Nitric oxide generation in a rat model of acute portal hypertension

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Objective—To document blood nitric oxide concentrations in the portal vein and systemic circulation in a rat model of acute portal hypertension and compare values with a control group and a sham surgical group.

Animals—Thirty male Sprague-Dawley rats (weight, 354 ± 71 g) were assigned at random to a control (group 1, n = 10), sham surgery (group 2, 10), or portal hypertension group (group 3, 10). Rats were cared for, and the study was performed in a manner consistent with the National Institutes of Health, Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Acts.

Study design—Surgery order was assigned randomly, and general anesthesia induced by administration of isoflurane in oxygen via tank induction. Anesthesia was maintained with isoflurane and oxygen delivered via a Bain non-rebreathing system and a snug-fitting mask taped to the face. Once the facemasks were in place, vaporizers were set at 2.5% for all rats, and adjusted as needed to maintain a surgical plane of anesthesia thereafter. Body temperature was maintained using a warm water circulating blanket and warm water bottles.

Rats in all 3 groups underwent surgical placement of jugular and carotid catheters, and rats in groups 2 and 3 underwent surgical placement of a portal vein catheter. A 22-gauge catheter was placed aseptically into the right jugular vein and secured to the vessel using tissue glue. The left carotid artery was catheterized aseptically with a 22-gauge catheter that was secured to the vessel using 4-0 suture and tissue glue. In groups 2 and 3, a standard ventral midline abdominal approach was performed, and a strand of 4-0 silk suture was passed around the portal vein at the hepatic hilus, but was not immediately tightened. An indwelling 24-gauge catheter that was secured to the vessel using 4-0 suture and tissue glue. In groups 2 and 3, a standard ventral midline abdominal approach was performed, and a strand of 4-0 silk suture was passed around the portal vein at the hepatic hilus, but was not immediately tightened. An indwelling 24-gauge catheter was placed aseptically into the right jugular vein and secured to the vessel using tissue glue. The left carotid artery was catheterized aseptically with a 22-gauge catheter that was secured to the vessel using 4-0 suture and tissue glue.

Procedure—Following induction of anesthesia, catheters were placed surgically in the carotid artery, jugular, and portal veins of group 2 and 3 rats and in the carotid artery and jugular vein of group 1 rats. Baseline heart and respiratory rates, rectal temperature, and vascular pressure measurements were obtained, and blood was drawn from all catheters for baseline nitric oxide (NO) concentrations. Acute portal hypertension was induced in the group 3 rats by tying a partially occluding suture around the portal vein and a 22-gauge catheter. The catheter was then removed, resulting in a repeatable degree of portal vein impingement. After catheter placement, all variables were remeasured at 15-minute intervals for 3 hours.

Results—Blood nitric oxide concentrations were greater in all vessels tested in group 3 than in group 2 rats.

Conclusions and Clinical Relevance—Acute portal hypertension in this experimental model results in increased concentrations of NO in the systemic and portal circulation. On the basis of information in the rat, it is possible that increased NO concentrations may develop in dogs following surgical treatment of congenital portosystemic shunts if acute life-threatening portal hypertension develops. Increased NO concentrations may contribute to the shock syndrome that develops in these dogs. (Am J Vet Res 2000;61:1173–1177)

During the last several years, nitric oxide (NO) has been identified as a fundamental biologic messenger in various physiologic functions, including nerve transmission, vascular dilatation, long-term memory, and immune system defense. In certain instances, however, NO production can become excessive. Excessive NO can be associated with adverse actions, and may play a role in tissue injury associated with various pathologic conditions. Excessive NO may be responsible for many of the pathologic changes found during endotoxic and septic shock in animals and humans.

Portosystemic shunts (PSS) are abnormal vessels connecting the portal vein with the systemic circulation allowing visceral blood to enter the systemic circulation prior to hepatic filtration. Diversion of blood from the liver permits toxic substances to enter the systemic circulation (resulting in many of the clinical signs in patients with PSS) and also leads to hepatic atrophy because hepatatrophic substances do not reach the liver. Surgery is the treatment of choice, and involves isolation and ligation or partial ligation of the abnormal vessel, or placement of an ameroid constrictor device around the shunt to permit slow occlusion of the shunting vessel. When ligating or partially ligating the abnormal vessel, portal pressures are monitored closely to avoid life-threatening acute portal hypertension. Despite efforts to prevent acute portal hypertension, 14 to 25% of dogs undergoing PSS surgery die presumably from portal hypertension (14%, ameroid constrictor method; up to 25%; traditional ligation or partial attenuation method). Death may be caused by visceral hypoxia, sloughing of intestinal mucosa, and endotoxic or septic shock.

