Inhaled Nitric Oxide Prevents Experimental Platelet Activating Factor–Induced Shock

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Objective: To investigate whether inhaled nitric oxide (INO) can prevent platelet activating factor (PAF)—induced pulmonary hypertension and shock.

Design: Randomized controlled animal trial.

Setting: Laboratory.

Subjects: Yorkshire swine.

Interventions: Animals received general anesthesia and invasive hemodynamic monitoring, then PAF only, 2.5 μg/kg intravenously over 45 minutes (PAF group, n=9) or PAF in addition to INO, 20 ppm (PAF-INO group, n=6).

Main Outcomes: Vascular pressures (mean arterial and mean pulmonary), vascular resistance indexes (systemic and pulmonary), cardiac indexes, and oxygen delivery and oxygen consumption.

Results: Mean arterial pressures, cardiac indexes, and oxygen delivery and consumption were significantly higher in the PAF-INO group. Mean pulmonary arterial pressures and systemic and pulmonary vascular resistance indexes were significantly lower in the PAF-INO group. There were 4 deaths (44%) in the PAF group vs none (0%) in the PAF-INO group (P=.10).

Conclusion: The use of INO prevents pulmonary hypertension, circulatory failure, and death during PAF-induced shock.

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Despite major advances in critical care and antimicrobial therapy, septic shock remains an entity with high morbidity and mortality rates. In humans, septic shock is characterized by an early hypodynamic phase, in which cardiac output is elevated and systemic vascular resistance (SVR) is depressed. During refractory sepsis, a hypodynamic phase ensues and is characterized by systemic hypotension, pulmonary hypertension, depressed cardiac function, and progressive multiplet system organ failure. Patients who reach this terminal phase of septic shock commonly succumb to their illness.

Platelet activating factor (PAF) has been shown to be a critical mediator in septic shock and multiple-system organ failure.1-3 In swine, PAF infusion results in hypodynamic shock with significant pulmonary hypertension.4 This model reproduces the right ventricular dysfunction seen in human sepsis.5-10 If the pulmonary hypertension component contributes significantly to this form of shock, resolution of the pulmonary hypertension should lead to improved hemodynamic status and perfusion. In this study, a group of swine in PAF-induced shock were treated with the selective pulmonary vasodilator and inhaled nitric oxide (INO) and compared with an untreated group in similar shock.

Results: Four animals died in the PAF group (44% mortality) vs none in the PAF-INO group (P=.10). Three of the four animals died within 15 minutes of beginning the PAF infusion with an acute cor pulmonale and cardiogenic shock picture (low MAP, low cardiac index, high CVP, and high PVRI). The fourth animal died after progressive decline in MAP and cardiac index and persistent elevation in PVRI 90 minutes af-

See Materials and Methods on next page
MATERIALS AND METHODS

ANIMAL PREPARATION

Fifteen adolescent Yorkshire swine weighing 17 to 22 kg were used. The animals were first examined by a veterinarian and housed for 2 days prior to experimentation to exclude underlying illness or infection. On the day of experimentation, the animals were first sedated with intramuscular atropine (0.04 mg/kg), xylazine (3.5 mg/kg), and ketamine (12.5 mg/kg) prior to halothane induction and endotracheal intubation. A 12F temperature probe (Hewlett-Packard 21090A, Hewlett-Packard, Andover, Mass) was inserted in the rectum, a veterinary pulse oximeter probe (Nellcor Inc, Hayward, Calif) was placed on the tongue or snout, and electrocardiogram limb leads were affixed. Under continuous halothane anesthesia (0.8%-1%), a cutdown on the right external jugular vein allowed placement of a double-lumen 7F central venous catheter (Cook Inc, Bloomington, Ind) in the superior vena cava. A continuous infusion of normal saline (4 mL/kg per hour) was then started and continued for the duration of the experiment. A femoral cutdown was then performed. A femoral artery catheter was placed for continuous systemic blood pressure monitoring and arterial blood gas analyses. A 6F or 7F pulmonary artery catheter (American Edwards Laboratories, Irvine, Calif) was introduced through the femoral vein. Through a 4-cm vertical paramedian left lower-quadrant incision, the urinary bladder was identified and catheterized with a Foley catheter secured with a purse string suture. This completed the surgical procedure.

