polygraph.\(^*\) Cardiac output (CO) was determined by use of a thermodilution technique. The mean value of 3 replications was used for each determin-

Pulmonary vascular resistance index (PVR) was calcu-

where $Q_{pv}$ and $Q_{hep}$ were the blood flow to the portal vein and common hepatic artery, respectively.\(^*\) Total mesenteric resistance (Rtm) was calculated as follows: $R_{tm} = \frac{\left(\text{MAP} - \text{PCWP}\right) \times 80}{\text{CI}}\.$$ Tonometer—Saline solution (2.5 mL) was placed in the silicone balloon of the tonometer; the tonometer then was allowed to equilibrate for ≥ 30 minutes. Thereafter, 1 mL of saline solution was aspirated; this volume represented the dead space of the tonometer. The remaining 1.5 mL was then immediately aspirated, and the PCO\(_2\) of the aliquot was determined in a blood gas analyzer.\(^*\) The correction factor listed by the manufacturer was used to adjust the measured PCO\(_2\) in the aliquot of saline solution.\(^*\)

Oxygen metabolism—The PO\(_2\), PCO\(_2\), and oxygen saturation were measured in samples of arterial, mixed-venous, and portal venous blood by use of a blood gas analyzer. Arterial oxygen content (CaO\(_2\)) was calculated as follows:

$$\text{CaO}_2 = \left(\text{SaO}_2 \times \text{hemoglobin concentration} \times 1.34\right) + \left(0.0031 \times \text{PO}_2\right),$$

where SaO\(_2\) is arterial blood oxygen saturation. Hemoglobin concentration was measured by use of an electronic cell counter.\(^*\) Systemic DO\(_2\) (DO\(_2\)sys) was calculated as follows:

$$\text{DO}_2\text{sys} = \text{CaO}_2 \times \text{CI} \times 10.$$
Mixed-venous oxygen content (CvO₂) was calculated as follows:

\[ \text{CvO}_2 = (\text{SvO}_2 \times \text{hemoglobin concentration} \times 1.34) + (0.0031 \times \text{PvO}_2) \]

where \(\text{SvO}_2\) is mixed-venous blood oxygen saturation and \(\text{PvO}_2\) is mixed-venous PO₂. Systemic VO₂ (VO₂sys) was calculated as follows:

\[ \text{VO}_2\text{sys} = (\text{CaO}_2 - \text{CvO}_2) / \text{CI} \times 10 \]

The systemic oxygen extraction ratio was derived as VO₂sys/DO₂sys.

Mesenteric DO₂ (DO₂mes) was calculated as follows:

\[ \text{DO}_2\text{mes} = \text{CaO}_2 - \text{CvO}_2 \]

where Qmes is blood flow to the mesenteric artery. The CI was calculated as follows:

\[ \text{CI} = \frac{\text{MAP}}{\text{HR}} \]

where MAP is mean arterial pressure and HR is heart rate. The SvO₂ is mixed-venous blood oxygen saturation and \(\text{PvO}_2\) is mixed-venous PO₂.

Results—All data were reported as mean ± SEM values for variables of systemic hemodynamics in 6 dogs receiving a continuous infusion of a low dose of lipopolysaccharide (LPS) solution or 6 dogs receiving a continuous infusion of saline (0.9% NaCl) solution (control group).

