

Blockade of the action of nitric oxide in human septic shock increases systemic vascular resistance and has detrimental effects on pulmonary function after a short infusion of methylene blue

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Abstract

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To investigate the role of nitric oxide in human sepsis, ten patients with severe septic shock requiring vasoactive drug therapy and mechanical ventilation were enrolled in a prospective, open, non-randomized clinical trial to study the acute effects of methylene blue, an inhibitor of guanylate cyclase. Hemodynamic and metabolic variables were measured before and 20, 40, 60, and 120 min after the start of a 1-h intravenous infusion of 4 mg/kg of methylene blue. Methylene blue administration caused a progressive increase in mean arterial pressure (60 [55-70] to 70 [65-100] mmHg, median [25-75th percentiles]; $P < 0.05$), systemic vascular resistance index (649 [479-1084] to 1066 [585-1356] $\text{dyne s}^{-1} \text{cm}^{-5} \text{m}^{-2}$; $P < 0.05$) and the left ventricular stroke work index (35 [27-47] to 38 [32-56] $\text{g m}^{-1} \text{m}^{-2}$; $P < 0.05$) from baseline to 60 min. The pulmonary vascular resistance index increased from 150 [83-207] to 186 [121-367] $\text{dyne s}^{-1} \text{cm}^{-5} \text{m}^{-2}$ after 20 min ($P < 0.05$). Mixed venous saturation decreased from 65 [56-76] to 63 [55-69]% ($P < 0.05$) after 60 min. The $\text{PaO}_2/\text{FiO}_2$ ratio decreased from 168 [131-215] to 132 [109-156] mmHg ($P < 0.05$) after 40 min. Arterial lactate concentration decreased from 5.1 ± 2.9 to 4.5 ± 2.1 mmol/l, mean \pm SD ($P < 0.05$) after 60 min. Heart rate, cardiac filling pressures, cardiac output, oxygen delivery and consumption did not change. Methylene blue administration was safe and no adverse effect was observed. In severe human septic shock, a short infusion of methylene blue increases systemic vascular resistance and may improve myocardial function. Although there was a reduction in blood lactate concentration, this was not explained by an improvement in tissue oxygenation, since overall oxygen availability did not change. However, there was a significant increase in pulmonary vascular tone and a deterioration in gas exchange. Further studies are needed to demonstrate if nitric oxide blockade with methylene blue can be safe for patients with septic shock and, particularly, if it has an effect on pulmonary function.

Key words

- Vasodilation
- Myocardial depression
- Septic shock
- Methylene blue
- Nitric oxide
- Guanylate cyclase

Introduction

The mortality rate from septic shock remains unacceptably high despite the progress in medicine over the last decades. Human septic shock is characterized by a profound arterial vasodilation with a decreased vascular reactivity to catecholamines and a high cardiac output despite the presence of myocardial depression (1). The failure to restore arterial pressure after fluid administration and the use of vasoactive drugs in the first 24 h is related to a poor outcome (1,2).

There are several lines of evidence indicating that an excessive liberation of nitric oxide is implicated in these hemodynamic alterations (3-6). *In vitro*, the exposure to endotoxin and several cytokines induces several cells to produce nitric oxide (7-10). Nitric oxide may also mediate the sepsis-induced myocardial depression (11-13). In experimental studies, the decreased response to vasopressors is associated with an increased nitric oxide production (14-16). The hemodynamic changes seen in septic shock patients may also be related to overproduction of nitric oxide (17,18). Blood concentrations of nitrites/nitrates, the stable by-products of nitric oxide, are increased in severely septic patients (19,20). The serum concentration of L-arginine, a precursor of nitric oxide, is reduced and its administration provokes vasodilation in these patients (17).