The animal was then transferred to volume-controlled ventilation (Servo 900C, Siemens-Elema, Solna, Sweden) and ventilation parameters set as follows: fractional inspired oxygen, ie, FiO2, 0.25; respiratory rate, 12; tidal volume, 10 to 12 mL/kg to maintain PaCO2 at 35 to 45 mm Hg. Continuous balanced anesthesia was maintained with halothane (0.8%-1%) and pancuronium (0.2 mg/kg per hour). Animals were allowed a 1-hour stabilization period before being randomized to the protocol described below. At the termination of the experiment, animals that survived were killed with intravenous injection of pentobarbital sodium (120 mg/kg). The protocol was approved by the Childrens Hospital, Los Angeles, Animal Care Committee and adhered to the National Institutes of Health guidelines on the care and handling of experimental animals.

EXPERIMENTAL PROTOCOL

After baseline hemodynamic parameters were recorded (T0), animals were randomized to 1 of 2 groups. The first group (PAF group, n=9) received a PAF bolus (1-α-phosphatidylycholine-β-acetylcholine-γ-O-alkyl] in chloroform solution (Sigma Chemical, St Louis, Mo) at a dose of 2.5 µg/kg over 45 minutes. The second group received the same dose of PAF in addition to inhaled nitric oxide (INO) (Gilmore Liquid Air, San Gabriel, Calif) at a dose of 20 ppm, starting simultaneously with the PAF infusion and continuing for 2 hours (PAF-INO group, n=6). Animals were followed up for 3 hours after the PAF infusion started. The solution was stored at −10°C. Prior to each experiment, 500 µg of PAF was mixed with 25 mL of normal saline to produce PAF at a 20-µg/mL solution and allowed to reach room temperature. This solution was then administered as a bolus at a rate of 2.5 µg/kg per hour for 30 minutes, followed by

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Platelet activating factor is a biologically active phospholipid found as an inactive precursor in the plasma membranes of immune and endothelial cells. Ample investigative evidence has implicated PAF as a critical, and perhaps the most potent, mediator of septic shock. Acute circulatory collapse, characterized by increased oxygen extraction, metabolic acidosis, and death, was seen in dogs after PAF infusion. In the same animal, Yamanaka et al showed a biphasic hypotensive response to PAF. The first phase was characterized by decreased SVR and increased cardiac output, while the second phase showed the reverse hemodynamic profile, in addition to increased PVR. This is very similar to the biphasic hemodynamic profile seen in human sepsis. Immune and endothelial cells stimulated by tumor necro-

ter the start of the PAF infusion. The potency of the PAF infusion and the early high mortality rate necessitated starting the INO simultaneously with the PAF infusion in the PAF-INO group.

Data for all continuous variables are reported as mean±SEM. Table 1 shows the hemodynamic parameters of the 2 groups at baseline (T0) and at each subsequent experimental hour (T1, T2, and T3). In all animals, PAF was given as a bolus over 45 minutes starting at T0. In group 2, INO was started at T0 and discontinued immediately after T2 values were recorded. There were no significant differences in CVP or PCWP at any time point between the 2 groups.

Table 2 shows the oxygenation and perfusion values of the 2 groups at the same time points. There was no significant difference in weight between the 2 groups (PAF group, 18.84±8.2 kg; PAF-INO group, 19.33±7.0 kg; P=.66). In this model, PAF infusion did not result in significant leukocytosis, hemoconcentration, acidosis, or hyperglycemia. There were no significant differences between the 2 groups with respect to the initial or final white blood cell count, hemoglobin concentration, hematocrit index, or platelet count. There were also no significant differences between the 2 groups with respect to the final levels of total CO2, glucose, blood urea nitrogen, or creatinine. Inhaled NO therapy did not result in significant methemoglobinemia (PAF group, 1.24%±0.04% of total hemoglobin; PAF-INO group, 1.17%±0.15% of total hemoglobin; P=.66). During INO therapy, NO2 levels did not rise over 1 ppm.