Although not documented in dogs with PSS, acute portal hypertension may result in formation and release of NO, which may contribute to the shock syndrome in some patients. The purpose of this study was to use a rat model of acute portal hypertension to simulate a life-threatening situation similar to that seen clinically in some dogs, and to measure and compare blood NO concentrations in control, sham operated, and acute portal hypertension rats.

Materials and Methods

Rats—Thirty male Sprague-Dawley rats (weight, 354 ± 71 g) were assigned at random to a control (group 1, n = 10), sham surgery (group 2, 10), or portal hypertension group (group 3, 10). Rats were cared for, and the study was performed in a manner consistent with the National Institutes of Health, Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Acts.

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Rats in all 3 groups underwent surgical placement of jugular and carotid catheters, and rats in groups 2 and 3 underwent surgical placement of a portal vein catheter. A 22-gauge catheter was placed aseptically into the right jugular vein and secured to the vessel using tissue glue. The left carotid artery was catheterized aseptically with a 22-gauge catheter that was secured to the vessel using 4-0 suture and tissue glue. In groups 2 and 3, a standard ventral midline abdominal approach was performed, and a strand of 4-0 silk suture was passed around the portal vein at the hepatic hilus, but was not immediately tightened. An indwelling 24-gauge catheter...
catheter was placed in the portal vein approximately 2 cm caudal to the hepatic hilus and advanced so that its tip was 0.5 to 1 cm caudal to the silk suture. The catheter was secured to the vessel using tissue glue. Intermittent injection caps were attached to each catheter. After catheter placement, pressure measurements were obtained from all vessels using a pressure transducer, and rectal temperature, heart rate, and respiratory rate were recorded. Blood was drawn (0.1 ml or 100 µl) from each vessel for NO analysis, and an equal volume of saline (0.9% NaCl) solution was administered to minimize blood pressure changes caused by the sampling process. Prior to collection of each blood sample, 0.2 ml blood was aspirated from the catheter (to empty the catheter of saline-diluted blood) in a separate syringe and was then given back to the rat after collection of the NO sample and prior to administration (and flushing of the catheter) of the 0.9% NaCl. Sample order was carotid artery, jugular vein, and portal vein (in group 2 and 3 rats), and samples were taken 5 minutes apart (to permit the NO analyzer to process each sample completely before the next sample was delivered). On completion of baseline data collection, portal hypertension was induced in group 3 rats by snugly tying the preplaced silk suture around the portal vein (the tip of the catheter previously placed in the portal vein was lying 0.5 to 1 cm caudal to the silk suture and was not disturbed during ligature manipulations). Once the ligature was tied, the catheter was removed. Portal pressures were recorded immediately on removal of the catheter and refilling of the portal vein with blood as well as 1 minute later. In group 2 rats, the suture was manipulated and then removed. Heart rate, respiratory rate, rectal temperature, and vascular pressures were recorded, and blood was collected for NO determinations at 15-minute intervals until 3 hours after induction of portal hypertension (or removal of the silk suture in group 2 rats). In group 1 rats, an identical protocol was followed except that blood was collected only from the carotid and jugular vessels. Sampling methods were identical to those described for baseline data collection. Materials used for catheter placement, blood collection, and fluid administration were sterile and pyrogen free. Following collection of the 180-minute sample, rats were euthanatized, using a barbiturate overload (0.25 ml, IV).

Determination of NO concentrations—Nitr oxide concentrations were determined, using an ozone chemiluminescent NO analyzer. The analyzer was combined with a reduction assembly, which reduces all nitrates, nitrites, and NO concentrations in the biologic system. The chemiluminescence assay is specific, as few other biologic compounds exist as gases and interact with ozone.

