Infusion of methylene blue in human septic shock: A pilot, randomized, controlled study

Mikhail Y. Kirov, MD; Oleg V. Evgenov, MD; Natalia V. Evgenov, MD; Elena M. Egorina, MD; Mikhail A. Sovershaev, MD; Baldur Sveinbjørnsson, PhD; Eduard V. Nedashkovsky, MD, PhD; Lars J. Bjertnaes, MD, PhD

Objective: **To evaluate the effects of continuous infusion of methylene blue (MB), an inhibitor of the nitric oxide pathway, on hemodynamics and organ functions in human septic shock.**

Design: **Prospective, randomized, controlled, open-label, pilot study.**

Setting: **Multidisciplinary intensive care unit of a university hospital.**

Patients: **Twenty patients with septic shock diagnosed <24 hrs before randomization.**

Interventions: **Patients were randomized 1:1 to receive either MB (MB group, n = 10) or isotonic saline (control group, n = 10), adjunctive to conventional treatment. MB was administered as an intravenous bolus injection (2 mg/kg), followed 2 hrs later by infusion at stepwise increasing rates of 0.25, 0.5, 1, and 2 mg/kg/hr that were maintained for 1 hr each. During infusion, mean arterial pressure was maintained between 70 and 90 mm Hg, while attempting to reduce concurrent adrenergic support.**

Measurements and Main Results: **Hemodynamics and organ function variables were assessed over a 24-hr period, and the survival rate at day 28 was noted. Infusion of MB prevented the stroke volume and the left-ventricular stroke work indexes from falling and increased mean arterial pressure. Compared with the control group, MB reduced the requirement for norepinephrine, epinephrine, and dopamine by as much as 87%, 81%, and 40%, respectively. Oxygen delivery remained unchanged in the MB group and decreased in the control group. MB also reduced the body temperature and the plasma concentration of nitrates/nitrites. Leukocytes and organ function variables such as bilirubin, alanine aminotransferase, urea, and creatinine were not significantly affected. Platelet count decreased in both groups. Five patients treated with MB survived vs. three patients receiving conventional treatment.**

Conclusions: **In human septic shock, continuously infused MB counteracts myocardial depression, maintains oxygen transport, and reduces concurrent adrenergic support. Infusion of MB appears to have no significant adverse effects on the selected organ function variables. (Crit Care Med 2001; 29:1860–1867)**

KEY WORDS: severe sepsis; septic shock; human; nitric oxide; nitric oxide synthase; methylene blue; vasopressor agents; hemodynamics; heart; organ dysfunction syndrome.

Sepsis is a common cause of morbidity and mortality in critically ill patients. Its pathogenesis includes a severe systemic inflammatory response that may be caused by a variety of microorganisms. Bacterial endotoxin stimulates different types of cells to release cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interferon-γ, as well as other inflammatory mediators that activate the inducible isoform of nitric oxide synthase (iNOS) (1, 2). This isoform escalates production of nitric oxide (NO). Activating the soluble guanylate cyclase of smooth muscle cells, NO increases the generation of cyclic guanosine monophosphate (cGMP). In hyperdynamic sepsis, excessive formation of NO and cGMP is associated with profound vasodilatation, hyporeactivity to catecholamines, and myocardial depression. In addition, large amounts of NO may modify gene expression, mediate oxidative and nitrosative stress, and lead to cytotoxic effects. These changes may cause tissue hypoxia and result in multiple organ failure and increased mortality. In contrast, the production of minute amounts of NO by the constitutive endothelial isoform of NO synthase (eNOS) is responsible for regulation of basal vascular tone and other physiologic effects (1–4).

Inhibition of excessively produced NO and cGMP may eventually prevent the detrimental hemodynamic effects associated with septic shock (4). Used for many years in the treatment of methemoglobinemia (5), methylene blue (MB) also has been found to counteract NO-induced effects by inhibiting soluble guanylate cyclase (6), eNOS (7), and iNOS (8). Recent experimental studies have revealed that MB reverses endotoxin-induced hypotension and antagonizes the hyporeactivity to vasoconstrictors, simultaneously normalizing plasma concentrations of the end-products of NO, nitrates/nitrites (NO_x), and cGMP (9–12). Several uncontrolled investigations on patients with septic shock requiring adrenergic support also indicate that MB, administered as a bolus or short-term infusion, restores mean arterial pressure (MAP) by increasing systemic vascular resistance. Moreover, MB improves myocardial con-

From the Departments of Anesthesiology (OVE, NVE, LJB) and Experimental Pathology (BS), Faculty of Medicine, University of Tromsø, Tromsø, Norway; and the Department of Anesthesiology, Northern State Medical University, Arkhangelsk, Russia (MYK, EME, MAS, EVN)

Supported, in part, by the Research Council of Norway (grant 120473/730), Sparebank1 Nord-Norge, Tromsø, and the Mariane and Rolf Bjørns Fund, Tromsø, Norway.

Address requests for reprints to: Lars J. Bjertnaes, MD, PhD, Department of Anesthesiology, Faculty of Medicine, University of Tromsø, N-9037 Tromsø, Norway. E-mail: lars.bjertnaes@rito.no

tractility and oxygen delivery (13–18). Although the beneficial effects of MB are transient, they can be reproduced by the administration of a second dose, as shown by Preiser et al (13). Unfortunately, most clinical reports on the use of MB on septic shock patients are anecdotal and provide no conclusive evidence regarding hemodynamics, gas exchange, or other organ functions. One investigation even reports worsening of arterial oxygenation after bolus injection of MB (15).

Continuous infusion of MB has been described only in endotoxemic sheep and in one case report of human septic shock (9, 19). Inspired by the latter investigations, the purpose of the present study was to evaluate the effects of continuously infused MB on hemodynamics, gas exchange, and other organ function variables in humans suffering from septic shock.

MATERIALS AND METHODS

Patient Population and Study Design. The Regional Committee on Medical Research Ethics, the University of Tromsø, Tromsø, Norway, and the Ethics Committee of the Northern State Medical University, Arkhangelsk, Russia, approved the study. Written informed consent was obtained from the patients wherever possible, or from the next of kin of the patients.

Twenty patients admitted to the intensive care unit of the City Hospital #1 of Arkhangelsk during 1998–2000 were enrolled. The patients were diagnosed with severe sepsis and septic shock according to the modified criteria of the American College of Chest Physicians and the Society of Critical Care Medicine consensus conference (20). The diagnosis of severe sepsis was based on either clinical evidence of infection or positive blood culture and, as a minimum, two indicators of the systemic inflammatory response syndrome. The latter includes fever (body temperature >38°C) or hypothermia (<36°C), tachycardia (>90 beats/min), tachypnea (>20 breaths/min), hyperventilation ($Paco_2$ <32 torr [4.3 kPa]), and a requirement for mechanical ventilation. Abnormal white blood cell count (>12 × 10^9/L or <4 × 10^9/L) or immature neutrophils (>10%) also belong to the typical signs. A further prerequisite for the diagnosis was an acute onset of end-organ dysfunction unrelated to the primary septic focus and not caused by any underlying chronic disease. In the present study, we evaluated the end-organ dysfunction according to the criteria used by Grover et al (21).