COMMENT

The last decade has witnessed a flurry of research into the mechanisms of septic shock and potential therapies. Most recently, the roles of PAF and NO have attracted significant attention because of the possibility of immediate therapeutic interventions based on blocking the action or production of these 2 substances.

Platelet activating factor is a biologically active phospholipid found as an inactive precursor in the plasma membranes of immune and endothelial cells. Ample investigative evidence has implicated PAF as a critical, and perhaps the most potent, mediator of septic shock. Acute circulatory collapse, characterized by increased oxygen extraction, metabolic acidosis, and death, was seen in dogs after PAF infusion. In the same animal, Yamanaka et al showed a biphasic hypotensive response to PAF. The first phase was characterized by decreased SVR and increased cardiac output, while the second phase showed the reverse hemodynamic profile, in addition to increased PVR. This is very similar to the biphasic hemodynamic profile seen in human sepsis. Immune and endothelial cells stimulated by tumor necro-
5.0 μg/kg per hour for 15 minutes. This total dose and rate of infusion were found to approximate the median lethal dose (LD₅₀) for PAF during pilot experiments to establish our model.

MONITORING

The weight of each animal (in kilograms) was recorded just prior to experimentation. During the course of the experiment, electrocardiogram, heart rate, mean arterial pressure (MAP), mean pulmonary arterial pressure (MPAP), central venous pressure (CVP), and temperature were continuously monitored by a temperature and pressure bedside monitor (Hewlett-Packard 78534C) and recorded at baseline and every 30 minutes. Pulmonary capillary wedge pressure (PCWP) and thermodilution cardiac output were measured and recorded at baseline and every 30 minutes by computer (9520A Cardiac Output Computer, American Edwards Laboratories). Systemic (SVR) and pulmonary vascular resistance (PVR) indexes (SVRI and PVRI, respectively) were calculated by the following formulas:

(1) \[ SVRI = \frac{\text{[MAP} - \text{CVP}] \times 80}{\text{(Cardiac Output)}/\text{Weight}} \]

and

(2) \[ PVRI = \frac{\text{[MPAP} - \text{PCWP}] \times 80}{\text{(Cardiac Output)}/\text{Weight}} \]

Hemoglobin saturation with oxygen was continuously monitored by pulse oximeter (model N-100, Nellcor Inc) and recorded at baseline and every 30 minutes. Arterial and mixed venous blood gases were measured at baseline and every hour with a blood analyzer (model 170 pH/Blood Gas Analyzer, Corning, Mayfield, Mass). Oxygen delivery, oxygen consumption, and extraction ratio were calculated by standard formulas at baseline and every hour. Hourly urine output was recorded at baseline and for the duration of the experiment. During INO therapy, NO and nitric oxide (NO) were continuously monitored on line by an electrochemical analyzer (Pulmonary II, Pulmonox, Tofield, Alberta).

A complete blood cell count was obtained at baseline and at the end of each experiment; Na⁺, K⁺, Cl⁻, total CO₂, glucose, blood urea nitrogen, creatinine, and methemoglobin levels were obtained at the end of each experiment.

STATISTICAL ANALYSIS

The differences in continuous outcome variables were compared between the PAF and PAF-INO groups at baseline and at each subsequent hour, using a combination of the F test and Student t test. The F test was used for any significant difference in the variance of the outcome variable between the 2 groups. A Student t test, assuming either equal or unequal variance, was then used to test the difference in the outcome variable itself between the 2 groups. Data from animals that did not survive the experiment were excluded from the above analysis. The Fisher exact test was used to test the difference in the mortality rates between the groups. A P value of ≤ .05 was considered statistically significant. All analyses were performed using Microsoft Excel 3.0 Analysis Tools (Microsoft Inc, Hayward) and were reviewed by an independent statistician.
citation and required catecholamine therapy. Right ventricular dysfunction during sepsis has various causes, pulmonary hypertension being a leading one. A right ventricle that is unable to pump blood against an increased afterload dilates acutely and fails. This acute cor pulmonale picture quickly leads to left ventricular failure by severely reducing left ventricular preload and restricting left ventricular pumping action through intrapericardial pressure effects. Pulmonary hypertension may also increase ventilation-perfusion mismatch and the shunt fraction, leading to hypoxia.