Table 1—Mean ± SEM values for variables of systemic hemodynamics in 6 dogs receiving a continuous infusion of a low dose of lipopolysaccharide (LPS) solution or 6 dogs receiving a continuous infusion of saline (0.9% NaCl) solution (control group).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Baseline</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (%)</td>
<td>LPS</td>
<td>100 ± 0</td>
<td>100.0 ± 3.0</td>
<td>98.7 ± 3.5</td>
<td>91.7 ± 3.6</td>
<td>99.8 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100 ± 0</td>
<td>103.3 ± 1.4</td>
<td>102.5 ± 2.3</td>
<td>99.5 ± 3.6</td>
<td>99.5 ± 2.7</td>
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<tr>
<td>CI (%)</td>
<td>LPS</td>
<td>100 ± 0</td>
<td>112.5 ± 4.6</td>
<td>117.2 ± 5.1</td>
<td>96.5 ± 8.1</td>
<td>70.7 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100 ± 0</td>
<td>94.3 ± 3.9</td>
<td>95.7 ± 4.9</td>
<td>97.2 ± 4.7</td>
<td>80.0 ± 5.5</td>
</tr>
<tr>
<td>MAP (%)</td>
<td>LPS</td>
<td>100 ± 0</td>
<td>94.0 ± 2.1</td>
<td>92.5 ± 1.6</td>
<td>95.8 ± 1.5</td>
<td>94.3 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100 ± 0</td>
<td>98.3 ± 1.5</td>
<td>98.0 ± 1.3</td>
<td>99.3 ± 1.8</td>
<td>92.2 ± 2.8</td>
</tr>
<tr>
<td>MPAP (%)</td>
<td>LPS</td>
<td>100 ± 0</td>
<td>91.8 ± 6.9</td>
<td>94.3 ± 2.9</td>
<td>117.3 ± 6.4</td>
<td>138.6 ± 16.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100 ± 0</td>
<td>110.3 ± 5.7</td>
<td>110.7 ± 6.3</td>
<td>124.2 ± 9.2</td>
<td>116.8 ± 8.8</td>
</tr>
<tr>
<td>CVP (%)</td>
<td>LPS</td>
<td>100 ± 0</td>
<td>102.7 ± 26.7</td>
<td>105.5 ± 31.6</td>
<td>136.2 ± 46.8</td>
<td>130.5 ± 44.0</td>
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<tr>
<td></td>
<td>Control</td>
<td>100 ± 0</td>
<td>84.8 ± 14.6</td>
<td>58.3 ± 15.4</td>
<td>118.2 ± 27.9</td>
<td>91.7 ± 24.3</td>
</tr>
<tr>
<td>PCWP (%)</td>
<td>LPS</td>
<td>100 ± 0</td>
<td>100.2 ± 9.8</td>
<td>90.8 ± 4.2</td>
<td>92.0 ± 5.7</td>
<td>82.8 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100 ± 0</td>
<td>97.5 ± 4.2</td>
<td>98.2 ± 11.1</td>
<td>92.7 ± 4.9</td>
<td>80.7 ± 5.2</td>
</tr>
<tr>
<td>SVRI (%)</td>
<td>LPS</td>
<td>100 ± 0</td>
<td>86.5 ± 4.1</td>
<td>80.5 ± 2.4</td>
<td>117.7 ± 7.2</td>
<td>145.2 ± 14.6</td>
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<tr>
<td></td>
<td>Control</td>
<td>100 ± 0</td>
<td>101.3 ± 4.0</td>
<td>101.7 ± 5.1</td>
<td>103.8 ± 4.4</td>
<td>121.8 ± 11.5</td>
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<tr>
<td>PVRI (%)</td>
<td>LPS</td>
<td>100 ± 0</td>
<td>72.8 ± 14.3</td>
<td>89.8 ± 10.7</td>
<td>178.2 ± 19.8</td>
<td>301.0 ± 66.6</td>
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<tr>
<td></td>
<td>Control</td>
<td>100 ± 0</td>
<td>125.0 ± 11.0</td>
<td>126.0 ± 10.2</td>
<td>158.5 ± 16.5</td>
<td>196.2 ± 19.3</td>
</tr>
</tbody>
</table>

Results are expressed as a percentage of values determined immediately before start of the infusions (time 0 [baseline]). HR = Heart rate. CI = Cardiac index. MAP = Mean arterial pressure. MPAP = Mean pulmonary arterial pressure. CVP = Central venous pressure. PCWP = Pulmonary capillary wedge pressure. SVRI = Systemic vascular resistance index. PVRI = Pulmonary vascular resistance index.

Within a time point for each variable, value differs significantly (P < 0.05) from the value for the control group. *Within a row, value differs significantly (P < 0.05) from baseline value.
Hepatosplanchnic hemodynamics—In the LPS group, blood flow in the cranial mesenteric artery rapidly increased by 1 hour after the start of the LPS infusion (176.5 ± 21.0%), which differed significantly from the baseline value (P = 0.002) and the corresponding value for the control group (P = 0.008). Blood flow in this artery remained increased until 2 hours after onset of the infusion (167.3 ± 17.6%), which differed significantly (P = 0.004) from the baseline value and the corresponding value for the control group. This was followed by a gradual decrease in blood flow (Figure 1). Blood flow in the portal vein increased by 2 hours after the start of the LPS infusion (109.0 ± 5.3%), which was significantly (P = 0.029) different from the corresponding value for the control group. This was followed by a decrease at 8 hours after the start of the LPS infusion (66.0 ± 7.8%), which differed significantly from the values at baseline (P = 0.003) and at 8 hours for the control group (P = 0.010). Blood flow in the common hepatic artery reached a peak increase 2 hours after the start of the LPS infusion (132.8 ± 9.2%), which differed significantly (P = 0.010) from the corresponding value for the control group; this was followed by a gradual decrease in blood flow. The Qtm rapidly increased by 1 hour after the start of the LPS infusion (115.8 ± 6.8%), which differed significantly (P = 0.029) from the corresponding value for the control group. The Qtm remained increased until 2 hours after the start of the LPS infusion (70.8 ± 6.7%), which differed significantly from the baseline value (P = 0.007) and the value for the control group at 8 hours after the start of the infusion (P = 0.024; Figure 2). The Rtm decreased by 2 hours after the start of the LPS infusion (82.8 ± 4.5%), which differed significantly (P = 0.004) from the corresponding value for the control group. This was followed by an increase at 8 hours after the start of the LPS infusion (134.8 ± 10.1%), which differed significantly from the baseline value (P = 0.004) and the value for the control group at 8 hours after the start of the infusion (P = 0.016).

