Gut and Liver

The Organs Responsible for Increased Nitric Oxide Production After Trauma-Hemorrhage and Resuscitation

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Objective: To determine which organs produce the increased levels of nitric oxide (NO) seen after hemorrhage and resuscitation.

Animals and Interventions: Adult male rats underwent laparotomy (ie, trauma induced) and were bled to and maintained at a mean arterial pressure of 40 mm Hg until 40% of the maximum bleedout volume was returned in the form of Ringer lactate. The rats were then resuscitated with Ringer lactate, 4 times the maximum bleedout volume for 1 hour. Sham-operated animals underwent only the surgical procedure.

Main Outcome Measures: Plasma levels of nitrate/nitrite (NO$_3^-$/NO$_2^-$, stable products of NO) were measured by colorimetric assay at the maximum bleedout volume; at the end of hemorrhage; at the end of resuscitation; and 1.5, 4, 8, and 24 hours after resuscitation. In additional rats, the heart, liver, small intestine, kidneys, and spleen were harvested 4 hours after resuscitation for the measurement of NO$_3^-$/NO$_2^-$ levels. Moreover, tissue perfusion was determined in the above-mentioned organs by radioactive microspheres 4 hours after resuscitation in other groups of animals.

Results: Plasma levels of NO$_3^-$/NO$_2^-$ were similar to those of sham-operated animals during hemorrhage and at the end of resuscitation. One and a half hours after the end of resuscitation, however, NO production increased significantly. The peak levels of plasma NO$_3^-$/NO$_2^-$ occurred at 4 hours, and the levels remained elevated even 24 hours after resuscitation. Tissue NO$_3^-$/NO$_2^-$ levels were significantly increased in the liver, small intestine, and spleen 4 hours after resuscitation. In contrast, the levels of NO$_3^-$/NO$_2^-$ were similar to those of sham-operated animals in the heart and kidneys at all times. Blood flow in the heart was maintained after hemorrhage, whereas hepatic, intestinal, splenic, and renal perfusion decreased significantly.

Conclusions: The gut and liver seem to be the sites responsible for the increased NO production seen after trauma and hemorrhage. The overproduction of NO is most likely caused by up-regulation of inducible NO synthase. Thus, attempts to reduce NO production using specific inhibitors for inducible NO synthase might be helpful for improving hepatic and intestinal functions after trauma and hemorrhagic shock.

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SEVERE HEMORRHAGIC shock is characterized by circulatory failure with hypotension and loss of vascular reactivity to a-drenergic agonists. This leads to tissue damage, multiple organ failure (MOF), and finally death. However, the precise mechanism underlying cardiovascular dysfunction and the subsequent MOF remains unknown. Based on a variety of studies, overproduction of nitric oxide (NO) caused by activation of the L-arginine–NO synthase (NOS) pathway is involved in the sequence of events leading to MOF after hemorrhagic shock. Cuzzocrea et al suggest that overproduction of NO may be responsible for the occurrence of MOF after zymosan-induced peritonitis. Two types of NOS have been characterized. Under physiologic conditions, NO release from vascular endothelial cells (through constitutive NOS [cNOS]) regulates vascular tone, blood pressure, and tissue perfusion. After various pathophysiological conditions such as prolonged hemorrhage and sepsis, however, an inducible form of NOS (iNOS) may produce a large amount of NO, which has been implicated in producing cell and organ dysfunctions seen in those circumstances. It has been shown that tumor necrosis factor and interleukin 1 (IL-1) can stimulate iNOS expression, leading to an overproduction of NO in vascular smooth muscle cells, endothelial cells, and macrophages in vitro and in various organs in vivo. Moreover, proinflammatory cytokines such as tumor necrosis factor, IL-1, and IL-6 were elevated after trauma-hemorrhage as early as 45 minutes after the induction of hypotension. These cytokines are involved in pathophysiological responses after trauma-
hemorrhage, possibly through up-regulation of NO production. Although inadequate perfusion has been postulated to produce cell and organ dysfunctions and although splanchnic circulation is thought to be at particularly high risk because of its tendency to be selectively hypoperfused under the shock state, it remains unknown whether altered tissue perfusion after hemorrhage has any effects on NO production. The aim of this study was to determine whether NO production is up-regulated after trauma-hemorrhage and resuscitation and, if so, which organs contribute to the increased NO production.