The inhibition of nitric oxide synthase in septic animals or patients prevents or corrects hypotension (3,4,19,21,22). However, a blockade of such magnitude may be deleterious. The cardiac output decreased in several studies due to an excessive systemic vasoconstriction (17,22) and the worsening in hemodynamic status increased the mortality in animal studies (5,6). In addition, pulmonary vascular resistance is often increased during sepsis and a further increase in pulmonary arterial pressure by nitric oxide synthesis inhibitors could precipitate right heart

failure. Moreover, nitric oxide has several potential beneficial effects: a) it plays a role in host defense against bacteria, and b) seems to play a role in keeping adequate blood flow and cellular function in the hepatosplanchnic and renal systems (23,24). Nevertheless, these studies raise concern about the safety of nitric oxide synthase inhibition. On the other hand, methylene blue, an inhibitor of soluble guanylate cyclase, may be a safer option by suppressing the action of nitric oxide without major side effects. In experimental studies, methylene blue attenuated vasodilation and myocardial dysfunction and increased mesenteric blood flow (11,14,25). Three studies on septic shock patients have reported an increase in blood pressure and in myocardial function after a bolus infusion of methylene blue without acute toxicity (26-28). However, Gachot et al. (27) reported a reduction in the arterial oxygen tension/inspired oxygen concentration ($\text{PaO}_2/\text{FiO}_2$) ratio and an increase in arterial carbon dioxide tension (PaCO_2), and these side effects raise concern about the safety of methylene blue administration in patients with lung injury.

The aim of the present study was to examine the role of nitric oxide in human septic shock. We investigated the acute effects of a 1-h infusion of 4 mg/kg of methylene blue on cardiovascular performance and gas exchange.

Material and Methods

Patients

Informed consent was obtained from each patient's next-of-kin after explanation of the purpose of the study, which was approved by the hospital Ethics Committee. We prospectively studied 10 adult patients with severe septic shock (Table 1). Sepsis was defined on the basis of at least three of the following criteria: a) temperature of $>38^\circ$ or $<36^\circ\text{C}$; b) white blood cell count of $>12,000$ cells/ mm^3 , $<4,000$ cells/ mm^3 or $>10\%$ of immature forms;

c) heart rate of >90 beats/min; d) respiratory rate of >20 breaths/min or mechanical ventilation. The source of infection had to be documented by clinical and/or radiographic features and be confirmed by local positive cultures and/or blood cultures in all patients. Shock was defined by hypotension (systolic blood pressure <90 mmHg or a decrease >40 mmHg) despite adequate fluid administration (pulmonary artery occlusive pressure (PAoP) >12 mmHg) along with signs of hypoperfusion (lactic acidosis, oliguria or an acute alteration in mental status); therefore, adrenergic support was always required. Patients were treated according to our Intensive Care Unit (ICU) standard treatment protocol. Each patient was mechanically ventilated and had an arterial line and a 7-Fr pulmonary artery balloon-tip catheter (Swan-Ganz Catheter, Baxter Edwards/Spectramed, Deerfield, IL, USA).

Pregnant patients, resuscitated patients and patients whose treatment was modified during the 2-h observation period, except for vasoconstrictor drugs, were excluded from the study.

Measurements and study protocol

Arterial pressure, pulmonary arterial pressure, pulmonary artery occlusion pressure, right atrial pressure, cardiac output (Bese,

Belo Horizonte, MG, Brazil), hemoglobin and methemoglobin (Technicon H.3 RTX, Bayer, Leverkusen, Germany), and arterial and mixed venous blood gases (gas analyzer 278, Ciba-Corning, San Diego, CA, USA) were obtained at baseline, and 20, 40, 60, and 120 min later. Arterial blood lactate concentration (normal, <2.2 mmol/l) was determined by an enzymatic technique (Cobas Mira Plus, Roche, Indianapolis, IN, USA) at baseline, and 60 and 120 min later. Cardiac output was determined by the thermodilution technique (the mean of five injections of 10 ml of cooled water (0-5°C) with injection performed at the end of inspiration). Oxygen-derived parameters were calculated using standard formulas. In each patient, 4 mg/kg of methylene blue was infused over 60 min via a central venous catheter. In view of the expected vasoconstriction caused by methylene blue, the protocol allowed adjustment of vasoconstrictor (dopamine and norepinephrine) doses if mean arterial pressure exceeded 90 mmHg or decreased to less than 60 mmHg. The infusion of fluids and ventilator settings were not changed during the observation period.

Statistical analysis

One-way repeated measures analysis of

Table 1 - Patient characteristics.

SAPS, Simplified acute physiology score; M, male; F, female; AST, aspartate aminotransferase.