Standard curves were generated, using sodium nitrate and sodium nitrite standards to determine the precision and accuracy of the reduction assembly and chemiluminescence analyzer. Association between standard concentrations and peak area was linear for the entire range of standard concentrations ($r^2 > 0.955$). The coefficient of variation for each of the controls was low control (9.56 µM, 7%), low medium control (14.94 µM, 10.7%), high medium control (23.24 µM, 14.6%), and high control (69.73 µM, 7.6%). The manufacturer’s lowest limit of detection is 1 PM of NO with sample sizes as small as 1 µl.

Statistical analysis—A computer software program was used for all statistical analyses. Because several rats died before the end of the study period (resulting in missing data points thereafter), baseline values of each variable (NO concentration, vascular pressure, heart rate, respiratory rate, and rectal temperature) were subtracted from the final value obtained (either prior to cardiac arrest or the end of the study) in each rat of each group to determine the change that had developed over time, and the mean and standard deviation for each group was determined. Additionally, baseline values for each variable were compared between groups to ascertain similarity between groups at the beginning of the study. Statistical analysis of this data was performed, using one-way ANOVA with a Tukey multiple-comparison test and an unpaired Student t-test for portal vein pressure and blood NO concentration in the portal vein. A value of $P < 0.05$ was considered significant.

Results

All group 1 rats survived the entire study period. One group 2 rat was euthanatized after 120 minutes when the portal vein catheter became dislodged, resulting in severe hemorrhage. In group 3, 5 of 10 rats survived the entire study period, while 1 rat died after 165 minutes, 2 died after 150 minutes, 1 died after 135 minutes, and 1 died after 90 minutes. All of these rats presumably died of complications associated with portal hypertension (ie, cyanotic intestine and cardiac arrest).

Baseline values for all variables, except rectal temperature and heart rate (ie, respiratory rate, vascular pressures, and NO concentrations), were not statistically different between groups (Table 1). Baseline rectal temperatures were significantly ($P = 0.002$) higher in group 1 rats than groups 2 and 3. Baseline heart rates were higher ($P = 0.01$) in group 1 rats than in group 3 rats.

Evaluating change in variables over time (baseline values subtracted from ending values) revealed no significant differences in respiratory rates between groups 1 and 3. Baseline rectal temperatures were significantly ($P = 0.002$) higher in group 1 rats than groups 2 and 3. Baseline heart rates were higher ($P = 0.01$) in group 1 rats than in group 3 rats.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 (n = 10)</th>
<th>Group 2 (n = 10)</th>
<th>Group 3 (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>277 ± 21*</td>
<td>246 ± 23</td>
<td>237 ± 24</td>
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<tr>
<td>RR (breaths/min)</td>
<td>48 ± 9</td>
<td>46 ± 8</td>
<td>42 ± 9</td>
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<td>Rectal temperature (°C)</td>
<td>97 ± 1.2</td>
<td>98.1 ± 1.7</td>
<td>94.8 ± 1.1</td>
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<td>Carotid artery (mm Hg)</td>
<td>77.2 ± 12.8</td>
<td>70.5 ± 10.5</td>
<td>67.7 ± 18.9*</td>
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<td>Jugular vein (mm Hg)</td>
<td>21.2 ± 2.0</td>
<td>17.1 ± 1.3</td>
<td>17.4 ± 2.0*</td>
</tr>
<tr>
<td>Portal vein (mm Hg)</td>
<td>—</td>
<td>—</td>
<td>19 ± 2.0</td>
</tr>
<tr>
<td>Carotid artery (µM NO)</td>
<td>10.4 ± 6.5</td>
<td>9.1 ± 2.7</td>
<td>13.2 ± 7.7</td>
</tr>
<tr>
<td>Jugular vein (µM NO)</td>
<td>10.1 ± 4.3</td>
<td>8.4 ± 2.9</td>
<td>7.0 ± 2.5</td>
</tr>
<tr>
<td>Portal vein (µM NO)</td>
<td>—</td>
<td>—</td>
<td>11.2 ± 5.2</td>
</tr>
</tbody>
</table>

*Significant difference ($P ≤ 0.05$) between groups 1 and 3. †Significant difference between group 1 and other groups. ‡Significant difference between groups 2 and 3.

**Group 1 = Control group. Group 2 = Sham surgical group. Group 3 = Acute portal hypertension group.**
*Represents a significant difference at \( P \leq 0.05 \) between the decreased NO concentrations over time in the other groups.