Septic shock was defined as severe sepsis associated with MAP of <70 mm Hg for at least 30 mins despite fluid resuscitation, or with requirement for infusion of dopamine >5 μg/kg/min and/or norepinephrine >0.05 μg/kg/min and/or epinephrine >0.05 μg/kg/min for at least 30 mins to maintain MAP between 70 and 90 mm Hg. The fluid resuscitation was considered adequate when additional infusion caused no further increase in cardiac index (CI), and pulmonary artery occlusion pressure remained between 8 and 18 mm Hg. We infused dobutamine when required to maintain CI >3.5 L/min/m^2.

Patients were eligible to enter the study if they fulfilled the above criteria for severe sepsis diagnosed <72 hrs, and septic shock diagnosed <24 hrs before randomization, received mechanical ventilation, and had the pulmonary artery catheters in place. Patients who were <18 yrs of age, pregnant, or receiving corticosteroids, immunosuppressants, or chemotherapy, and those with a known irreversible underlying disease such as end-stage neoplasms were not included.

Randomization Procedure and Therapeutic Protocol. When a patient with septic shock was admitted to the intensive care unit, the attending physician contacted the trial coordinator to discuss eligibility. After meeting the inclusion criteria, written consent was obtained. The patients were then randomized 1:1, using unmarked, sealed envelopes containing the instruction to administer either MB (methylthionine; Nycomed, Oslo, Norway) (MB group, n = 10), or a corresponding volume of isotonic saline (control group, n = 10). The order of envelopes was determined blindly by a member of the department's secretarial staff who played no other role in the study. The study was to begin within 2 hrs after randomization.

MB was diluted by isotonic saline from 10 to 5 mg/mL and administered via a dedicated central venous catheter. Intravenous bolus injection of MB (2 mg/kg for 15 mins) was followed 2 hrs later by an infusion at stepwise increasing rates of 0.25, 0.5, 1, and 2 mg/kg/hr that were maintained for 1 hr each. Vasopressor therapy was adjusted to maintain MAP within the range of 70 to 90 mm Hg. If MAP exceeded 90 mm Hg, norepinephrine or epinephrine were tapered off in steps of 0.03 μg/kg/min and dopamine of 1 μg/kg/min, respectively, every 15 mins. Norepinephrine was weaned first, followed by epinephrine and dopamine. If urine output was <0.5 mL/kg/hr, dopamine in low dose (2–4 μg/kg/min) was the drug of choice until normalization of the diuresis. If CI exceeded 3.5 L/min/m^2, dobutamine was tapered off in steps of 1 μg/kg/min every 15 mins.

Bacterial and fungal cultures (respiratory secretion, blood, urine, peritoneal fluid, and wound discharge) were routinely assayed. Bacterial infections were treated with selective antibiotics, with preference given to third-generation cephalosporins, quinolones, or carbapenems. The concomitant therapy included substitution of fluids, mechanical ventilation, anticoagulants, sedation, nutritional support, and hemodialysis in cases of acute renal failure. If necessary, all patients received surgery and advanced cardiac life support.

Measurements. The patients were monitored by means of a pulmonary artery balloon floatation catheter (131HF7; Baxter, Irvine, CA), a radial artery catheter, and a central venous catheter. Pressures were measured at the end of expiration with the zero reference point at the mid-chest level in the supine position. Serial measurements of MAP, pulmonary artery occlusion pressure, heart rate, mean central venous pressure, and mean pulmonary arterial pressure were taken. Cardiac output was measured in triplicate by injection of 10 mL of 5% dextrose at room temperature into the proximal port of the pulmonary artery catheter, and computed by a hemodynamic monitor (SMU 612; Hellige, Freiburg, Germany). CI, systemic vascular resistance index (SVRI), pulmonary vascular resistance index (PVRI), stroke volume index (SVI), left-ventricular stroke work index (LVSWI), and right-ventricular stroke work index (RVSWI) were calculated using standard formulas. Body temperature was measured by means of the thermistor probe of the pulmonary artery catheter. Arterial and mixed venous blood gases and methemoglobin were determined using a blood gas analyzer (ABL 520; Radiometer, Copenhagen, Denmark). Inspired oxygen concentration (F_{IO_2}) was noted from an oxygen blender (7200; Puritan-Bennett, Carlsbad, CA). Oxygen delivery index (DO_2I), oxygen consumption index (VO_2I), and oxygen extraction ratio (O_2ER) were also calculated by commonly used formulas. Peak airway pressure, positive end-expiratory pressure, quasi-static pulmonary compliance, minute volume of ventilation, and respiratory rate were measured using a 7200 ventilator (Puritan-Bennett). Quasi-static pulmonary compliance was estimated as the ratio of the tidal volume to the plateau airway pressure. Hemodynamic and respiratory parameters, gas exchange, oxygen-derived variables, and vasopressor infusion rates were registered at baseline (0 hr) and subsequently at 1-hr intervals during the first 6 hrs, and finally at 24 hrs.

The Simplified Acute Physiology Score (SAPS) II (22) and the Sepsis-Related Organ Failure Assessment (SOFA) score (23) were assessed upon inclusion into the study. Durations of septic shock, vasopressor support, mechanical ventilation, hospital and intensive care unit stays, number of organ dysfunctions at 24 hrs, details of sedation and fluid therapy from 0 to 24 hrs, and survival rate at day 28 were recorded as well. The duration of septic shock was defined as the period from the onset of shock to its resolution. We identified resolution with MAP >70 mm Hg for at least two consecutive hours, in parallel with discontinuation of vasopressor support or requirement for infusion of dopamine <5 μg/kg/min and/or norepinephrine <0.05 μg/kg/min and/or epinephrine <0.05 μg/kg/min.

Leukocytes, platelets, plasma concentrations of TNF-α, bilirubin, alanine aminotrans-

ferase, creatinine, and urea were determined at 0, 6, and 24 hrs. The bioactivity of TNF-α was assessed by its cytotoxic effect on the fibrosarcoma cell line WEHI 164 clone 13 with rhTNF-α as a standard (24). The TNF-α specificity of the assay was verified using a polyclonal antibody against rhTNF-α, which neutralized the cytotoxic effect detected in plasma. Viability of the target cells in the TNF-α bioassay was determined by incubation with MTT tetrazoleum salt, which is converted to insoluble purple formazan by living cells (25). MTT was purchased from Sigma (St. Louis, MO), rhTNF-α and polyclonal antibody against rhTNF-α were purchased from R&D Systems (Abingdon, UK). Total plasma NO_X concentration was measured employing a commercially available kit (850–001-KI01; Cayman, Ann Arbor, MI). In addition, we measured TNF-α and NO_X in the plasma from six healthy volunteers. Plasma concentration of MB was measured according to the method of DiSanto and Wagner (26).