Patient	Sepsis source	Sex	Age (years)	SAPS II	Microorganism	Creatinine (mg/dl)	AST (mg/dl)	Time of shock (days)	Outcome
1	Peritonitis	M	55	94	Non-identified	5.8	519	1	Died
2	Pancreatitis	F	68	76	Non-identified	2.9	18	3	Died
3	Leptospirosis	M	30	94	Leptospira interrogans	5.8	167	3	Died
4	Urinary	F	56	76	Escherichia coli	2.0	23	1	Died
5	Pneumonia	F	35	65	Enterobacter cloacae	0.4	52	1	Died
6	Pneumonia	M	44	44	Serratia marcescens	1.0	33	2	Survived
7	Pneumonia	M	33	65	Staphylococcus aureus	4.6	88	1	Died
8	Pneumonia	M	91	73	Staphylococcus aureus	3.3	22	4	Died
9	Pneumonia	M	50	55	Pseudomonas aeruginosa	2.6	37	1	Died
10	Pneumonia	M	44	51	Pseudomonas aeruginosa	1.8	72	4	Died

variance (ANOVA) or Friedman repeated measures ANOVA on ranks was used when appropriate and $P < 0.05$ was considered statistically significant. The Student-Newman-Keuls test was used for multiple comparisons. Data are reported as mean \pm SD or median (25-75th percentiles).

Results

Table 1 shows patient characteristics. All patients had an acute lung injury and seven fulfilled the criteria for acute respiratory distress syndrome (29). Eight patients had elevated creatinine with a mean concentration of 3.01 ± 1.79 mg/dl. Nine patients subsequently died in the ICU with multiple organ failure and 8 of them died more than 24 h after the end of the infusion. The mean simplified acute physiology score II (SAPS II) was 69.3 ± 15.9 (30).

The course of hemodynamic parameters and the effect of vasoactive drugs are shown in Table 2. Heart rate and filling pressures

were unchanged. Methylene blue administration resulted in a significant increase in mean arterial pressure and in systemic vascular resistance index throughout the observation period (Figure 1). In two patients vasopressor doses were reduced to keep mean arterial pressure at a level not exceeding 90 mmHg. There was a slight but not significant increase in mean pulmonary artery pressure. The mean pulmonary resistance index was significantly higher only at 20 min. Cardiac index did not change, but the left ventricular stroke work index increased at 40 and 60 min (Figure 2).

The course of metabolic variables and blood oxygenation is shown in Table 3. Oxygen delivery was unaffected. The oxygen consumption and thus extraction ratio increased but the difference was not significant. Mixed venous saturation decreased significantly at 40 and 60 min (Figure 3). The $\text{PaO}_2/\text{FiO}_2$ ratio significantly decreased at 40 min, remained lower during the infusion period and recovered at 120 min (Figure 3).

Table 2 - Time course of hemodynamic parameters.

HR, Heart rate; CI, cardiac index; PAoP, pulmonary artery occlusive pressure; RAP, right atrial pressure; MAP, mean arterial pressure; MPAP, mean pulmonary artery pressure; LVSWI, left ventricular stroke work index; RVSWI, right ventricular stroke work index; SVRI, systemic vascular resistance index; PVRI, pulmonary vascular resistance index. * $P < 0.05$ compared with time 0 (Friedman repeated ANOVA on ranks). Data are reported as mean \pm SD or median (25-75th percentiles).