Tissue blood flow—Intestinal tissue blood flow began to decrease 4 hours after the start of the LPS infusion and was significantly decreased by 8 hours after the start of the LPS infusion (88.5 ± 3.0%), compared with the baseline value (P = 0.002) or the value for the control group at 8 hours after the start.

Table 2—Mean ± SEM values for variables of systemic oxygen metabolism in dogs receiving a continuous infusion of a low dose of LPS solution or saline solution (control group).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Time (h)</th>
<th>Baseline</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{DO}_2 \text{sys} ) (%)</td>
<td>LPS</td>
<td>100 ± 0</td>
<td>106.8 ± 6.7</td>
<td>108.5 ± 5.1</td>
<td>74.2 ± 4.7</td>
<td>68.5 ± 6.8</td>
<td>6.3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100 ± 0</td>
<td>87.8 ± 3.1</td>
<td>83.7 ± 3.5</td>
<td>81.5 ± 3.5</td>
<td>71.0 ± 4.3</td>
<td>6.5%</td>
<td></td>
</tr>
<tr>
<td>( \text{VO}_2 \text{sys} ) (%)</td>
<td>LPS</td>
<td>100 ± 0</td>
<td>63.6 ± 28.6</td>
<td>91.7 ± 22.6</td>
<td>71.2 ± 21.6</td>
<td>90.3 ± 26.6</td>
<td>17.6%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100 ± 0</td>
<td>72.7 ± 18.3</td>
<td>64.2 ± 13.7</td>
<td>69.8 ± 14.2</td>
<td>86.3 ± 16.9</td>
<td>13.2%</td>
<td></td>
</tr>
<tr>
<td>( \text{O}_2 \text{ERsys} ) (%)</td>
<td>LPS</td>
<td>100 ± 0</td>
<td>55.8 ± 13.6</td>
<td>74.5 ± 15.6</td>
<td>114.2 ± 19.7</td>
<td>143.8 ± 40.1</td>
<td>28.6%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>100 ± 0</td>
<td>91.7 ± 23.3</td>
<td>87.7 ± 21.8</td>
<td>100.2 ± 24.8</td>
<td>127.0 ± 21.2</td>
<td>20.1%</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as a percentage of baseline values.

\( \text{DO}_2 \text{sys} \) = Systemic oxygen delivery, \( \text{VO}_2 \text{sys} \) = Systemic oxygen consumption, \( \text{O}_2 \text{ERsys} \) = Systemic oxygen extraction ratio.

See Table 1 for remainder of key.

Figure 1—Mean ± SEM blood flow in the cranial mesenteric artery (CMA; A), portal vein (PV; B), and common hepatic artery (HA; C) in dogs receiving a continuous infusion of a low dose of lipopolysaccharide (LPS) solution (circles) or saline (0.9% NaCl) solution (control group; squares). Results are expressed as a percentage of values determined immediately before start of the infusions (time 0 [baseline]). There were 6 dogs in each group. *Within a group, value differs significantly (P < 0.05) from the baseline value. **Within a time point, value differs significantly (P < 0.05) from the corresponding value for the control group. ***Within a time point, value differs significantly (P < 0.05) from the value for the control group.
of the infusion (P = 0.016; Figure 3). Hepatic tissue blood flow was increased by 1 hour after the start of the LPS infusion (112.3 ± 3.5%), which differed significantly (P = 0.019) from the corresponding value for the control group; this was followed by a gradual decrease. There were no significant changes in hepatic tissue blood flow for the control group throughout the experiment.

**Mesenteric oxygenation**—The D\textsubscript{O\textsubscript{2}}\textsubscript{mes} was increased by 1 hour after the start of the LPS infusion (181.8 ± 19.7%), which differed significantly from the baseline value (P = 0.002) and the corresponding value for the control group (P = 0.004). The D\textsubscript{O\textsubscript{2}}\textsubscript{mes} remained increased until 2 hours after the start of the LPS infusion (177.3 ± 27.8%), which differed significantly (P = 0.004) from the baseline value and the corresponding value for the control group; this was followed by a decrease to the baseline value (Figure 4). The V\textsubscript{O\textsubscript{2}}\textsubscript{mes} increased, although the values did not change significantly.