RESULTS

PLASMA LEVELS OF NO\textsubscript{3}/NO\textsubscript{2}−

The results shown in Figure 1 indicate that NO production was similar to that of sham-operated animals during hemorrhage and immediately after the completion of fluid resuscitation. One and a half hours after resuscitation, plasma levels of NO\textsubscript{3}/NO\textsubscript{2}− increased significantly compared with sham values (23.9±3.2 vs 2.9±1.0 nmol/mL). The peak NO production occurred 4 hours after the completion of fluid resuscitation (82.2±11.8 vs 4.4±1.3 nmol/mL), and the production of NO remained elevated 8 and 24 hours after resuscitation (19.7±3.4 vs 3.5±1.0 and 22.4±5.2 vs 4.0±1.3 nmol/mL, respectively; Figure 1).

TISSUE LEVELS OF NO\textsubscript{3}/NO\textsubscript{2}−

The results shown in Figure 2 indicate that 4 hours after the completion of fluid resuscitation, NO\textsubscript{3}/NO\textsubscript{2}− concentrations were significantly increased in the liver (63.2±15 vs 19.6±14 nmol/g), small intestine (43±12 vs 8±4 nmol/g), and spleen (38±7 vs 14.4±6.7 nmol/g) compared with sham values. The levels of NO\textsubscript{3}/NO\textsubscript{2}− were similar to sham levels in the heart and kidneys. By multiplying the NO\textsubscript{3}/NO\textsubscript{2}− concentration per gram of tissue with the weight of each organ (indicated in Table 1), we calculated the total content of NO\textsubscript{3}/NO\textsubscript{2}− in each organ to determine which organ contributes to the increased plasma level of NO\textsubscript{3}/NO\textsubscript{2}−. Although NO\textsubscript{3}/NO\textsubscript{2}− concentrations remained significantly increased in the spleen, the results in Figure 3 indicate that the liver (686±138 vs 210±98 nmol) and small intestine (258±91 vs 51±13 nmol) are the major sources of the increased plasma levels of NO\textsubscript{3}/NO\textsubscript{2}−.

ORGAN BLOOD FLOW

The results in Table 2 indicate that blood flow in the liver, kidneys, spleen, and small intestine decrease significantly 4 hours after hemorrhage and fluid resuscitation compared with that of sham-operated animals. The decreased hepatic perfusion was caused by the
through 30-kd molecular weight cutoff filters (Centricon 30, Amicon Inc, Beverly, Mass). Nitrate in the sample was first converted to nitrite by the addition of nitrate reductase. The presence of nitrite was detected by the addition of Griess reagents and was quantitatively measured by analysis with a spectrophotometer (Bio-Tek EL 311 Microplate Autoreader, Bio-Tek, Winooski, Vt) at 550 nm. Tissue samples were homogenized in phosphate-buffered saline solution and centrifuged at 10,000g for 20 minutes. The supernatant was ultracentrifuged at 42,000g for 45 minutes at 4°C in a Beckman Avanti J-25i centrifuge (Beckman Instruments Inc, Fullerton, Calif) and then filtered through a 30-kd molecular weight cutoff filter. The presence of nitrite was then determined as described above.

**BLOOD FLOW DETERMINATION**

In additional groups of animals (n = 8 per group), blood flow in the heart, kidneys, liver, spleen, and small intestine was determined by radioactive microsphere technique. Four hours after the completion of fluid resuscitation, hemorrhaged or sham-operated rats were anesthetized with methoxyflurane, the left carotid artery was cannulated, and the catheter was inserted into the left ventricle by following the left ventricle pulse pressure tracing. The exact position of the catheter in the ventricle was verified at the autopsy. Microspheres labeled with strontium-85 (diameter, 15 µm; specific activity, 4.6 × 10^8 Bq; Du Pont/ NEN, Billerica, Mass) were suspended in 10% dextran solution containing 0.05% polyoxyethylene sorbitan 80 surfactant to prevent aggregation and dispersed with a Vortex shaker for 3 minutes before injection. A 0.3-ml suspension of microspheres was injected into the left ventricle for 20 seconds. A reference blood sample was withdrawn from the femoral artery starting 10 seconds before the onset of microsphere injection and continuing for 90 seconds at a rate of 0.7 mL/min. After the microsphere injection, the left ventricular catheter was flushed with 1 mL of isotonic sodium chloride solution for 60 seconds. Blood pressure was monitored before and after the microsphere injection to ensure that this procedure did not affect mean arterial pressure. At the end of the experiment, the rats were killed with an overdose of pentobarbital sodium. After death, the organs were harvested, rinsed with isotonic sodium chloride solution, blotted, and weighed, and the radioactivity level was counted on a gamma counter (model 1470 Wizard, Wallac, Gaithersburg, Md). The reference sample was transferred to a vial and counted. Organ blood flows (in milliliters per minute per 100 g tissue) were calculated using the following equation:

\[
\text{Organ Blood Flow} = \frac{(\text{RBF} \times \text{Ct})}{\text{Cr}} \times 100,
\]

where RBF is reference blood sample withdrawal rate (0.7 mL/min); Ct, count per minute per gram of tissue; and Cr, count per minute in the reference blood sample. Portal blood flow was calculated as the sum of the blood flow to the stomach, spleen, pancreas, small intestine, large intestine, and mesentery. Total hepatic blood flow was calculated as the sum of portal blood flow and hepatic arterial blood flow.

**STATISTICAL ANALYSIS**

All data are expressed as mean±SEM. Differences between experimental groups were considered to be statistically significant if P<.05 as determined by 1-way analysis of variance and Tukey or unpaired Student t tests.

The only known endogenous source of NO \textsuperscript{3}−/NO \textsuperscript{2}− in mammalian tissues is through the conversion of L-arginine to NO by NOS. Nitric oxide degrades to NO \textsuperscript{2}−,

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**Figure 1.** Alterations in plasma nitrate/nitrite levels after trauma-hemorrhage and fluid resuscitation. Plasma levels of nitrate/nitrite were determined at maximum bleedout (MB); at the end of hemorrhage (EH); at the end of resuscitation (ER); and 1.5, 4, 8, and 24 hours after the completion of fluid resuscitation. There were 8 to 10 animals in each group at each time point. Data are represented as mean±SEM and compared by 1-way analysis of variance and Tukey tests. Asterisk indicates P<.05 compared with sham-operated animals.

**Figure 2.** Alterations in tissue nitrate/nitrite levels 4 hours after the completion of trauma-hemorrhage and fluid resuscitation. There were 8 to 10 animals in each group at each time point. Data are represented as mean±SEM and compared by unpaired Student t test. Asterisk indicates P<.05 compared with sham-operated animals.
which is then converted to NO3− when it reacts with hemoglobin.15,16 In this study, we examined circulating and tissue levels of NO3−/NO2−, the stable end products of NO, to estimate NO production after trauma-hemorrhage and resuscitation. Two types of NOS have been identified, the constitutive form and the inducible form. Through the constitutive pathway (ie, via the activation of cNOS), a small amount of NO is generated by vascular endothelial cells in a regulated manner, which seems to play a critical role in the control of local perfusion and blood pressure.17,18 Previous studies1 demonstrated a significant increase in the plasma level of NO3−/NO2− within the first 2 hours of onset of hemorrhage, and it has been postulated that the early increase of NO after hemorrhagic shock might be caused by activation of cNOS. However, this issue remains controversial because several authors2,3,19-21 reported an impairment in the release of NO from endothelial cells after hemorrhage and resuscitation. Results of studies by Thiemermann et al1 indicate that approximately 4 hours after the onset of hemorrhage, there was increased iNOS messenger RNA (mRNA) expression in the aorta, lung, liver, and spleen and that aortic segments isolated from rats after hemorrhage were associated with hyporeactivity to adrenergic agonists and increased iNOS activity in vitro. Kelly et al,22 using a preheparinized model of severe hemorrhagic shock without subsequent resuscitation, showed that iNOS expression increased in the lungs and liver 5 hours after the onset of hemorrhage. Because the intestine was not examined for the expression of iNOS mRNA in that study,22 it remains unclear whether the intestine is also involved in producing NO after hemorrhagic shock. Although the previously mentioned studies indicate that iNOS mRNA expression is increased in certain tissues after hemorrhage, such studies did not examine whether tissue levels of NO3−/NO2− also increased under those conditions. The aim of our study, therefore, was to determine not only the time course of NO production but also whether the peak production of NO was associated with increased tissue levels of NO3−/NO2−.