Statistical Analysis. Continuous data are expressed as mean ± SD. For each continuous variable, normality was checked. Data were assessed by two-way analysis of variance. If the *F* value was statistically significant, an unpaired two-tailed Student's *t*-test or paired Student's *t*-test with Bonferroni correction was used to evaluate differences between groups and within groups toward the baseline values, respectively. Analysis of the discrete data were performed by chi-square test or Fisher's exact test when the numbers were small. A *p* value of <.05 was regarded as statistically significant, or otherwise, not significant (NS).

Table 1. Characteristics of patients with septic shock randomized to receive methylene blue (MB group; n = 10) or isotonic saline (C group; n = 10) in addition to conventional therapy at inclusion to the study

	MB Group	C Group	*p*
Age, yrs	55.3 ± 20.9	59.4 ± 14.5	.62
Gender, females/males	5/5	4/6	
SAPS II, points	57.8 ± 16.3	57.7 ± 18.2	.98
SOFA, points	10.1 ± 2.1	10.5 ± 3.7	.77
No. of organ dysfunctions	2.4 ± 0.5	2.4 ± 1.0	.96
Primary illness			
Medical	6	5	
Surgical	4	5	
Underlying infections			
Nosocomial pneumonia	1	1	
Community-acquired pneumonia	5	4	
Pancreonecrosis	2	1	
Peritonitis	2	3	
Urinary infection	0	1	
Gram-positive infection	3	3	
Gram-negative infection	4	5	
Polymicrobal infection	3	2	
Positive blood cultures	4	4	
Adrenergic support			
Dobutamine	4	4	
Dopamine	5	7	
Epinephrine	7	6	
Norepinephrine	5	5	

SAPS, Simplified Acute Physiology Score; SOFA, Sepsis-Related Organ Failure Assessment score. Data are mean ± SD or number of patients.

RESULTS

At the study entry and baseline, no intergroup differences were observed with respect to demographic data, SAPS II and SOFA scores, number of organ dysfunctions, hemodynamics, respiratory parameters, and laboratory variables (Tables 1–4, Figs. 1–3).

Hemodynamics. As depicted in Figure 1, infusion of MB prevented SVI and LVSWI from falling. At the end of infusion, MB enhanced LVSWI by 32% as compared with baseline ($p = .04$). In contrast, in the control group, SVI and LVSWI decreased to nadirs of 32% and 40%, respectively, below their respective baseline values ($p < .05$). From 3 hrs, SVI, LVSWI, and RVSWI in the MB group were 30% to 80% higher than the controls ($p < .05$). As shown in Table 2, heart rate, mean pulmonary arterial pressure, central venous pressure, pulmonary artery occlusion pressure, CI, SVRI, and PVRI all displayed no significant inter-

Table 2. Hemodynamics in septic shock patients randomized to receive methylene blue (MB group; n = 10) or isotonic saline (C group; n = 10) in addition to conventional therapy

	Group	0 Hr	1 Hr	2 Hrs	3 Hrs	4 Hrs	5 Hrs	6 Hrs	24 Hrs
MAP, mm Hg	C	79.6 ± 12.1	82.5 ± 17.2	78.8 ± 11.6	72.2 ± 13.5	70.9 ± 17.6	72.1 ± 18.0	71.8 ± 20.3	69.9 ± 18.4
	MB	76.3 ± 16.0	86.5 ± 13.6	77.2 ± 8.9	79.0 ± 10.4	77.5 ± 7.0	87.4 ± 19.9	91.4 ± 11.0[a,b]	86.6 ± 15.3[b]
HR, beats/min	C	125 ± 23	123 ± 25	127 ± 25	125 ± 22	127 ± 23	131 ± 22	133 ± 20	127 ± 25
	MB	130 ± 26	135 ± 27	129 ± 24	124 ± 21	122 ± 21	125 ± 25	117 ± 26	112 ± 14[a]
PAP, mm Hg	C	19.5 ± 4.4	20.3 ± 2.5	20.1 ± 3.2	17.9 ± 3.3	19.4 ± 3.9	20.5 ± 3.1	19.5 ± 4.2	21.6 ± 3.2
	MB	21.3 ± 3.2	21.5 ± 2.4	19.3 ± 1.8	20.9 ± 3.0	21.5 ± 3.0	21.6 ± 3.1	21.9 ± 2.3	21.2 ± 2.4
CVP, mm Hg	C	6.5 ± 3.2	6.5 ± 2.4	6.1 ± 2.1	5.9 ± 1.9	5.7 ± 2.8	5.8 ± 2.8	5.6 ± 2.7	6.0 ± 3.2
	MB	5.9 ± 3.7	5.2 ± 3.2	4.7 ± 3.0	4.9 ± 3.1	4.8 ± 3.1	5.4 ± 3.7	4.9 ± 3.7	5.3 ± 4.1
PAOP, mm Hg	C	9.2 ± 1.8	10.1 ± 1.7	9.9 ± 2.2	9.8 ± 2.8	10.2 ± 3.9	10.6 ± 3.8	10.7 ± 4.2	11.0 ± 4.0
	MB	8.9 ± 2.8	8.6 ± 2.1	8.2 ± 2.8	8.5 ± 1.6	9.1 ± 2.2	8.7 ± 1.7	9.2 ± 2.9	9.3 ± 3.5
CI, L/min/m^2	C	4.7 ± 1.7	4.2 ± 1.3	3.9 ± 0.9	3.4 ± 1.1[a]	3.6 ± 1.1	3.6 ± 1.1	3.5 ± 1.1[a]	3.5 ± 1.2[a]
	MB	4.6 ± 1.4	4.4 ± 1.4	4.5 ± 1.4	5.0 ± 2.2	4.8 ± 1.7	4.6 ± 1.6	4.6 ± 1.3	4.2 ± 1.4
SVRI, dyne·sec/cm^5/m^2	C	1371 ± 421	1530 ± 466	1617 ± 539	1629 ± 457	1520 ± 502	1519 ± 458	1571 ± 491	1444 ± 358
	MB	1302 ± 389	1565 ± 349[a]	1357 ± 323	1368 ± 512	1348 ± 415	1536 ± 507	1631 ± 494[a]	1686 ± 648
PVRI, dyne·sec/cm^5/m^2	C	195 ± 95	202 ± 85	213 ± 126	199 ± 90	206 ± 84	209 ± 48	203 ± 74	237 ± 122
	MB	211 ± 143	239 ± 140	195 ± 125	208 ± 171	207 ± 159	215 ± 154	212 ± 104	225 ± 118

MAP, mean arterial pressure; HR, heart rate; PAP, mean pulmonary arterial pressure; CVP, central venous pressure; PAOP, pulmonary artery occlusion pressure; CI, cardiac index; SVRI, systemic vascular resistance index; PVRI, pulmonary vascular resistance index.

