Use of Methylene Blue in Sepsis: A Systematic Review

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A systematic review of the literature was conducted to determine if the administration of methylene blue in humans improves hemodynamic status and/or outcome in patients with septic shock. Studies were identified from MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials database. The review included human studies of patients with septic shock treated with methylene blue in which hemodynamic variables or mortality rates were reported. An electronic form was used to extract items including study design, population characteristics, intervention details, and outcomes. No meta-analysis was performed. Methylene blue administration in patients with septic shock increases mean arterial pressure and systemic vascular resistance while decreasing vasopressor requirements. Increased pulmonary vascular resistance has been reported with bolus administration but might be avoided by continuous infusion. No other ill effects were reported. Effects on mortality have not been adequately evaluated in the literature.

Key words: *septic shock, hypotension, nitric oxide, methylene blue*

Despite advances in diagnosis and treatment, mortality rates associated with sepsis and septic shock remain unacceptably high [1]. Widespread vasodilation and a decrease in myocardial contractility result in hypotension. Unresponsive hypotension is present in half of the patients who die from sepsis and is the number one cause of death in the first week after diagnosis; in the second week, multiple organ failure resulting from prolonged hypotension and maldistribution of blood flow is the leading cause of death [2].

The mechanism of widespread vasodilation involves the activation of the soluble intracellular enzyme guanylate cyclase (GC) by nitric oxide (NO), resulting in the production of cyclic guanosine monophosphate (cGMP). Initially discovered as an endothelium-derived relaxing factor in blood vessels, NO is made by the enzyme nitric oxide synthase (NOS) [3,4]. There are 2 general subtypes of NOS: (1) constitutive (cNOS) is constantly active and is further subdivided into neuronal (nNOS) and endothelial (eNOS); (2) inducible (iNOS) is activated under the influence of endotoxin and cytokines.

In vitro studies have shown that, when exposed to lipopolysaccharides (LPS), isolated macrophages increase production and release of cytokines such as tumor necrosis factor, interleukins, and platelet-activating factor [5]. These activate iNOS in endothelium and vascular smooth muscle, resulting in a drastic increase in NO and subsequently cGMP. Ultimately, this pathway leads to vasodilation, myocardial depression, and increased vascular permeability.

It has been suggested that the inhibition of NO generation might be a treatment option for sepsis and septic shock. One way of doing this is via competitive antagonism of NOS; analogues of L-arginine (a substrate for NOS action) such as N^G-monomethyl-L-arginine (L-NMMA) have been used [6]. In animal studies, these analogues have been shown to significantly increase mean arterial pressure (MAP) in LPS-induced sepsis [7,8]. However, these studies also showed no ultimate improvement in the circulatory state of the animals; in fact, a study in canines demonstrated increased mortality [9]. In humans, recent phase III clinical trials have demonstrated a correlation in NO inhibition with increased overall mortality rate [10]. One explanation for this observation is that NO has roles in numerous other potentially beneficial pathways, and global NOS inhibition can result in deleterious effects.

Efforts have shifted to investigating methods of inhibiting only the inducible form of NOS as well as targeting the generation of cGMP itself [5]. Methylene blue (MB) is a chemical dye that easily crosses

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cell membranes, inhibits iNOS, and is capable of inhibiting the GC enzyme in vascular smooth muscle cells [11]. Animal studies have provided evidence of hemodynamic benefits from MB infusion during endotoxic shock; MAP and peripheral vascular resistance were consistently increased with little effect on cardiac output in rat, rabbit, and dog models of sepsis [12,13]. There is also evidence that MB attenuates the inhibition of mitochondrial function as well as decreases acute lung injury in sepsis [14,15]. MB has been used clinically for many years in the treatment of methemoglobinemia, malaria, and pharmacologically induced priapism, and its use has been shown to be safe in humans [12].

Much is still unknown about the use of MB in septic humans. The scarcity of large population-based, controlled studies makes MBs effectiveness difficult to assess, and the dosage and duration of administration have not been well addressed [16]. We know of no systematic reviews previously published on this topic.

The purpose of this study is to systematically review the literature to determine if the administration of MB improves hemodynamic status and/or outcome in patients with septic shock.

Materials and Methods

Four search strategies were used to identify potentially relevant studies. The MEDLINE (1996 to July 2005) database was searched by combining results from the keyword search of *methylene blue* with the results from exploding the medical subject heading of *sepsis*. The EMBASE (1980-2005 week 27) was searched using the keywords *methylene blue* and *septic shock* or *sepsis* or *septicemia*. The Cochrane Central Register of Controlled Trials (3rd quarter 2005) was searched using the keywords *methylene blue* and *septic shock* or *sepsis* or *septicemia*. References from the included articles were searched for relevant citations.