Our septic model using PAF infusion was characterized by systemic hypotension, pulmonary hypertension, decreased cardiac output, increased SVR, decreased oxygen delivery and consumption, and high mortality. These are all stigmata of the late or hypodynamic phase of human septic shock, making this model very relevant to that clinical situation. Therapeutic intervention with selective pulmonary vasodilation using PAF-INO dramatically reduced PVR, in turn resulting in an improved systemic hemodynamic profile and perfusion.

The improvement in cardiac index lasted beyond the PAF-INO treatment period. Death resulting from acute, fulminant cor pulmonale was also prevented by PAF-INO. In our model, severe right ventricular dysfunction, rather than

### Table 1. Systemic and Pulmonary Hemodynamic Outcomes

<table>
<thead>
<tr>
<th>Hemodynamic Parameter/Experimental Group†</th>
<th>Time Points, h</th>
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<tbody>
<tr>
<td></td>
<td>T₀</td>
</tr>
<tr>
<td>CI, ml-min⁻¹·kg⁻¹</td>
<td></td>
</tr>
<tr>
<td>PAF</td>
<td>81.6±4.7</td>
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<tr>
<td>PAF-INO</td>
<td>83.6±3.9</td>
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<tr>
<td>MAP, mm Hg</td>
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<tr>
<td>PAF</td>
<td>67±4</td>
</tr>
<tr>
<td>PAF-INO</td>
<td>58±2</td>
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<tr>
<td>SVRI, dyn·sec·cm⁻²·kg⁻¹</td>
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<tr>
<td>PAF</td>
<td>172±7</td>
</tr>
<tr>
<td>PAF-INO</td>
<td>136±13</td>
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<tr>
<td>MPAP, mm Hg</td>
<td></td>
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<tr>
<td>PAF</td>
<td>15.0±0.4</td>
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<tr>
<td>PAF-INO</td>
<td>17.0±0.7</td>
</tr>
<tr>
<td>PVRI, dyn·sec·cm⁻²·kg⁻¹</td>
<td></td>
</tr>
<tr>
<td>PAF</td>
<td>17.0±2.2</td>
</tr>
<tr>
<td>PAF-INO</td>
<td>16.0±1.2</td>
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<tr>
<td>HR, beats/min</td>
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</tr>
<tr>
<td>PAF</td>
<td>74±2</td>
</tr>
<tr>
<td>PAF-INO</td>
<td>97±1</td>
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</tbody>
</table>

*CI indicates cardiac index; PAF, platelet activating factor; INO, inhaled nitric oxide; MAP, mean arterial pressure; SVRI, systemic vascular resistance index; MPAP, mean pulmonary arterial pressure; PVRI, pulmonary vascular resistance index; and HR, heart rate. Values are mean±SEM.
†PAF group (n=9) includes only animals that survived (n=5); PAF-INO group (n=6).
‡P<.05 compared with PAF group.

### Table 2. Oxygenation and Perfusion Outcomes

<table>
<thead>
<tr>
<th>Oxygenation and Perfusion Parameter/Experimental Group†</th>
<th>Time Points, h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₀</td>
</tr>
<tr>
<td>Pao₂, mm Hg</td>
<td></td>
</tr>
<tr>
<td>PAF</td>
<td>107±6</td>
</tr>
<tr>
<td>PAF-INO</td>
<td>113±3</td>
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<tr>
<td>DO₂, mL O₂·min⁻¹</td>
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<tr>
<td>PAF</td>
<td>200±11</td>
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<tr>
<td>PAF-INO</td>
<td>208±18</td>
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<tr>
<td>VO₂, mL O₂·min⁻¹</td>
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<tr>
<td>PAF</td>
<td>80±16</td>
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<tr>
<td>PAF-INO</td>
<td>77±6</td>
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<tr>
<td>Extraction ratio, %</td>
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<tr>
<td>PAF</td>
<td>40±7</td>
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<tr>
<td>PAF-INO</td>
<td>38±5</td>
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<tr>
<td>Urose, output, mL·kg⁻¹·h⁻¹</td>
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</tr>
<tr>
<td>PAF</td>
<td>1.72±0.18</td>
</tr>
<tr>
<td>PAF-INO</td>
<td>1.47±0.11</td>
</tr>
</tbody>
</table>