	Methylene blue (4 mg/kg; 1-h infusion period)				
	0 min	20 min	40 min	60 min	120 min
HR (beats/min)	107 \pm 20	113 \pm 20	110 \pm 24	113 \pm 21	117 \pm 24
CI (l min ⁻¹ m ⁻²)	5.7 (4.1-7.7)	4.7 (3.9-6.8)	5.6 (4.0-6.9)	5.5 (3.8-7.1)	5.6 (3.7-6.4)
PAoP (mmHg)	13 (12-16)	15 (13-16)	14 (14-16)	13 (13-15)	12 (10-19)
RAP (mmHg)	15 \pm 3	15 \pm 2	14 \pm 4	12 \pm 5	12 \pm 6
MAP (mmHg)	60 (55-70)	63 (60-80)*	70 (65-80)*	70 (65-100)*	78 (55-90)*
MPAP (mmHg)	26 (21-30)	28 (26-32)	30 (25-33)	29 (25-30)	25 (16-29)
LVSWI (g m ⁻¹ m ⁻²)	35 (27-47)	36 (19-40)	40 (31-55)*	38 (32-56)*	36 (25-45)
RVSWI (g m ⁻¹ m ⁻²)	8 \pm 5	9 \pm 5	10 \pm 5	10 \pm 4	8 \pm 3
SVRI (dyne s ⁻¹ cm ⁻⁵ m ⁻²)	649 (479-1084)	910 (544-1327)*	888 (628-1190)*	1066 (585-1356)*	871 (678-1317)*
PVRI (dyne s ⁻¹ cm ⁻⁵ m ⁻²)	150 (83-207)	186 (121-367)*	198 (104-236)	166 (114-248)	162 (102-240)
Treatment ($\mu\text{g kg}^{-1} \text{min}^{-1}$)					
Dopamine	12.8 \pm 6.3	12.8 \pm 6.3	12.8 \pm 6.3	12.8 \pm 6.3	12.8 \pm 6.3
Dobutamine	8.3 \pm 1.7	8.3 \pm 1.7	8.3 \pm 1.7	8.3 \pm 1.7	8.3 \pm 1.7
Norepinephrine	0.69 \pm 0.19	0.69 \pm 0.19	0.67 \pm 0.2	0.67 \pm 0.2	0.72 \pm 0.18

PaCO₂ increased but the difference was not significant. Arterial blood lactate concentrations decreased significantly from 5.2 ± 2.9 mmol/l at baseline to 4.5 ± 2.1 mmol/l at 60 min ($P < 0.05$).

Discussion

The major finding of this pilot study is that infusion of methylene blue in severe septic shock patients with respiratory failure had beneficial systemic effects on arterial pressure, systemic vascular resistance and left ventricular stroke work, but detrimental effects on pulmonary hemodynamics and possibly on gas exchange.

The antivasodilatory effect of methylene blue was not followed by the decrease in cardiac output that often occurs with various specific competitive L-arginine antagonists (17,18,31). The different effects of inhibitors of nitric oxide production and methylene blue on cardiac output may be due to a more intense vasoconstriction induced by the former than by the latter, but the cause is not known (32). However, methylene blue may act not only by inhibiting activation of guanylate cyclase (33), but also by inhibiting nitric oxide synthesis (25,34-36). Although cardiac output did not increase significantly, the increase in left ventricular stroke work in the presence of unchanged filling pressures, despite an increase in systemic vascular resistance, suggests an improvement in myocardial function following methylene blue administration. Our finding is in agreement with previous studies using methylene blue in septic shock patients (26,28). *In vivo* and *in vitro* studies have shown that exposure to endotoxin-activated macrophages, cytokines or serum from septic patients causes depressant effects on myocytes that are reversed by L-arginine analogs and methylene blue (11-13). Furthermore, our observations are in agreement with these studies, in that the release of nitric oxide is implicated in sepsis-induced myocardial depression and is par-

tially improved by methylene blue.

In our study, we observed a reduction in blood lactate concentrations, as also observed by others (26). This reduction further sug-