The mesenteric oxygen extraction ratio decreased immediately during the first hour after the start of the LPS infusion, which was followed by a gradual increase; however, the values did not change significantly. There were no significant changes in mesenteric oxygenation for the control group throughout the experiment.

**Tonometry**—In the LPS group, intramucosal P\textsubscript{CO\textsubscript{2}} was increased by 2 hours after the start of the infusion (122.2 ± 5.9%), which differed significantly from the baseline value (P = 0.009) or the corresponding value for the control group (P = 0.010). Intramucosal P\textsubscript{CO\textsubscript{2}} continued to increase throughout the experiment (Figure 5). There were no significant changes in tonometry for the control group throughout the experiment.
Discussion

We evaluated specific characteristics of hepatosplanchnic circulation during a septic condition by simultaneously determining systemic and hepatosplanchnic blood flow, systemic and intestinal oxygenation, and tonometry by use of a low-dose LPS continuous-infusion technique in dogs. The main finding of the study reported here was that hepatosplanchnic blood flow indicated a hyperdynamic state during the acute phase. Moreover, intestinal tissue oxygenation was disordered even when hepatosplanchnic blood flow was increased in the hyperdynamic state.

After the start of the LPS infusion, blood flow increased in the cranial mesenteric artery, common hepatic artery, and portal vein. These results revealed that hepatosplanchnic blood flow increased during the hyperdynamic state of early sepsis. The main finding of the study reported here was that hepatosplanchnic blood flow indicated a hyperdynamic state during the acute phase. Moreover, intestinal tissue oxygenation was disordered even when hepatosplanchnic blood flow was increased in the hyperdynamic state.

As a result of the increase in hepatosplanchnic blood flow, hepatic tissue blood flow increased significantly and intestinal tissue blood flow also increased. These increases in hepatosplanchnic perfusion are associated with 3 mechanisms. First, hepatosplanchnic perfusion increases secondarily along with an increase in the CI. Second, the increase in \( V_O_2 \) increases the oxygen requirement, and blood flow may increase as a result. Third, in the hepatosplanchnic vascular bed, vasoactive factors such as prostacyclin and nitric oxide may be released from endothelial cells in response to stimulation by LPS, resulting in vasodilation. In the study reported here, Rtm decreased when hepatosplanchnic perfusion increased; this finding is consistent with the third mechanism.

Interestingly, intramucosal \( PCO_2 \), which indicates intestinal tissue oxygenation, started to increase 2 hours after the start of LPS infusion; this was also the time when blood flow increased to the intestine and there was an increase in splanchnic \( DO_2 \). Analysis of these results indicates that intestinal tissue oxygenation is disordered and anaerobic metabolism is induced even when blood flow is increased. Our results are in accordance with those of a study of induced endotoxin shock in pigs and another study conducted by our laboratory group in which we evaluated intestinal ischemia-reperfusion in dogs.

A countercurrent shunt in the microvilli may cause the disorder in intestinal tissue oxygenation during the hyperdynamic state. Intestinal villi have a countercurrent system, and hypoxia in the tip of a vil-lus develops easily during low oxygen flow or hypoxia. There are species differences in the countercurrent exchange mechanism of oxygen. This mechanism is reportedly important in the long, slender villi of cats but not in the broad, wide villi of rabbits. The effect of this mechanism has been reported in dogs, which have long, densely packed villi, but the existence of a countercurrent exchange mechanism of oxygen in dogs is controversial. In a study in which investigators induced endotoxemia in dogs, there was a decrease in intramucosal pH, which is an indicator of intestinal tissue oxygenation as well as intramucosal
E-selectin, and P-selectin). These mediators may also induce hypoxia. 

During the latter half of the experimental period, CO decreased and SVRI and PVRI increased. In addition, hepatosplanchnic blood flow decreased and Rtm increased. At the same time, systemic blood pressure was maintained. These results could be attributable to a combination of decreased cardiac function related to myocardial dysfunction directly induced by LPS as well as to the effect of a local vasoconstrictive mediator, such as endothelin-1 or thromboxane. In addition to the decrease in blood flow to the splanchnic region, the microvascular disturbance induced by inflammatory mediators appears to further disorder oxygenation of intestinal tissues.

Analysis of results of the study reported here revealed that hepatosplanchnic blood flow entered a hyperdynamic state during the early stages of sepsis and intestinal tissue oxygenation was threatened even when hepatosplanchnic blood flow increased or was maintained. This latter finding suggests that in septic patients, improvement and maintenance of hepatosplanchnic blood flow and intestinal tissue oxygenation related to inflammatory mediators are important for the prevention of multiple organ dysfunction syndrome originating from the hepatosplanchnic region.

References

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