A large amount of NO can be produced by cells via the inducible pathway. Inducible NOS has been identified in macrophages, endothelial cells, and several other cell populations, such as vascular and nonvascular smooth muscle cells, cardiomyocytes, and fibroblasts. Expression of iNOS can be stimulated by bacterial products such as endotoxin or by various proinflammatory cytokines such as interferon gamma, IL-1, and tumor necrosis factor.10,18,19 Because vascular decompensation occurs because of increased NO production, NO released from iNOS might be involved in the delayed peripheral vascular failure seen after severe hemorrhagic shock.1,23

Our results demonstrated an increase in plasma levels of NO3−/NO2− starting 1.5 hours after the completion of fluid resuscitation (ie, approximately 4 hours after the onset of hemorrhagic shock). The peak NO production occurred 4 hours after the completion of fluid resuscitation (ie, approximately 6.5 hours after the onset of hemorrhagic shock) and remained elevated at 24 hours. No change in plasma levels of NO3−/NO2− was detected during hemorrhagic shock or immediately after the completion of resuscitation. Results of previous studies19,20 have demonstrated an early endothelial cell dysfunction (ie, reduced release of NO from vascular cNOS) after trauma-

Table 1. Wet Weight of Various Organs After Sham Operation or Hemorrhage and Resuscitation

<table>
<thead>
<tr>
<th>Organ</th>
<th>Wet Weight, g</th>
<th>Sham</th>
<th>Hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>1.01 ± 0.03</td>
<td>1.03 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Kidneys</td>
<td>2.44 ± 0.11</td>
<td>2.47 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>10.71 ± 0.18</td>
<td>10.88 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>6.37 ± 0.21</td>
<td>6.43 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.75 ± 0.03</td>
<td>0.76 ± 0.04</td>
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</tbody>
</table>

* Organ wet weight was determined 4 hours after the completion of fluid resuscitation in hemorrhaged animals and sham-operated animals. Data are presented as mean ± SEM, with 8 to 10 rats in each group. Analysis with unpaired Student t test indicated that there was no significant difference in organ wet weight between the 2 groups of animals.

Table 2. Alterations in Organ Blood Flow After Trauma-Hemorrhage and Resuscitation

<table>
<thead>
<tr>
<th>Organ</th>
<th>Organ Blood Flow, mL/min per 100 g</th>
<th>Sham</th>
<th>Hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>544 ± 63</td>
<td>436 ± 50</td>
<td></td>
</tr>
<tr>
<td>Kidneys</td>
<td>985 ± 60</td>
<td>731 ± 71†</td>
<td></td>
</tr>
<tr>
<td>Hepatic arterial blood flow</td>
<td>22 ± 2</td>
<td>30 ± 5</td>
<td></td>
</tr>
<tr>
<td>Portal blood flow</td>
<td>134 ± 5</td>
<td>90 ± 7†</td>
<td></td>
</tr>
<tr>
<td>Total hepatic blood flow</td>
<td>156 ± 6</td>
<td>121 ± 11†</td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>122 ± 8</td>
<td>81 ± 6†</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>172 ± 27</td>
<td>46 ± 4†</td>
<td></td>
</tr>
</tbody>
</table>

* Organ blood flow was determined by radioactive microsphere technique 4 hours after the completion of trauma-hemorrhage and resuscitation. Results are presented as mean ± SEM, with 8 animals in each group. The results were compared by unpaired Student t test.
†P < .05 compared with sham-operated animals.
hemorrhage and resuscitation. Therefore, considering the delayed but prolonged increase in plasma levels of NO$_3^-$/NO$_2^-$, we suggest that the increase in NO production in the present study may be caused by up-regulation of iNOS. Four hours after the completion of resuscitation, concentrations of NO$_3^-$/NO$_2^-$ were significantly increased in the liver, small intestine, and spleen compared with those of sham-operated animals. In view of the small weight of the spleen (approximately 0.75 g), it is unlikely that this organ was a major source for the increase in plasma NO$_3^-$/NO$_2^-$ levels. In contrast, the liver (approximately 11.00 g) and small intestine (approximately 6.50 g) may represent the major organs responsible for the increased circulating levels of NO$_3^-$/NO$_2^-$ after hemorrhagic shock and resuscitation. Tissue levels of NO$_3^-$/NO$_2^-$ determined 4 hours after the completion of resuscitation only. The reason for selecting this time point was that peak circulating levels of NO$_3^-$/NO$_2^-$ were observed at this interval after hemorrhage and resuscitation. Whether tissue levels of NO$_3^-$/NO$_2^-$ remain markedly elevated at subsequent intervals after hemorrhage and resuscitation requires future investigation.