*PAF indicates platelet activating factor; INO, inhaled nitric oxide; DO₂, oxygen delivery; and VO₂, oxygen consumption. Values are mean±SEM.
†PAF group (n=9) includes only animals that survived (n=5); PAF-INO group (n=6).
‡P<.05 compared with PAF group.
enhanced NO production, appears to be the critical cause of shock and death secondary to PAF infusion. This finding, in combination with the previous studies mentioned above, would indicate that the hypotensive mechanisms of PAF may be species dependent. We have previously shown that swine in severe cardiogenic shock secondary to acute hypoxic cor pulmonale can be resuscitated with INO as a sole agent. Recent studies showing therapeutic hemodynamic benefits of INO in other gram-positive and gram-negative septic shock models are also in concert with the results reported here.

Therefore, measures to prevent hyperproduction of NO may be of benefit during early or hyperdynamic sepsis, as long as the systemic vasopressor effect is not offset by significant pulmonary vasoconstriction. In late or hypodynamic sepsis, diagnosing and treating pulmonary hypertension and right ventricular dysfunction may be the key to improving hemodynamic profile, oxygenation, and prognosis.

The clinical correlate of the findings reported here is obvious. Patients suffering from hypodynamic septic shock with significant pulmonary hypertension may benefit from INO. Randomized controlled human trials will be needed to define this patient population and elucidate any possible therapeutic benefits.

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Statistical assistance was provided by Linda S. Chan, PhD, Research and Biostatistics Division, Departments of Pediatrics and Emergency Medicine, Los Angeles County–University of Southern California Medical Center.

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REFERENCES


DISCUSSION

Robert S. Sawin, MD, Seattle, Wash: This group has used the model of septic shock, which represents a very severe challenge to these animals with a 44% mortality rate, apparently due to acute cor pulmonale, a nearly 50% reduction in cardiac index, a 100% increase in pulmonary artery pressure, and a 10-fold increase in pulmonary vascular resistance. These deleterious effects were nearly eliminated with the use of inhaled nitric oxide, and all of the animals treated with nitric oxide survived. You mentioned the biphasic nature of the septic shock, the first phase, warm or hyperdynamic shock, and the second phase, cold, hypodynamic shock. It would appear that the model...
you used with the PAF infusion is replicating only the physiology of the hypodynamic shock. What is known about endogenously PAF levels in serum or pulmonary vasculature during sepsis, that is, is the PAF activity a biphasic response or is the response to an increased PAF level a biphasic reaction? As an extension of that, why didn’t the animals in your study demonstrate a hyperdynamic phase prior to the profound hypodynamic shock?

Your results suggest that the mortality associated with the PAF-induced shock is predominantly related to pulmonary hypertension, which can be completely overcome by inhaled nitric oxide. You mentioned in the manuscript paper that there is a lot of conflicting data in the literature regarding the role of NO in the hyperdynamic phase of shock. If NO is going to be used clinically for the treatment of septic shock, will the timing of NO inhalation be crucial, that is, if we use it early in the hyperdynamic phase of shock, would it be detrimental, or would you predict that it will prevent the progression to hypodynamic physiology?

Over the past several years much of us in pediatrics have been excited about the use of NO for treating pulmonary hypertension, especially in neonates. But our experience at Children’s Hospital in Seattle with this treatment has been somewhat sobering. Over the first several hours, many of the children have an excellent response to nitric oxide, but eventually they seem to develop a tachyphylaxis requiring higher doses and eventually they become unresponsive to that treatment. Have you repeated these sorts of studies in these animals for a longer time to see if the therapeutic response to NO can be maintained? This paper suggests that nitric oxide may well become a portion of our armamentarium against sepsis, and I look forward to hearing about more work from your group.

Kenneth Waxman, MD, Orange, Calif: To suggest a clinical trial is quite premature because the actions of nitric oxide and of PAF are much more complicated than just pulmonary vasconstriction and vasodilatation. Both have many other effects. For example, both nitric oxide and PAF effect WBC activation. The effects of inhaled nitric oxide on leukocyte activation are not well understood; the effects of this in sepsis are even less predictable.