[a]$p < .05$ from intragroup baseline; [b]$p < .05$ between groups. Data are mean ± SD.

Table 3. Respiratory variables, gas exchange, and oxygen-derived variables in septic shock patients randomized to receive methylene blue (MB group; n = 10) or isotonic saline (C group; n = 10) in addition to conventional therapy

	Group	0 Hr	1 Hr	2 Hrs	3 Hrs	4 Hrs	5 Hrs	6 Hrs	24 Hrs
Minute ventilation, L/min	C	11.0 ± 3.6	10.9 ± 3.7	10.9 ± 3.6	10.7 ± 3.7	10.8 ± 3.5	10.8 ± 3.5	10.7 ± 3.4	10.3 ± 3.2
	MB	10.0 ± 2.8	10.2 ± 2.7	10.2 ± 2.5	9.8 ± 2.4	9.6 ± 2.5	9.7 ± 2.5	9.6 ± 2.4	9.6 ± 2.3
Respiratory rate, breaths/min	C	18.7 ± 3.4	18.6 ± 3.5	18.6 ± 3.5	18.6 ± 3.5	18.6 ± 3.5	18.2 ± 2.9	18.7 ± 3.4	19.6 ± 3.8
	MB	18.5 ± 3.4	19.6 ± 3.0	19.4 ± 3.2	19.4 ± 3.2	19.1 ± 3.1	19.3 ± 3.1	19.3 ± 3.1	18.8 ± 3.6
P_{peak}, cm H_2O	C	19.7 ± 7.0	19.5 ± 7.3	19.2 ± 6.4	20.0 ± 5.5	20.7 ± 6.6	21.1 ± 6.0	21.1 ± 6.0	21.6 ± 6.3
	MB	21.1 ± 3.9	20.6 ± 3.9	20.4 ± 4.4	20.7 ± 4.5	21.6 ± 2.9	21.3 ± 3.5	20.8 ± 3.6	21.3 ± 6.0
PEEP, cm H_2O	C	4.2 ± 2.0	4.0 ± 2.0	4.1 ± 1.9	4.2 ± 2.4	4.1 ± 2.1	4.2 ± 2.1	4.3 ± 2.4	3.9 ± 2.1
	MB	4.7 ± 1.4	4.6 ± 1.6	4.6 ± 1.5	4.6 ± 2.1	4.2 ± 2.0	4.4 ± 1.9	4.2 ± 1.8	3.6 ± 1.8
C_{qs}, mL/cm H_2O	C	42.4 ± 8.7	40.2 ± 9.2	40.8 ± 9.5	41.3 ± 9.0	44.1 ± 6.9	44.4 ± 7.5	43.8 ± 7.0	43.5 ± 6.6
	MB	41.0 ± 12.8	44.5 ± 11.8	48.9 ± 11.9	47.7 ± 10.2	49.6 ± 10.7	48.1 ± 15.6	45.2 ± 12.0	47.7 ± 8.5
pHa	C	7.37 ± 0.09	7.38 ± 0.06	7.38 ± 0.05	7.37 ± 0.09	7.34 ± 0.11	7.34 ± 0.13	7.33 ± 0.15	7.36 ± 0.10
	MB	7.35 ± 0.11	7.35 ± 0.09	7.36 ± 0.09	7.38 ± 0.08	7.37 ± 0.10	7.37 ± 0.08	7.35 ± 0.12	7.37 ± 0.13
$Paco_2$, torr	C	36.8 ± 7.4	35.7 ± 4.2	34.2 ± 4.3	36.3 ± 6.5	35.8 ± 3.7	36.2 ± 5.9	38.1 ± 6.0	37.8 ± 5.3
	MB	36.8 ± 8.2	35.7 ± 7.6	34.9 ± 5.4	35.7 ± 4.9	36.9 ± 5.3	37.2 ± 4.5	36.8 ± 5.7	34.5 ± 4.6
Pao_2/Fio_2, torr	C	180 ± 125	185 ± 121	190 ± 125	187 ± 114	166 ± 112	193 ± 133	181 ± 110	194 ± 121
	MB	195 ± 78	194 ± 90	188 ± 101	205 ± 100	186 ± 93	188 ± 90	205 ± 106	234 ± 98
Sao_2, %	C	95.1 ± 3.6	94.1 ± 4.2	95.2 ± 3.8	93.2 ± 6.5	91.9 ± 5.8	93.1 ± 5.3	91.6 ± 6.3	93.1 ± 5.7
	MB	95.0 ± 4.1	93.1 ± 8.2	93.8 ± 4.6	95.6 ± 2.2	94.0 ± 4.5	92.2 ± 8.3	92.6 ± 8.6	93.9 ± 5.3
Svo_2, %	C	68.8 ± 10.4	64.3 ± 13.2	63.6 ± 13.6	61.9 ± 13.8	60.6 ± 14.6	64.1 ± 13.8	65.9 ± 15.2	67.2 ± 15.9
	MB	68.5 ± 13.7	65.8 ± 12.0	66.5 ± 9.9	68.9 ± 14.3	67.1 ± 14.1	67.3 ± 13.7	68.0 ± 12.5	65.9 ± 16.2
DO_2I, mL/min/m^2	C	704 ± 256	621 ± 237	565 ± 146	509 ± 181[a]	505 ± 176[a]	501 ± 168[a]	483 ± 191[a]	508 ± 199
	MB	761 ± 313	676 ± 227	722 ± 319	792 ± 415	740 ± 400	706 ± 365	695 ± 275	638 ± 289
VO_2I, mL/min/m^2	C	197 ± 99	179 ± 59	180 ± 65	159 ± 49	161 ± 71	152 ± 77	133 ± 68	135 ± 65
	MB	203 ± 69	183 ± 82	204 ± 69	204 ± 93	212 ± 126	178 ± 87	190 ± 107	179 ± 65[b]
O_2ER, %	C	28.0 ± 8.1	28.8 ± 11.0	31.8 ± 10.2	31.2 ± 9.6	31.0 ± 9.8	30.3 ± 9.8	27.5 ± 9.6	26.6 ± 10.6
	MB	26.7 ± 13.6	27.1 ± 13.1	28.3 ± 8.4	28.5 ± 14.9	25.8 ± 15.0	25.2 ± 11.9	27.3 ± 11.4	28.1 ± 15.2
Hemoglobin, g/dL	C	11.3 ± 2.3	11.3 ± 2.3	11.1 ± 2.3	11.2 ± 2.4	11.1 ± 2.5	11.1 ± 2.4	11.1 ± 2.5	11.1 ± 2.0
	MB	12.2 ± 2.7	12.0 ± 2.9	11.8 ± 2.6	11.8 ± 3.0	11.5 ± 2.7	11.6 ± 2.8	11.7 ± 3.0	11.4 ± 3.1
Methemoglobin, %	C	0.88 ± 0.06	0.78 ± 0.15	0.75 ± 0.24	0.88 ± 0.19	0.93 ± 0.30	0.80 ± 0.16	0.73 ± 0.17	0.78 ± 0.21
	MB	0.83 ± 0.30	1.06 ± 0.22[b]	1.23 ± 0.41[b]	0.99 ± 0.23	1.00 ± 0.15	0.91 ± 0.22	1.03 ± 0.26[b]	1.08 ± 0.36
Temperature, °C	C	37.5 ± 1.5	37.7 ± 1.4	37.9 ± 1.4	38.0 ± 1.3	38.0 ± 1.6	38.1 ± 1.6	38.1 ± 1.7	38.2 ± 1.4
	MB	37.4 ± 1.2	37.1 ± 1.3	37.2 ± 1.5	37.1 ± 1.5	37.1 ± 1.7	37.2 ± 1.9	37.1 ± 2.0	36.8 ± 1.1[b]
C_{MB}, μg/mL	MB	0.0 ± 0.0	1.18 ± 0.68[a]	0.88 ± 0.40[a]	1.44 ± 2.21[a]	5.07 ± 6.05[a]	6.91 ± 4.25[a]	7.35 ± 8.21[a]	0.27 ± 0.26[a]