Intracarotid arterial pressures were higher (\( P = 0.04 \), \( P = 0.03 \), and \( P = 0.01 \) respectively) in group 3 compared with group 2.

**Discussion**

In 1980, it was discovered that endothelial derived relaxing factor (EDRF) was released by endothelial cells, and that EDRF appeared to modulate endothelium-dependent vasodilatory responses. \(^{22}\) In 1987, it was demonstrated that EDRF was actually NO. \(^{23}\) Nitric oxide is a small diatomic free radical that plays an important role as a biologic messenger. \(^{24-26}\) Nitric oxide is synthesized by numerous cell types, including endothelial cells, platelets, neutrophils, endocardium, neurons, hepatocytes, adrenal glands, retina, mesangial cells, mast cells, chondrocytes, and others. \(^{22,27,28}\) Some of its many functions include neurotransmission, blood pressure control, blood clotting, and killing of tumor cells and intracellular parasites by the immune system. \(^{6}\)

Nitric oxide is synthesized from 1 of the terminal guanido nitrogen atoms of L-arginine by a distinct class of enzymes through a process that incorporates molecular oxygen. \(^{32-34}\) These enzymes are broadly classified as constitutive (endothelial and neuronal) and inducible nitric oxide synthases (NOS). \(^{4,27,28}\) The constitutive enzyme synthesizes and releases NO within seconds in response to ligand-receptor coupling or other stimuli that cause an acute extracellular calcium entry or elevated cytosolic calcium. \(^{4,27,28}\) Thus, the constitutive enzyme is highly dependent on calcium, calmodulin, and NADPH for its activity. \(^{4,27,28}\) Inducible nitric oxide synthase is functionally calcium independent. \(^{4,27,28}\) The inducible enzyme requires some time to be expressed, and once synthesized, it releases NO until the cell dies or until substrate or cofactors are depleted. \(^{4,27,28}\) Positive promoters of inducible NOS include endotoxin, TNF, IL-1, IL-2, and interferon-\( \beta \). \(^{4,27,28}\) During endotoxemia, inducible NOS is synthesized, leading to overproduction of NO in vascular endothelial and smooth muscle cells. \(^{6}\) Excessive NO results in hypotension as the result of widespread relaxation of vascular smooth muscle and the inability of smooth muscle to respond to therapeutic agents used to raise blood pressure. \(^{6}\)

Historically, NO measurement has proved problematic because of lack of a sensitive assay. The chemiluminescence assay (with sample reduction) has proven to be approximately 100-fold more sensitive than the Griess reaction (a commonly used assay). \(^{21,35}\) In addition to measuring NO, the chemiluminescence assay can be used to measure the intermediate and end-products of NO oxidation (nitrate, nitrite, and S-nitrosothiol). \(^{21,35}\) Because NO oxidation is quite rapid
in the presence of oxygen and oxygen-derived species, measuring nitrate, nitrite, and S-nitrosothiol is important for NO determinations in biologic systems.

The portal vein constriction model used in our study proved to be satisfactory for simulating the acute portal hypertension situation. Life-threatening portal hypertension was produced by the model as was confirmed by the observation that 50% of group 3 animals died during the study period (presumably of complications associated with portal hypertension). Although this model relied on prehepatic portal hypertension rather than increased hepatic portal blood flow as the mechanism of portal hypertension (as suspected in the clinical situation in dogs), both mechanisms result in splanchnic pooling and hypoxia of the viscera that may ultimately lead to sloughing of intestinal mucosa and endotoxic or septic shock. Differences may also exist in that the clinical patient with PSS may have variations in biosynthesis of NO and other inflammatory mediators during acute portal hypertension caused by the chronicity of portal venous blood shunting (as compared with this model of acute portal hypertension).

In our study, baseline values of variables were subtracted from the final value obtained either prior to cardiac arrest or the end of the study in each rat in each group to determine the change that occurred over time. This study design was implemented (rather than comparing individual time points) because the rats in group 3 were not expected to survive the entire length of study (if they developed severe portal hypertension) and because the purpose of the study was to compare the change in NO concentration (and other parameters) occurring between groups over time.