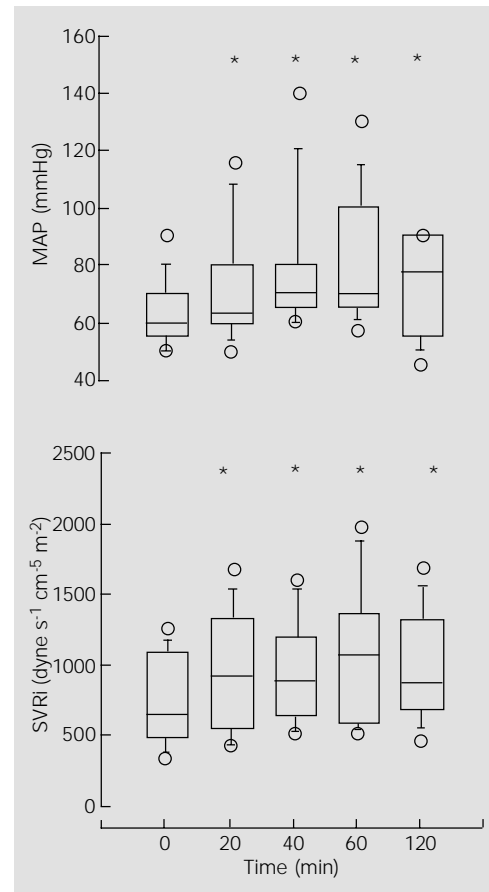


Figure 1 - Time course of mean arterial pressure (MAP) (top) and systemic vascular resistance index (SVRI) (bottom) after intravenous administration of methylene blue (4 mg/kg) after baseline (time 0) in 10 septic shock patients. Mean arterial pressure significantly increased from baseline at 20, 40, 60 and 120 min ($*P < 0.05$ compared to time 0). The box plot lines indicate the median and the 25th and 50th percentiles and error bars indicate the 10th and 90th percentiles. The circles indicate the extreme values.

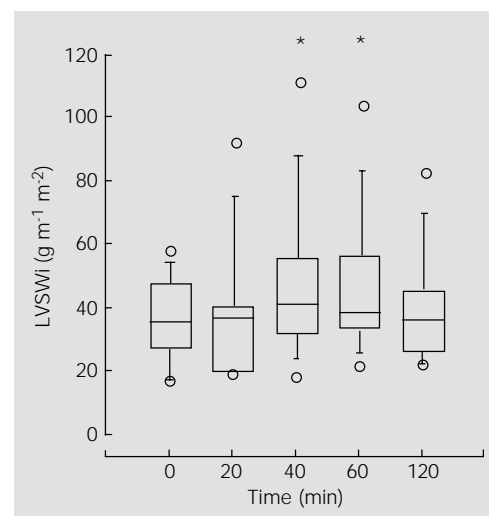


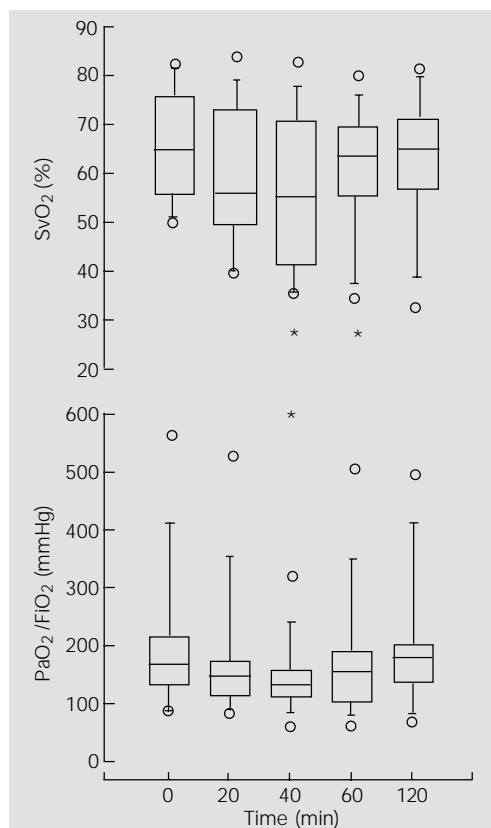
Figure 2 - Time course of left ventricular stroke work index (LVSWI) after intravenous administration of methylene blue (4 mg/kg) after baseline (time 0) in 10 septic shock patients. Left ventricular stroke work index significantly increased from baseline at 40 and 60 min ($*P < 0.05$ compared to time 0). The box plot lines indicate the median and the 25th and 50th percentiles, and error bars indicate the 10th and 90th percentiles. The circles indicate the extreme values.

Table 3 - Metabolic variables.

DO₂, Oxygen delivery; VO₂, oxygen consumption; O₂ER, oxygen extraction ratio; PaO₂, arterial oxygen tension; SaO₂, arterial oxygen saturation; PvO₂, mixed venous oxygen tension; SvO₂, mixed venous oxygen saturation; PaO₂/FiO₂, arterial oxygen tension/inspired oxygen concentration ratio; PaCO₂, arterial carbon dioxide tension; HCO₃⁻, bicarbonate. Data are reported as mean ± SD or median (25-75th percentiles) *P<0.05 compared with time 0 (Friedman repeated measures ANOVA on ranks). +P<0.05 compared with time 0 (one-way repeated measures ANOVA).