H. Atik, MD, Idyllwild, Calif: My understanding of the beneficial effects of nitric oxide is 2-fold. One is vasodilatation, as the author described, through the endothelial system and relaxation of smooth muscle. The other one is the inhibition of platelet release and platelet aggregation. I wonder if the authors would make any comments whether or not they have studied platelet function and whether there is any justification for trying other antiplatelet agents in their model.

James W. Holcroft, MD, Sacramento, Calif: Inhaled nitric oxide blocks hypoxic pulmonary vasconstriction. On the one hand that could well be good, as the authors pointed out, because it would lessen the hindrance that the pulmonary vasculature imposes on the right ventricle and thereby allow the cardiovascular system to respond favorably to the septic insult. But, on the other hand, hypoxic pulmonary vasconstriction has been with us for many millions of years as a key defensive process in any ill patient in responding to collapse or flooding of alveoli. Have the authors measured shunt fractions or alveolar arterial O2 gradients or PaO2/FIO2 indices to see if they changed in response to the NO administration?

John K. MacFarlane, MD, Vancouver, British Columbia: Perhaps I missed it, but when exactly was the nitric oxide delivered? When was delivered at the same time as the insult or shortly thereafter? If you have information on when it becomes effective or perhaps when it is ineffective could you share it with us?

Dr Atkinson: Dr Sawin, you asked about the clinical measurement of platelet activating factor in these patients who are undergoing late phases of sepsis, and I think it is a biphasic response. There is an early peak of PAF in patients who are septic, which then rapidly declines with the metabolism of the product, and then there is a sustained elevation that persists through the later phases of shock. That is important in this model. In this model, we are using pharmacological doses of platelet activating factor and not levels that you would commonly see in vivo.

As far as the use and timing of the nitric oxide administration in the hyperdynamic early phases of shock vs the later hypodynamic phases, it is unlikely that nitric oxide−induced pulmonary vasodilatation would be effective in the hypodynamic phase in preventing progression. It is of interest though that it may be useful to use nitric oxide in combination with very powerful vaspressors such as a N-AME CN-arginine methyl chloride, which is a product that is a nitric oxide inhibitor which can’t be used because it causes such hypoxia from pulmonary vasoconstriction, but if you combine that with inhaled nitric oxide, it might be a very powerful agent to use early in the vasodilatory phases of shock.

I share your experience with newborn application of nitric oxide. It has not been as useful as we had hoped that it would be for patients who have just pulmonary hypertension and not necessarily the same as in this discussion with sepsis and all of the other factors that are going on at that time.

Dr Waxman pointed out that a clinical trial may be premature. That is very true. Many of the agents that we test as new agents for treatment of complex diseases suffer from this same problem: that there are many factors that may be going on that we do not understand. There may be many effects of the drug that we are going to trial that may not be apparent at the time that we initiate that trial. But there is on the horizon a time when we might want to apply nitric oxide to the treatment of sepsis, particularly in patients in whom we can demonstrate a proven pulmonary hypertension and right heart failure component.

Dr Atik has been one of my mentors and has long guided me in thinking about platelets and platelet aggregation. In this particular study, we did measure the platelet counts and those did not decline. We did not have any formal platelet aggregation studies or any other more sophisticated measures of platelet activity.

Dr Holcroft I think correctly pointed out that there may be adverse effects of nitric oxide blocking the alveolar hypoxic response in the lungs, and that those may have a detrimental effect on the patients’ ability to oxygenate. That is a valid consideration. We did not measure any changes in the VQ fractions in these animals. It would be something that might be useful to look at in the future, particularly if we were using a lung injury type of model of sepsis.

Dr MacFarlane asked an important question about the timing of the nitric oxide delivery. The nitric oxide was started at the same time as the platelet activating factor was infused. This was necessitated by the severity of this insult and the fact that the pigs would probably not survive to receive the nitric oxide if we didn’t initiate it at the time of the infusion.

That leaves open a clinical question about what are the consequences of this study as far as being able to use nitric oxide therapeutically. Will it work when it is somewhat after the fact, after the patient produces the PAF as a cytokine that produces these syndromes? That question would need to be addressed in subsequent studies.