Only human studies were considered for inclusion. The condition treated had to meet 1 of the following definitions: (1) sepsis, defined by presence of systemic inflammatory response syndrome with demonstrated infection; (2) septic shock, defined by presence of hypotension (systolic arterial pressure <90 mm Hg) and signs of tissue hypoperfusion (oliguria, arterial lactate >2.5 mmol/L); or (3) refractory septic shock, defined by the presence of arterial hypotension persisting despite fluid administration and simultaneous intravenous infusion of 2 or more vasoactive drugs for 1 hour. Case reports, case series, and controlled trials were eligible for inclusion. Studies involving patients younger than 16 years of age were excluded.

Two independent reviewers assessed the titles and abstracts using the inclusion/exclusion criteria. If there was insufficient information to determine inclusion, the full text was reviewed. Articles selected by either or both of the reviewers were included in this study. Data extraction was performed using a standard electronic form.

Results

The initial search produced 125 potentially relevant articles; 14 articles met the inclusion criteria. One was excluded because participants' ages fell below 16 yrs. In all cases, the reviewers agreed on the studies to be included.

Two studies were randomized, controlled trials, one involving 20 patients and the other 30 patients (Table 1). In the study by Kirov et al [17], 20 patients with septic shock were randomized 1:1 to receive either isotonic saline or MB as an intravenous bolus injection (2 mg/kg), followed 2 hours later by an infusion at increasing rates of 0.25, 0.5, 1, and 2 mg/kg/h, each maintained for 1 hour. Hemodynamic variables were followed over 24 hours, and the investigators noted increased mean arterial pressures in those who received MB. Stroke volume and left-ventricular stroke work indexes were maintained with MB administration. Oxygen delivery was unchanged in the MB group, whereas it decreased in the control group. Those who received MB had reduced requirements for norepinephrine, epinephrine, and dopamine by 87%, 81%, and 40%, respectively. Twenty-eight days after the beginning of the study, survival rates were 50% in the MB group and 30% in those who received conventional treatment, but these results did not achieve statistical significance. No adverse effects were noted. Overall, the investigators concluded that infused MB counteracted myocardial depression, maintained oxygen transport, and reduced concurrent adrenergic support.

Memis and colleagues' [26] prospective, randomized, controlled trial had a slightly larger patient population. Thirty patients with sepsis were randomized 1:1 to receive isotonic saline or an infusion of MB (0.5 mg/kg/h) for 6 hours. The main purpose of the study was to examine the effects of MB on plasma levels of cytokines in severe sepsis. Mean arterial pressure and heart rate were reported at 24 and 48 hours post-treatment. The investigators found significantly increased mean arterial pressure in the MB group compared with control, with no significant adverse effects noted.

Table 1. Summary of Significant Results of Controlled Studies

Study (Study Design)	No. of Patients	Vasopressor Use (Pre-MB Administration)	MB Administration	Significant Outcomes (Compared With Controls)
Kirov et al 2001 [17] (Prospective, randomized, double-blinded, placebo-controlled)	Control (N = 10) Treatment (N = 10)	D >5 μg/kg/min NE >0.05 μg/kg/min E >0.05 μg/kg/min	(1) 2 mg/kg bolus over 15 min (2) Stepwise 1-h infusions of 0.25, 0.5, 1, and 2 mg/kg/h	MAP ↑ NE ↓ by 87% E ↓ by 81% D ↓ by 40% MPAP stable Oxygen delivery stable
Memis et al 2002 [18] (Prospective, randomized, placebo-controlled)	Control (N = 15) Treatment (N = 15)	None	0.5 mg/kg/h infusion over 6 h	MAP ↑

MB = methylene blue; D = dopamine; NE = norepinephrine; E = epinephrine; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure.

The remaining studies in this review were case reports or case series of patients in septic shock (Table 2). The studies that followed mean arterial pressures and/or systemic vascular resistance (SVR) all reported a significant increase in those variables for patients who received MB. Three of the studies also noted a significant decrease in the need for vasopressors. Five of the studies reported an increase in mean pulmonary artery pressure or pulmonary vascular resistance. The studies were too heterogeneous in their design and dosing to allow for meta-analysis.

Discussion

The majority of the studies were observational, with 2 relatively small controlled trials. We were unable to perform a meta-analysis, but a number of observations can be made about the results.

All of the studies demonstrated that MB administration results in an increase in systemic vascular resistance, reflected by an increase in mean arterial pressure and/or a decrease in vasopressor requirements. There is ample evidence of the role of NO in endotoxin- and tumor necrosis factor–induced vasodilation and decreased sensitivity to vasopressors [19,20]. Inhibition of NOS, or downstream of the NOS pathway, represents a potential method of counteracting the severe hypotension of septic shock.