P_{peak}, peak airway pressure; PEEP, positive end-expiratory pressure; C_{qs}, quasi-static compliance; pHa, arterial pH; DO_2I, oxygen delivery index; VO_2I, oxygen consumption index; O_2ER, oxygen extraction ratio; C_{MB}, plasma concentration of MB.

[a] $p < .05$ from intragroup baseline; [b] $p < .05$ between groups. Data are mean ± SD.

group differences. However, toward the end of MB infusion, MAP increased both in comparison to its own intragroup baseline ($p = .018$) and to the control group ($p = .017$). The latter effect of MB remained unchanged at 24 hrs ($p = .04$). Throughout the study, patients in both groups had tachycardia. In the MB group, heart rate decreased by 14% below baseline at 24 hrs ($p = .04$). CI remained nearly unchanged in patients receiving MB, but decreased by 30% to 40% in the control group ($p < .05$). In the MB group, SVRI rose by approximately 20% above baseline at 1 and 6 hrs ($p < .02$).

Respiratory Parameters, Gas Exchange, and Oxygen-Derived Variables. No significant changes were observed within or between the groups with respect to minute ventilation, respiratory rate, peak airway pressure, positive end-expiratory pressure, and quasi-static pulmonary compliance (Table 3). Arterial pH, $Paco_2$, Pao_2/Fio_2, Sao_2, and Svo_2 also remained unchanged. In the MB group, DO_2I and VO_2I remained close to baseline throughout the study. In contrast, in the control group, DO_2I declined gradually to 69% of baseline ($p < .05$). At 24 hrs, VO_2I was higher in the MB group ($p = .019$). No differences were noticed regarding hemoglobin concentration and O_2ER. When compared with the controls, the methemoglobin concentration rose in the MB group at 1, 2, and 6 hrs ($p < .05$) and the body temperature decreased at 24 hrs ($p = .02$).

Inotropic and Vasopressor Support. As depicted in Figure 2, MB reduced the requirement for norepinephrine, epinephrine, and dopamine by as much as 87%, 81%, and 40%, respectively, in comparison with the control group ($p < .05$). The intergroup difference in the infusion rate of norepinephrine occurred from 1 hr throughout the study and of epinephrine at 6 hrs ($p < .05$). As compared with baseline, MB reduced the requirement for epinephrine and norepinephrine by as much as two- and fivefold, respectively ($p < .05$). With regard to dopamine, differences were seen at 3 and 24 hrs, respectively ($p < .05$). At 6 and 24 hrs, the infusion rate of dobutamine also was significantly lower in the MB group.

Laboratory Assessment. No significant intergroup differences were seen in the organ function variables (Table 4). At 6 hrs, the platelet count demonstrated a trend to a larger reduction in the MB group ($p = .14$). When compared with baseline, the platelet count fell by 33% in the MB group and by 26% in the control group at 24 hrs ($p < .05$). Leukocytes, bilirubin, and alanine aminotransferase rose in the control group and decreased in the MB group (NS), whereas creatinine and urea remained unchanged. At baseline, plasma NO_X was 46.4 ± 19.0 μmol/L in the patients vs. 12.4 ± 3.9 μmol/L in the healthy volunteers ($p = .002$), and TNF-α was 57.7 ± 44.2 pg/mL vs. 2.9 ± 1.0 pg/mL ($p = .004$) in patients and volunteers, respectively. After 4 hrs of MB infusion (Fig. 3), NO_X fell to a nadir 26%

Table 4. Selected organ function variables and plasma concentrations of tumor necrosis factor (TNF)-α in septic shock patients randomized to receive methylene blue (MB group; n = 10) or isotonic saline (C group; n = 10) in addition to conventional therapy

	Group	0 Hr	6 Hrs	24 Hrs
Platelets, 10^9/L	C	137 ± 47	115 ± 81	101 ± 42[a]
	MB	215 ± 91	162 ± 112	145 ± 78[a]
Leukocytes, 10^9/L	C	9.4 ± 7.0	12.5 ± 11.0	13.0 ± 10.9
	MB	10.4 ± 7.9	8.6 ± 5.7	7.5 ± 3.8
Bilirubin, μmol/L	C	21.2 ± 9.8	24.6 ± 14.8	32.6 ± 20.5
	MB	21.2 ± 11.7	18.7 ± 12.6	18.1 ± 10.7
Alanine aminotransferase, IU/L	C	29.7 ± 9.4	34.1 ± 15.9	58.6 ± 41.0
	MB	42.4 ± 22.8	32.2 ± 17.7	25.0 ± 15.0
Creatinine, μmol/L	C	164 ± 81	166 ± 110	186 ± 102
	MB	154 ± 89	161 ± 85	169 ± 139
Urea, mmol/L	C	13.0 ± 4.3	14.5 ± 4.8	15.6 ± 5.0
	MB	12.4 ± 6.0	14.2 ± 5.1	15.1 ± 4.7
TNF-α, pg/mL	C	55.4 ± 34.9	44.3 ± 22.2	63.6 ± 28.5
	MB	60.0 ± 56.3	30.2 ± 37.2	26.4 ± 23.6

[a] $p < .05$ from intragroup baseline. Data are mean ± SD.