The precise mechanism and cell populations responsible for the increased NO production after trauma-hemorrhage and resuscitation remain unknown. One possible mechanism could be the decreased perfusion of the small intestine because it has been shown that hypoxia induced by low flow conditions augments proinflammatory cytokine release from the liver and gut. Moreover, preliminary results indicate that splenic peritoneal macrophages from rats subjected to hypoxia express iNOS and release a large amount of NO$_3^-$/NO$_2^-$. Thus, the decreased blood flow in the liver, small intestine, and spleen after hemorrhage, as demonstrated in the present study (Table 2), may induce a local increase of cytokines that could, in turn, up-regulate iNOS expression, leading to a prolonged increase in tissue and plasma levels of NO$_3^-$/NO$_2^-$. Further support for this hypothesis comes from previous studies that demonstrate that plasma levels of tumor necrosis factor significantly increase as early as 45 minutes after the onset of hypotension and remain elevated up to 6 hours after hemorrhage. Therefore, the increased circulating levels of tumor necrosis factor and IL-1 could stimulate iNOS, leading to the increased plasma level of NO$_3^-$/NO$_2^-$. It is thus possible that the enhanced formation of NO might be involved in vascular decompensation, organ injury, and the pathophysiological origin of the systemic inflammatory response. Additional support for this hypothesis comes from the fact that many of the cellular elements of the bowel (endothelium, smooth muscle, macrophages, and enterocytes) are capable of expressing iNOS in vitro. Furthermore, studies have demonstrated up-regulation of iNOS in the gut of rats exposed to infection or proinflammatory cytokines. Similarly, hepatocytes and Kupffer cells represent a large tissue mass with the potential to produce massive amounts of NO after specific stimulation. Further studies are required to determine which cell populations in the liver and small intestine are responsible for the up-regulated NO production after trauma-hemorrhage and resuscitation.

Although studies have shown that alveolar and interstitial macrophages produce NO during acute endotoxemia, and this molecule has been implicated as a mediator of acute lung injury in animal models of endotoxemia, it remains unknown whether the lung plays any significant role in producing NO during and after trauma and hemorrhagic shock. Because the lung was not examined in our study, whether pulmonary tissues are the major source of the elevated levels of NO$_3^-$/NO$_2^-$ under such conditions remains to be determined. In addition, although several studies have suggested that overproduction of NO after various adverse circulatory conditions may be detrimental, studies by Harbrecht et al have indicated that inhibition of NO synthesis by a nonspecific NOS inhibitor after hemorrhagic shock increases hepatic damage. In line with this observation, several studies have demonstrated that acetylcholine-stimulated (ie, endothelium-dependent) vascular relaxation is depressed after hemorrhagic shock. Because acetylcholine-induced relaxation is mediated by endothelium-derived NO (ie, cNOS-derived NO), it seems that reduction of cNOS-derived NO is detrimental. In light of this, we propose that enhancing endothelial cNOS activity and reducing iNOS activity by specific pharmacological agents may be a useful adjunct for the management of traumatized hosts. Tissue levels of NO$_3^-$/NO$_2^-$ may not be the precise marker of local production of NO; further studies are needed to confirm that the increased tissue levels of NO$_3^-$/NO$_2^-$ are solely caused by up-regulation of iNOS by using Western blot analysis, immunohistochemistry, or enzymatic assay.

In summary, our study demonstrates that NO production increased significantly after trauma-hemorrhage and resuscitation. The liver and small intestine seem to be the major sources of the up-regulated NO production by a mechanism that likely involves the up-regulation of iNOS. The increased production of NO might be responsible for the delayed vascular failure and the organ injury seen after hemorrhagic shock.
The present study raises important questions for future research, such as whether attempts to decrease the level of up-regulated NO after trauma hemorrhage would be helpful for improving hepatic and intestinal functions under such conditions.