	Methylene blue (4 mg/kg; 1-h infusion period)				
	0 min	20 min	40 min	60 min	120 min
DO ₂ (ml min ⁻¹ m ⁻²)	663 ± 267	598 ± 261	626 ± 249	637 ± 275	661 ± 324
VO ₂ (ml min ⁻¹ m ⁻²)	182 ± 51	187 ± 52	207 ± 56	197 ± 37	207 ± 43
O ₂ ER (%)	31 ± 13	35 ± 13	37 ± 14	35 ± 13	36 ± 12
PaO ₂ (mmHg)	91 (73-131)	83 (71-98)	77 (62-109)*	84 (70-98)	90 (80-197)
SaO ₂ (%)	93 (90-98)	92 (85-96)	92 (80-96)	90 (88-96)	94 (91-99)
PvO ₂ (mmHg)	44 (39-53)	41 (38-50)	38 (36-47)	41 (36-48)	43 (40-50)
SvO ₂ (%)	65 (56-76)	56 (49-73)	55 (41-71)*	63 (55-69)*	65 (57-71)
PaO ₂ /FiO ₂ (mmHg)	168 (131-215)	145 (112-171)	132 (109-156)*	151 (101-188)	175 (133-201)
PaCO ₂ (mmHg)	47 (34-73)	49 (34-81)	50 (35-89)	49 (32-90)	40 (36-73)
pH	7.15 (7.03-7.25)	7.11 (6.96-7.22)	7.13 (6.98-7.26)	7.12 (6.97-7.24)	7.17 (7.01-7.25)
HCO ₃ ⁻ (mEq/l)	17.5 ± 4.7	18.2 ± 4.6	17.9 ± 4.7	18.0 ± 4.2	17.6 ± 4.6
Base-excess (mEq/l)	-12.2 ± 3.9	-12.1 ± 4.1	-12.6 ± 3.8	-12.2 ± 4.5	-12.0 ± 3.7
Lactate (mmol/l)	5.2 ± 2.9			4.5 ± 2.1+	4.6 ± 2.4

Figure 3 - Time course of mixed venous saturation (Sv) (top) and PaO₂/FiO₂ (bottom) after intravenous administration of methylene blue (4 mg/kg) after baseline (time 0) in 10 septic shock patients. Mixed venous saturation significantly decreased from baseline at 40 and 60 min (P<0.05). The PaO₂/FiO₂ ratio decreased significantly from baseline at 40 min (*P<0.05 from time 0). The box plot lines indicate the median and 25th and 50th percentiles, and error bars indicate the 10th and 90th percentiles. The circles indicate the extreme values.



gests that methylene blue was not deleterious to tissue perfusion and may have improved cellular oxygen utilization. However, an improvement in global tissue perfusion was unlikely since oxygen transport and consumption were unchanged. Lastly, the reduction in lactate concentrations could be secondary to the reductor properties of methylene blue (37).

To date, the effects of systemic use of nitric oxide inhibitors on individual organ systems are unclear. We may speculate that methylene blue possibly influenced the redistribution of blood flow among different organs. The effects of methylene blue on regional blood flow may be different (25) from those of nitric oxide synthase inhibitors (5,32,38-40). In endotoxemic dogs, low doses of methylene blue have been shown to increase splanchnic blood flow (25). However, several investigators reported that nitric oxide synthase inhibitors caused a dose-dependent reduction in mesenteric and renal blood flow, exacerbating organ vasoconstriction.

tion and ischemia and increasing mortality rate (5,6,32,38,39). We have studied the effects of the nitric oxide donor SIN-1 at low-to-moderate doses 1 h after endotoxic shock in dogs and we have observed a significant increase in cardiac index and superior mesenteric blood flow (41). These findings support the hypothesis that nitric oxide is essential to maintain organ blood flow, at least during early endotoxic shock. In this study, the administration of methylene blue reversed the effects of SIN-1 on cardiac index and regional blood flow. The effects of methylene blue on mesenteric blood flow were certainly not additive and deserve clarification.