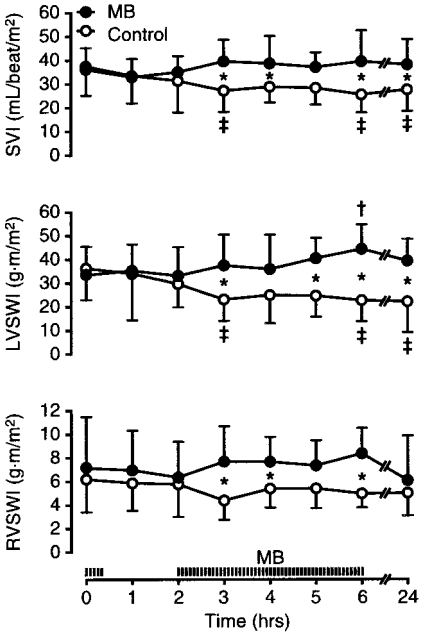

Figure 1. Cardiac function of patients with septic shock randomized to receive methylene blue (*MB* group, n = 10) or isotonic saline (control group, n = 10) in addition to conventional therapy. MB was administered as a bolus injection (2 mg/kg) after baseline measurements at 0 hrs, followed 2 hrs later by infusion at stepwise increasing rates of 0.25, 0.5, 1, and 2 mg/kg/hr that were maintained for 1 hr each. *SVI*, stroke volume index; *LVSWI*, left ventricle stroke work index; *RVSWI*, right ventricle stroke work index. Data are mean ± SD. †$p < .05$ from intragroup baseline in the MB group; ‡$p < .05$ from intragroup baseline in the control group; *$p < .05$ between the groups.

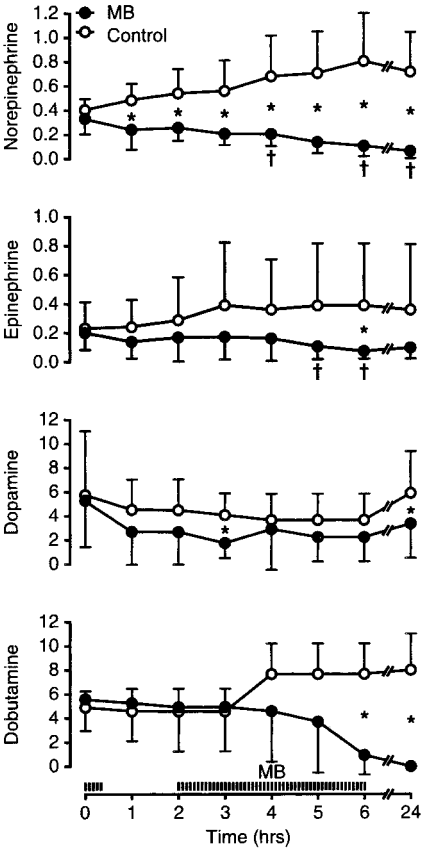

Figure 2. Adrenergic support to patients with septic shock randomized to receive methylene blue (*MB* group, n = 10) or isotonic saline (control group, n = 10) in addition to conventional therapy. Drug doses are presented in μg/kg/min. Data are mean ± SD. †$p < .05$ from intragroup baseline in the MB group; *$p < .05$ between the groups. For further information, see legend to Figure 1.

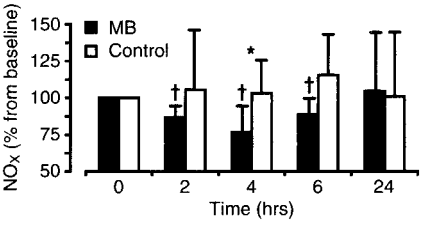

Figure 3. Plasma concentrations of nitrates and nitrites (NO_X) of patients with septic shock randomized to receive methylene blue (*MB* group, n = 10) or isotonic saline (control group, n = 10) in addition to conventional therapy. Data are mean ± SD. †$p < .05$ from intragroup baseline in the MB group; *$p < .05$ between the groups. For further information, see legend to Figure 1.

below that of the control group (p = .036). At 24 hrs, there was a tendency for reduced TNF-α in the MB group, as compared with the control group (Table 4; p = .08). The plasma concentration of MB increased gradually, peaking at 6 hrs (Table 3).

Clinical Characteristics. As shown in Table 5, no significant differences were noticed between the groups regarding clinical characteristics. Although MB led to resolution of shock in seven patients vs. three patients in the control group, the durations of the septic shock and the need for vasopressor support were only slightly lower in the MB group (NS). Seven patients in the control group and three in the MB group died of irreversible shock and multiple organ failure. Of those in the MB group achieving resolution of shock, one died of arrhythmia and one of respiratory failure. As compared with the number of organ dysfunctions at the entry of the study, an increased number was observed at 24 hrs in the control group (p = .01). Infusion of MB demonstrated no obvious signs of toxicity. However, the urine turned blue for a period of 2–4 days. In addition, a majority of the patients developed a noticeable blue-gray skin color persisting for 1–3 days.

DISCUSSION

The present study revealed that in human septic shock, continuously infused MB improves cardiovascular function and reduces the requirement for adrenergic support. Moreover, oxygen delivery and consumption are better maintained after MB.

As compared with the control group, the MB improvement of cardiac function was associated with increased SVI, LVSWI, and RVSWI in concert with a decrease in heart rate. This observation reflects an increase in the myocardial contractility in the presence of unchanged CI and filling pressure, consistent with previous reports (13, 16). In contrast, the decrease in SVI and LVSWI

Table 5. Clinical characteristics of septic shock patients randomized to receive methylene blue (MB group; n = 10) or isotonic saline (C group; n = 10) in addition to conventional therapy

	MB Group	C Group	*p*
Duration of septic shock, hrs	58.9 ± 36.7	66.3 ± 44.3	.73
Duration of vasopressor support, hrs	71.4 ± 34.2	93.3 ± 49.7	.54
Duration of mechanical ventilation, hrs	84.4 ± 43.9	75.1 ± 57.1	.72
No. of organ dysfunctions at 24 hrs	2.9 ± 0.9	3.2 ± 1.0	.49
Fluid therapy from 0 to 24 hrs, L	3.6 ± 1.4	3.7 ± 1.3	.93
Midazolam from 0 to 24 hrs, mg/hr	2.7 ± 1.4	2.7 ± 1.3	.94
Period of stay, days			
In intensive care unit	6.4 ± 4.0	6.1 ± 4.5	.69
In hospital	17.4 ± 15.5	16.1 ± 15.6	.87
Resolution of shock	7	3	.07
Survivors at day 28	5	3	.65

Data are mean ± SD or number of patients.

Continuously infused methylene blue as an adjuvant treatment to patients with septic shock counteracts myocardial depression, maintains oxygen transport, and reduces adrenergic support compared with conventional treatment alone.

in the control group indicates further progress of the myocardial depression induced by mediators of septic shock (4, 27), as documented by the increments in NO_X and TNF-α.

In the present study, MB decreased plasma NO_X and tended to reduce TNF-α concentrations, in agreement with the effect of other NOS inhibitors (28, 29). In a recent study on cultured rat cardiac myocytes exposed to TNF-α, IL-1β, and serum from septicemic patients, MB prevented myocyte depression and restored myocyte shortening velocity (30). Because MB also has been shown to reduce the endotoxin-induced TNF-α synthesis in Kupffer cells (31), it is conceivable that MB counteracts the detrimental effects of this cytokine on other target organs as well, including the heart. Although it was not addressed in the present study, the action of MB on the inflammatory response may be more complex. In our own experiments on endotoxemic sheep, we found that the MB improvement of the cardiopulmonary function was associated with a reduction of the arachidonic acid metabolites thromboxane B_2 and prostaglandin $F_{1\alpha}$ (9, 32), whereas TNF-α and IL-6 remained unchanged (Evgenov et al., unpublished observations). However, the latter sheep model did not produce a hyperdynamic state, which was observed in the majority of patients in the present study.