Groups were similar at the beginning of the study, with the exception of rectal temperature and heart rate. The lower rectal temperatures in groups 2 and 3 reflect the loss of body heat associated with abdominal surgery. The lower heart rate in group 3 rats may reflect lower rectal temperature in that group at the beginning of the study period or subtle differences in anesthetic depth.

As expected, portal venous pressures were higher in group 3 rats, compared with group 2 rats.Portal hypertension with pooling of blood in the splanchnic circulation and decreased venous return to the heart accounted for lower systemic arterial pressures, compared with the control group. The initial decrease in portal pressures in group 3 rats 1 minute after attenuation of the portal vein reflects the capacitance of the portal venous system.

Systemic arterial, central venous, and portal venous circulations in group 3 rats had elevated NO concentrations, compared with group 2 rats (Fig 1-3). In group 1 rats, NO concentrations decreased only slightly during the course of the study, whereas the NO concentrations in group 2 rats decreased more prominently over time, compared with baseline values. However, in group 3 rats, the NO concentrations increased over time, compared with baseline, presumably as the result of portal hypertension.

Portal hypertension can be speculated to result in increased NO production by 2 mechanisms. First, portal hypertension may have increased shear stress and altered oxygen tension in the portal venous system stimulating NO release by the endothelium (and potentially the hepatocytes). Of the physiologic stimuli that may stimulate NO release (changes in shear stress, oxygen tension, and calcium concentrations), shear stress appears most important, because of changes that develop in the endothelium in response to increased blood flow. However, vessels of small diameter release greater amounts of NO basally than those of greater diameter, and veins appear to synthesize less NO than arteries.

The second proposed mechanism for increased NO production secondary to portal hypertension in group 3 rats is suspected endotoxemia/endotoxic shock. Although endotoxemia/endotoxic shock was not documented for the rats of our study, endotoxemia appears to play a role in death of patients with acute portal hypertension. Evidence suggests that increased NO contributes to many pathologic features of septic and endotoxic shock. During the acute phase of shock, NO production increases as the result of activation of constitutive endothelial NOS, while later increased NO production is caused by activation of inducible NOS. Increased NO production seems to mediate the vascular hyporeactivity in conductance, resistance, and venous vessels observed during endotoxemia. Endotoxin does not cause hypotension in mice in which the inducible NOS gene has been inactivated (inducible NOS knock-out mice), lending more support to the role of NO in endotoxic shock.

The increase in NO production in group 3 rats is presumably the result of increased constitutive NOS activity, because NO concentrations increased early after induction of portal hypertension. As noted previously, the constitutive enzyme can be synthesized and released NO in a short period. However, increased NO production in group 3 rats may also reflect increased inducible NOS activity, although expression of inducible NOS generally requires approximately 2 hours to be expressed. Had the study period been extended, NO concentrations may have increased further as inducible NOS became expressed. Further studies are necessary to determine which isoform(s) of NOS are involved in acute portal hypertension.

It is unclear whether the increase in blood NO concentrations in group 3 rats contributed to or resulted from circulatory collapse and shock (and cardiac arrest in some rats). Of interest is the observation that mean blood concentrations of NO in all 3 vessels were higher in group 3 rats than group 2 rats over the course of the study beginning approximately 1 hour after the induction of portal hypertension and prior to the observation of severe clinical signs in that group. One could infer that increased concentrations of NO may have been contributory to the clinical signs rather than resultant from the clinical signs. In addition to having the highest NO concentrations, group 3 rats also had the lowest blood pressures. Because NO causes vasodilation, the high systemic concentrations of NO in the systemic circulation of group 3 rats likely contributed to the clinical signs. It is unlikely that isoflurane (a vasodilator) vaporizer settings contributed to the lower blood pressures in group 3 rats, because vaporizer settings were the same for all groups of rats until rats in
group 3 began having signs of circulatory collapse, at which time vaporizer settings were decreased in an effort to prevent early mortality.

In conclusion, the experimental model of acute portal hypertension resulted in increased concentrations of NO in the systemic and portal circulation of rats. If increased concentrations of NO develop in patients with acute portal hypertension, the overproduction of NO may contribute to the severe shock syndrome seen in some of these patients. Additional studies are needed to determine the role of NO in patients with acute portal hypertension, as well as the therapeutic role of NOS inhibitors (selective or nonselective) or NOS scavengers.

References