Studies examining the effects of non-selective nitric oxide inhibitors on pulmonary injury are controversial. L-NAME, a nitric oxide synthase antagonist, has been found to decrease lung wet-to-dry weight and broncho-alveolar lavage protein content in an isolated rat lung model of oxidant injury (42). In another study, in a murine model of endotoxemia, pretreatment with L-NAME resulted in significant pulmonary hypertension and increased lung neutrophilic infiltration (6). Similarly, in a recent study, L-NAME was associated with increased histologic evidence of interstitial lung inflammation in endotoxin-provoked rats (43). Therefore, the worsening of lung injury after nitric oxide blockade in septic lung injury may be due to a variety of mechanisms including excessive pulmonary vasoconstriction with inhibition of both constitutive and inducible nitric oxide production/action, leading to worsening of lung inflammation and/or lung ischemia.

We also observed significant alterations in blood gases after methylene blue infusion (25,27). There was a progressive decrease in the $\text{PaO}_2/\text{FiO}_2$ ratio and a small increase in PaCO_2 . In another study, these parameters were unchanged, but the patients had a less severe pulmonary injury (28). In contrast, all of our patients had severe lung injury and

seven of them acute respiratory distress syndrome (ARDS). Therefore, these potential side effects were clinically important in our subset of patients. Gachot et al. (27) speculated that these alterations may be due to an exaggerated increase in pulmonary artery vasoconstriction, thus worsening pulmonary ventilation perfusion mismatching and gas exchange. In our study, there was only a slight increase in pulmonary artery pressure and resistance. Thus, another likely explanation is that the decrease in PaO_2 may have been related to an increase in oxygen extraction ratio, which resulted in a decrease in mixed venous saturation. In addition, we cannot exclude that nitric oxide blockade may have induced bronchoconstriction, although this was not clinically observed. The interference of methylene blue in the reading of mixed venous saturation could also be considered an alternative unlikely explanation. Further work is required to examine the precise role of nitric oxide inhibition in lung injury.

The kidney eliminates methylene blue (44). The presence of renal failure in eight patients may have contributed to prolonging the effects of methylene blue on arterial pressure and vascular resistance even after 120 min. However, no acute toxic effects were noted and only the urine and some body secretions were colored blue. Methemoglobinemia, a potential side effect of high doses of methylene blue (45), was not observed in the present study.

The mortality rate was high, but all patients studied were severely ill and most of them had severe respiratory failure and significant renal failure. We believe that these deaths were probably not related to methylene blue infusion. First, it was a short-time infusion. Second, the main deleterious potential side effect, a reduction in $\text{PaO}_2/\text{FIO}_2$ ratio, was transient. Third, most patients died late with multiple organ failure that was already present upon admission to the study.

There is good evidence that an enhanced

formation of nitric oxide, particularly due to induction of inducible nitric oxide synthase, contributes to several of the pathophysiological events leading to hemodynamic disturbances and multiple organ failure in animal models of septic shock. Although non-selective nitric oxide inhibitors like methylene blue exert beneficial hemodynamic effects in animals and man with septic shock, these agents also seem to exert adverse effects due to the inhibition of endothelial nitric oxide synthase. The clinical data regarding effects and side effects of non-selective nitric oxide inhibition in patients with septic shock are limited. The finding that some S-substituted isothiourea derivatives are relatively selective inhibitors of inducible nitric oxide synthase clearly indicates that the development of highly selective inhibitors may be possible (46). These agents will be a useful tool to better understand the role of non-physi-

ologic nitric oxide production in host defense and the side effects of selective inhibition.

In conclusion, methylene blue infusion in patients with septic shock augments systemic vascular tone and may improve myocardial function. The effects of nitric oxide blockade seem to be deleterious to the pulmonary vascular tone and regulation of gas exchange during human septic shock. We do not recommend the use of methylene blue in the management of septic shock. Further studies are needed to demonstrate if nitric oxide blockade with methylene blue can be safe for patients with septic shock, particularly in terms of pulmonary function.

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