A recent randomized trial of MB in post–cardiac surgery vasoplegia demonstrated a statistically and clinically significant 31.4% absolute reduction in mortality [21]. Vasoplegia is characterized by hypotension, low filling pressures, normal-to-high cardiac index, low SVR, and significant vasopressor requirements. It is thought of as a type of "pure" systemic inflammatory response. The improvement in mortality and morbidity rates seen as a result of MB use supports the possibility that it might be useful in septic shock.

Even more encouraging would be demonstration of improved oxygen delivery or a reduction in mortality. These end points were often not reported, and the small sample sizes left the studies underpowered to detect clinically significant differences. It is more difficult to anticipate the effect of MB on oxygen delivery than blood pressure. Studies have shown that although low doses of iNOS inhibitors help restore myocardial contractility in hearts exposed to proinflammatory cytokines, higher doses decrease contractility. It has been proposed that low levels of NO increase myocardial contractility, whereas higher levels result in myocardial depression [22].

Global NO blockade can contribute to an increase in pulmonary vascular resistance [23], which worsens the pulmonary hypertension that can be associated with sepsis. Trials that used high, bolus doses of MB demonstrated an increase in pulmonary pressures, but this effect was absent in trials that used MB infusions. Some researchers have thus suggested that infusions at low doses should always be used for this reason [24]. Simultaneous treatment with inhaled NO might also be considered for this side effect of NO inhibition.

A multiple-center, randomized, placebo-controlled phase II trial of L-NMMA, a nonselective NO inhibitor, demonstrated improved resolution of shock in patients with severe sepsis [25]. A subsequent phase III trial of the same drug was stopped prematurely because of an increase in mortality in the treated group [26].

Table 2. Summary of Results of Uncontrolled Observational Studies

Study	No. of Patients	MB Administration	Significant Outcomes
Andresen et al 1996 [27]	14	1 mg/kg bolus over 15 min	MAP ↑ SVR ↑ MPAP ↑
Andresen et al 1998 [28]	10	1 mg/kg bolus over 15 min	MAP ↑ SVR ↑ MPAP ↑
Brown et al 1996 [29]	1	(1) 1.5 mg/kg bolus (2) 17 mg/h infusion	MAP ↑ D ↓ (to 3 μg/kg/min) NE ↓ (to 0 μg/kg/min)
Daemen-Gubbels et al 1995 [30]	9	2 mg/kg bolus over 20 min	MAP ↑ Oxygen delivery ↑
Donati et al 2002 [31]	15	3 mg/kg bolus over 10 min	MAP ↑ MPAP ↑ SVR ↑
Gachot et al 1995 [32]	6	3 mg/kg bolus over 10 min	MAP ↑ MPAP ↑ SVR ↑ PVR ↑
Grayling and Deakin 2003 [33]	1	(1) 2 mg/kg bolus (2) 1 mg/kg/h infusion	MAP ↑
Preiser et al 1995 [34]	14	2 mg/kg bolus over 15 min	MAP ↑ SVR ↑
Schneider 1995 [35]	2	(1) 1 mg/kg bolus (2) 2 mg/kg bolus (3) 3 mg/kg bolus	MAP ↑ SVR ↑ NE ↓ (to 0 μg/kg/min)
Weingartner et al 1999 [36]	10	4 mg/kg bolus over 1 h	MAP ↑ SVR ↑ PVR ↑
Zygun 2005 [37]	1	2 mg/kg bolus over 30 min	MAP ↑ NE ↓ (to 0.5 μg/kg/min)

MB = methylene blue; D = dopamine; NE = norepinephrine; E = epinephrine; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure; SVR = systemic vascular resistance; PVR = pulmonary vascular resistance.

A number of points should be considered in evaluating the impact that these studies should have on the pursuit of NO inhibition. L-NMMA is a nonselective NOS blocker, inhibiting both cNOS and iNOS; cNOS is a component of normal physiology, and its inhibition might understandably result in undesirable changes in vascular tone. The phase III trial used a higher dose and longer duration of treatment, different targets for mean arterial pressure, and different inclusion criteria. It is difficult to know whether the change from benefit in the phase II trial to harm in the phase III trial is attributable to sampling error or the changes in study protocol.

If NOS inhibition is going to be used to treat septic shock, patient selection, timing, dose, and the use of selective versus nonselective inhibitors will all be important considerations. Methylene blue has potential as a selective iNOS pathway inhibitor with a known safety profile, clinical experience, and low cost. More randomized, controlled trials of MB in patients with septic shock are needed to determine whether the benefits of iNOS pathway suppression and its resulting increase in blood pressure translate to mortality benefits.

Conclusions

The use of MB in patients with septic shock results in increased systemic vascular resistance and mean arterial pressure, but its effect on oxygen delivery and mortality is unknown. Further randomized, controlled trials are needed to better define the role of this drug in septic shock.

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