The improved myocardial function with MB may also partly result from increased sensitivity of the cardiovascular system to catecholamines, resulting from inhibition of excessively produced NO/cGMP (9–11). The latter assumption is consistent with *in vitro* findings after endotoxin shock in rats demonstrating that MB restores the contractions of rat aortic rings to norepinephrine (12). This effect of MB may have contributed to the reduction of adrenergic support in the present study as well as in other clinical investigations of MB (14, 18). However, no significant intergroup differences were seen regarding the duration of the adrenergic support and the septic shock, probably because of the limited period of MB administration.

As compared with healthy volunteers, the patients had a fourfold increase in circulating NO_X. A similar increase has been reported in a recent investigation of human septic shock demonstrating a negative correlation between plasma NO_X and SVRI (29). In the present study, most of the patients had low SVRI at inclusion. In contrast to previous investigations, where MB was administered as bolus injections (15–17), infusion of MB at rates of 0.25–1 mg/kg/hr caused less severe rises in SVRI and MAP. This may be explained by the fact that we reduced the infusion of adrenergic agents while keeping MAP within 70–90 mm Hg, whereas in other studies the infusion rate remained unchanged. The present investigation was also designed to avoid increases in PVRI and pulmonary arterial pressure, as previously reported after MB (15, 17). It has been shown that enhanced vasoconstriction after NOS inhibitors may reduce CI and even increase the mortality of patients with septic shock (21, 33, 34). Consequently, we included only patients with signs of hyperdynamic septic shock assuming that iNOS was activated.

The lack of effect of MB on gas exchange is consistent with the findings in other studies (13, 16, 17). Nevertheless, a study on septicemic rats demonstrated that MB might reduce the alveolar injury and improve the arterial oxygenation (35), possibly secondary to suppression of free radical formation (36). The latter observation also contrasts a finding of worsened gas exchange probably caused by pulmonary vasoconstriction following bolus injection of MB in human septic shock (15). Thus, if septic shock is complicated by pulmonary hypertension, inhibitors of the NO pathway should be administered with caution.

In the MB group, stable CI in the presence of unchanged oxygenation kept DO_2I above 600 mL/min/m^2. Such a level of DO_2I is assumed to be beneficial for survival in sepsis (37). This effect of MB may explain the trend to resolution of shock for a larger proportion of patients, although no significant statistical difference was noted with respect to the survival rate. In the control group, DO_2I decreased in parallel with a reduction of CI and VO_2I. The latter may indicate maldistribution of blood flow and derangement of metabolism (38) as documented by the progress of organ dysfunction in the control group of the present study.

The bolus dose of MB (2 mg/kg) was similar to the dose used in other clinical investigations (13, 16, 18) but lower than those employed in most experimental studies (9–11, 35). The same dose has been recommended for treatment of drug-induced methemoglobinemia, with a possibility of a repeat injection (5). Because the clinical effects of MB persist for 2–3 hrs (16, 17), we established a 2-hr interval between the injection and start of infusion. To minimize the risk of toxic effects, we did not continue administration of MB beyond 6 hrs. As evaluated by the changes in hemodynamics, the most

efficient infusion rates of MB were from 0.5 to 2 mg/kg/hr. However, infusion rates of 0.5–1 mg/kg/hr already resulted in a marked elevation of plasma MB. The latter rate is probably sufficient inasmuch as the plasma concentration of MB represents only one half of the whole blood concentration, and the half-life of intravenously administered MB is 102 mins (26, 39). The total accumulated dose of MB was 5.75 mg/kg, which is consistent with that used in a previous case of human septic shock treated with continuously infused MB (19). This is also close to the highest recommended daily dose of MB (5), and seven times lower than the 24-hr LD_{50} for sheep (39).

We observed no significant adverse effects of MB, as evaluated by the selected organ function variables. This is in agreement with previous clinical studies (13, 17). The rise in methemoglobin following MB did not exceed the values reported by other authors (16). The fact that MB tended to reduce platelet count when compared with the controls is consistent with a clinical report employing the NOS inhibitor, N^G-methyl-L-arginine hydrochloride (21). The latter finding is, most likely, caused by inhibition of the antiaggregatory effect of endothelial cGMP resulting in increased procoagulant activity (40). Although we found no clinical evidence of thromboses or bleeding complications, it would seem prudent in future studies to use the continuous infusion of MB at a rate of <2 mg/kg/hr to avoid coagulation disorders. The transient blue coloring of the skin and the urine is considered to be the result of reduction of the slowly excreted leuco form of MB in the tissues (5). The effect of MB on lowering body temperature observed in the present investigation is consistent with results obtained in sheep and rabbits exposed to endotoxin (9, 41). Apparently, MB influences the febrile reaction by modulation of the interaction between NO and the cyclooxygenase pathway (32, 41), or through reduced generation of oxygen free radicals (36).

In conclusion, continuously infused MB as an adjuvant treatment to patients with septic shock counteracts myocardial depression, maintains oxygen transport, and reduces adrenergic support compared with conventional treatment alone. Although MB may influence the coagulation profile, the infusion of MB appears to have no adverse effects on organ functions. Because of the pilot design and limited size of the present study, the heterogeneity of the patient population, and the short period of observation, our findings warrant confirmation by a larger clinical trial of MB infused in a dose-titrated manner.

ACKNOWLEDGMENTS

We thank Tormod Brenn, PhD, for reviewing the statistical methods used in the present study, Wenche H. Bakkelund for technical assistance, Rod Wolstenholme for preparation of the figures, and Peter McCourt, PhD, for linguistic corrections.