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REFERENCES


DISCUSSION

Mitchell Fink, MD, Boston, Mass: You are probably quite aware that NO is a remarkably versatile and potent signaling and effector molecule produced by many different kinds of mammalian cells. I want to emphasize that we are speaking about NO, which is actually nitrogen monoxide, one of the simplest stable molecules in nature, and not nitrous oxide, the gas commonly employed as an adjunct to other volatile anesthetics in the operating room.

In the past few years, severe hemorrhage has been shown to induce increased expression of one of the enzymes responsible for NO production, which, as you have heard, is iNOS, the inducible isofrom of nitric oxide synthase. Indeed, it has been proposed by some investigators that so-called irreversible shock following prolonged hemorrhagic hypotension is due to excessive production of the potent vasodilator NO. Other investigators actually have called this notion into question. The purpose of the present study from Dr Chaudry’s group was to determine which organs contribute to increased NO production following hemorrhage and resuscitation in rats. Nitric ox-
circulating nitrite plus nitrate levels increase to very high lev-

mans. For example, following the injection of endotoxin in rats,

different in a structural way from the iNOS promoter in hu-

a hair trigger in rats. The iNOS promoter in rats appears to be
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by infusion of the ion? The ion is nontoxic and that should be

tal animals when you artificially raise circulating nitrate levels

what happens to tissue nitrite and nitrate levels in experimen-

measures of tissue NO synthase activity? By the same token,

ing that tissue nitrite plus nitrate levels actually correlate with

ished washout of NO metabolites that were being produced.

so one wonders whether the findings simply reflect dimin-

in the present study also showed decrements in perfusion, and

fusion. Indeed, the organs showing increased levels of the ions

in the present study also showed decrements in perfusion, and

so one wonders whether the findings simply reflect diminish-

ished washout of NO metabolites that were being produced. 
Thus, I must ask the presenter whether there are any data show-

ing that tissue nitrite plus nitrate levels actually correlate with

measures of tissue NO synthase activity? By the same token,

what happens to tissue nitrite and nitrate levels in experimen-
tal animals when you artificially raise circulating nitrate levels

by infusion of the ion? The ion is nontoxic and that should be

a fairly straightforward experiment.

The second point is that the inducible isoform of NO syn-

thase is very tightly controlled in the human system but is on

a hair trigger in rats. The iNOS promoter in rats appears to be

different in a structural way from the iNOS promoter in hu-

mans. For example, following the injection of endotoxin in rats,
circulating nitrite plus nitrate levels increase to very high lev-

els. In contrast, in septic humans, although nitrite and nitrate

levels are elevated, the changes are much more modest. There-

fore, one is prompted to ask, “Do you think the results in this

present study in rats actually apply to hemorrhage shock in hu-

man beings?”

Dr Catania: To respond to some of the points raised and

some of the questions asked, in terms of Northern and West-

ern analysis of the tissues involved, that work is actually un-

under way as we speak. Unfortunately, the results were too pre-

liminary at the time of the abstract deadline to include them.

Some of our preliminary results do now in fact indicate that

Northern and Western blot show up-regulation of the induc-

ible NO synthase.

In terms of functional production, we were attempting to

look at a measure of functional NO production. It has been

shown in several different cytokine models that sometimes in-

duction of mRNA expression does not necessarily correlate with

functional expression of the active cytokine, and that was why

we chose to look at the nitrite and nitrate. We could have also

employed the cellular lysate (?) preparations and looked for

calcium-dependent and calcium-independent function. We sim-

ply chose what we thought was a quicker and easier method.

In terms of correlation between levels of activity of the en-

zyme and tissue levels, nothing in the literature at this point

shows a correlation. We feel that this is one of the first studies

that actually shows elevated tissue levels of nitrite and nitrate.
I think a very reasonable follow-up study would be to check
iNOS activity and correlate that with tissue levels.

And we also did not infuse the ion and simply check to

see if it accumulates in tissues. One wouldn’t expect that it would
accumulate in different tissues at different levels; however, it

has never actually been shown that is not the case.

And finally, in terms of applicability to the human organ-

ism, NO has been studied quite extensively. In fact, in the last

4 years, there have been over 8000 publications using models

as simple as tissue culture all the way through animal models

and human models. I think at this point in terms of nitric ox-

ide and its effects on hemorrhage and resuscitation, we are still

trying to get a handle on exactly how much of a role it plays,

and this basic science work is just intended to demonstrate that

there are organs that are adversely affected by trauma and hem-

orrhage and NO is present in these organs.