REFERENCES

1. Bone RC: The pathogenesis of sepsis. *Ann Intern Med* 1991; 115:457–469
2. Liaudet L, Soriano FG, Szabo C: Biology of nitric oxide signaling. *Crit Care Med* 2000; 28:N37–N52
3. Parrillo JE: Pathogenetic mechanisms of septic shock. *N Engl J Med* 1993; 328:1471–1477
4. Wolkow PP: Involvement and dual effects of nitric oxide in septic shock. *Inflamm Res* 1998; 47:152–166
5. Martindale W: The Extra Pharmacopoeia. 29th Edition. London, Pharmaceutical Press, 1989, pp 843–844
6. Gruetter CA, Kadowitz PJ, Ignarro LJ: Methylene blue inhibits coronary arterial relaxation and guanylate cyclase activation by nitroglycerin, sodium nitrate, and amyl nitrite. *Can J Physiol Pharmacol* 1981; 59:150–156
7. Mayer B, Brunner F, Schmidt K: Novel actions of methylene blue. *Eur Heart J* 1993; 14:22–26
8. Lomniczi A, Cebral E, Canteros G, et al: Methylene blue inhibits the increase of inducible nitric oxide synthase activity induced by stress and lipopolysaccharide in the medial basal hypothalamus of rats. *Neuroimmunomodulation* 2000; 8:122–127
9. Evgenov OV, Sager G, Bjertnaes LJ: Methylene blue reduces lung fluid filtration during the early phase of endotoxemia in awake sheep. *Crit Care Med* 2001; 29:374–379
10. Paya D, Gray GA, Stoclet JC: Effects of methylene blue on blood pressure and reactivity to norepinephrine in endotoxemic rats. *J Cardiovasc Pharmacol* 1993; 21:926–930
11. Zhang H, Rogiers P, Preiser J-C, et al: Effects of methylene blue on oxygen availability and regional blood flow during endotoxic shock. *Crit Care Med* 1995; 23:1711–1721
12. Wu CC, Chen SJ, Yen MH: Nitric oxide-independent activation of soluble guanylyl cyclase contributes to endotoxin shock in rats. *Am J Physiol* 1998; 275:H1148–H1157
13. Preiser J-C, Lejeune P, Roman A, et al: Methylene blue administration in septic shock: A clinical trial. *Crit Care Med* 1995; 23: 259–264
14. Driscoll W, Thurin S, Carrion V, et al: Effect of methylene blue on refractory neonatal hypotension. *J Pediatr* 1996; 129:904–908
15. Gachot B, Bedos JP, Veber B, et al: Short-term effects of methylene blue on hemodynamics and gas exchange in humans with septic shock. *Intensive Care Med* 1995; 21: 1027–1031
16. Daemen-Gubbels CR, Groeneveld PH, Groeneveld AB, et al: Methylene blue increases myocardial function in septic shock. *Crit Care Med* 1995; 23:1363–1370
17. Andresen M, Dougnac A, Diaz O, et al: Use of methylene blue in patients with refractory septic shock: impact on hemodynamics and gas exchange. *J Crit Care* 1998; 13:164–168
18. Schneider F, Lutun P, Hasselmann M, et al: Methylene blue increases systemic vascular resistance in human septic shock. *Intensive Care Med* 1992; 18:309–311
19. Brown G, Frankl D, Phang T: Continuous infusion of methylene blue for septic shock. *Postgrad Med J* 1996; 72:612–624
20. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: Definitions for sepsis and organ failures and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992; 20:864–874
21. Grover R, Zaccardelli D, Collice G, et al: An open-label dose escalation study of the nitric oxide synthase inhibitor, N^G-methyl-L-arginine hydrochloride (546C88), in patients with septic shock. *Crit Care Med* 1999; 27: 913–922
22. Le Gall JR, Lemeshow S, Saulnier F: A new Simplified Acute Physiology Score (SAPS II) based on an European/North American multicenter study. *JAMA* 1993; 270:2957–2963
23. Vincent J-L, Moreno R, Takala J, et al: The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. *Intensive Care Med* 1996; 22:707–710
24. Espevik T, Nissen-Meyer J: A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. *J Immunol Methods* 1986; 95:99–105
25. Mosmann T: Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; 65:55–63
26. DiSanto AR, Wagner JG: Pharmacokinetics of highly ionized drugs. I. Methylene blue—whole blood, urine, and tissue assays. *J Pharm Sci* 1972; 61:598–602
27. Grocott-Mason RM, Shah AM: Cardiac dysfunction in sepsis: new theories and clinical implications. *Intensive Care Med* 1998; 24: 286–295
28. Danner RL, Cobb J, Van Dervort AL: Nitric oxide as a mediator of hypotension and inflammation in sepsis. *In:* Role of Nitric Oxide in Sepsis and ARDS. Fink MP, Payen D (Eds). Berlin, Springer-Verlag, 1995, pp 155–180
29. Avontuur JA, Stam TC, Jongen-Lavrencic M, et al: Effect of L-NAME, an inhibitor of nitric oxide synthesis, on plasma levels of IL-6, IL-8, TNFα and nitrite/nitrate in human sep-

tic shock. *Intensive Care Med* 1998; 24: 673–679
30. Kumar A, Brar R, Wang P, et al: Role of nitric oxide and cGMP in human septic serum-induced depression of cardiac myocyte contractility. *Am J Physiol* 1999; 276:R265–R276
31. Harbrecht BG, Wang SC, Simmons RL, et al: Cyclic GMP and guanylate cyclase mediate lipopolysaccharide-induced Kupffer cell tumor necrosis factor-α synthesis. *J Leukoc Biol* 1995; 57:297–302
32. Evgenov OV, Eliassen LK, Bjertnaes LJ: Methylene blue impairment of lung fluid filtration in awake endotoxemic sheep is due to inhibition of cyclooxygenase products. Abstr. *Acta Anaesthesiol Scand* 1997; 41(Suppl 110):174
33. Avontuur JA, Tutein Nolthenius RP, Buijk SL, et al: Effect of L-NAME, an inhibitor of nitric oxide synthesis, on cardiopulmonary function in human septic shock. *Chest* 1998; 113:1640–1646
34. Grover R, Lopez A, Lorente J, et al: Multicenter, randomized, placebo-controlled, double blind study of the nitric oxide synthase inhibitor 546C88: Effect on survival in patients with septic shock. Abstr. *Crit Care Med* 1999; 27(Suppl):A33
35. Galili Y, Kluger Y, Mianski Z, et al: Methylene blue—A promising treatment modality in sepsis induced by bowel perforation. *Eur Surg Res* 1997; 29:390–395
36. Salaris SC, Babbs CF, Voorhees WD III: Methylene blue as an inhibitor of superoxide generation by xanthine oxidase. A potential new drug for the attenuation of ischemia/reperfusion injury. *Biochem Pharmacol* 1991; 42:499–506
37. Yu M, Burchell S, Hasaniya NW, et al: Relationship of mortality to increasing oxygen delivery in patients ≥50 years of age: A prospective, randomized trial. *Crit Care Med* 1998; 26:1011–1019
38. Abraham E, Shoemaker WC, Bland RD, et al: Sequential cardiorespiratory patterns in septic shock. *Crit Care Med* 1983; 11:799–803
39. Burrows GE: Methylene blue: Effects and disposition in sheep. *J Vet Pharmacol Ther* 1984; 7:225–231
40. Alheid U, Reichwehr I, Forstermann U: Human endothelial cells inhibit platelet aggregation by separately stimulating platelet cyclic AMP and cyclic GMP. *Eur J Pharmacol* 1989; 164:103–110
41. Lin JH, Lin MT: Nitric oxide synthase-cyclooxygenase pathways in organum vasculosum laminae terminalis: Possible role in pyrogenic fever in rabbits. *Br J Pharmacol* 1996; 118:179–185