Methylene Blue and Vasoplegia: Who, When, and How?

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Abstract: Systemic inflammatory response can be associated with clinically significant and, at times, refractory hypotension. Despite the lack of uniform definitions, this condition is frequently called vasoplegia or vasoplegic syndrome (VS), and is thought to be due to dysregulation of endothelial homeostasis and subsequent endothelial dysfunction secondary to direct and indirect effects of multiple inflammatory mediators. Vasoplegia has been observed in all age groups and in various clinical settings, such as anaphylaxis (including protamine reaction), sepsis, hemorrhagic shock, hemodialysis, and cardiac surgery. Among mechanisms thought to be contributory to VS, the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway appears to play a prominent role. In search of effective treatment for vasoplegia, methylene blue (MB), an inhibitor of nitric oxide synthase (NOS) and guanylate cyclase (GC), has been found to improve the refractory hypotension associated with endothelial dysfunction of VS. There is evidence that MB may indeed be effective in improving systemic hemodynamics in the setting of vasoplegia, with reportedly few side effects. This review describes the current state of clinical and experimental knowledge relating to MB use in the setting of VS, highlighting the potential risks and benefits of therapeutic MB administration in refractory hypotensive states.

INTRODUCTION

Systemic inflammatory response associated with profound vasodilation, known as vasoplegia or vasoplegic syndrome (VS), has been observed in a variety of settings. Despite lack of a uniform definition, the classic description of this phenomenon consists of a clinical state characterized by hypotension, low systemic vascular resistance and increased requirement for intravenous fluid and vasopressor administration [1-2]. This clinical entity has been most prominently associated with cardiopulmonary bypass (CPB) and severe sepsis, but has also been described in the setting of anaphylaxis and hemodialysis [2-5].

The incidence of vasoplegic syndrome (VS) varies, but may be as high as 10% in post-cardiac surgery patients and can reach 42% among patients following left ventricular assist device placement for end-stage heart failure [2, 6-7]. The exact incidence of vasoplegia in septic shock is not well defined, but VS may be present in approximately half of patients dying from sepsis [3, 5]. Vasoplegia has been also been noted in association with anaphylaxis and following the administration of protamine for reversal of systemic heparinization [8-12]. Vasoplegia has been observed in all age groups [13-14]. There is evidence that methylene blue (MB, Fig. **1**), an inhibitor of the vasodilatory effects of NO and other nitrovasodilators on endothelium and vascular smooth muscle, may be of therapeutic benefit in the setting of VS in a variety of clinical scenarios. This also suggests that dysfunction of a common pathophysiologic effector pathway may be responsible for VS, and that MB may offer potential therapy in refractory hypotension due to the activation of this pathway [1, 2, 4, 5, 15, 16].

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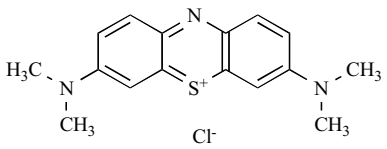

Fig. (1). Chemical structure of methylene blue.

This review will begin with an overview of the pathophysiology of VS, the various mechanisms believed to be involved in the vasoplegic response, and a description of what is known about the common end-effector pathway. This will be followed by a detailed discussion of MB pharmacology, dosing, routes of administration, and side effect profile. We will then focus on the potential role of MB as a therapeutic agent in various clinical settings, with emphasis on published clinical trials and experimental studies. A brief overview of nitric oxide synthase (NOS) inhibitors other than MB is also included, along with a short discussion of associated structure-function relationships.

OVERVIEW OF VASOPLEGIC SYNDROME: DEFINITIONS AND RISK FACTORS

It is generally agreed that vasoplegia or vasoplegic syndrome (VS) constitutes a pathophysiologic endothelial dysregulation state wherein persistent hypotension continues despite adequate fluid resuscitation and high-dose vasopressor administration. Despite that general agreement, the definition of VS varies depending on the literature source, and there is no established consensus definition of VS at present. While different definitions have been proposed for VS in the setting of cardiac surgery and sepsis, no formalized definitions for vasoplegia associated with other conditions, such as hemodialysis and anaphylaxis, exist. Consequently, a unified definition of VS is unlikely, as the various clinical settings associated with this phenomenon differ substantially. Table **1**

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Table 1. Definitions of Vasoplegia/Vasoplegic Syndrome (VS) According to Various Literature Reports

Author (Reference) and Year	Definition	Comment
Ozal *et al.* [17]	Arterial pressure <50 mmHg; Cardiac index >2.5 liters/min/m^2; Right atrial pressure <5 mmHg; Left atrial pressure <10 mmHg; Low systemic vascular resistance (<800 dynes/s/cm^5) during intravenous norepinephrine infusion.	The authors report experience in cardiac surgery patients.
Gomes *et al.* [18]	Perioperative vasodilatory shock accompanied by tachycardia and increased cardiac output in the absence of other causes of hypotension.	The authors report experience in cardiac surgery patients.
Donati *et al.* [3]	Systolic blood pressure <90 mmHg despite fluid loading titrated to achieve pulmonary artery occlusion pressure of 14-16 mmHg, and despite vasopressor agent infusion, including norepinephrine and/or dopamine infusion, started when urine output was <0.5 mL/kg/hr for a set period of time.	The authors report experience with MB in the setting of septic shock.

features the various published definitions of VS along with the corresponding citations [3, 17, 18].

Numerous factors have been identified as potential contributors to VS in different clinical settings. Perhaps the most is known about risks for VS in cardiac surgery patients. Independent risk factors for postoperative vasoplegia following cardiac surgery include preoperative intravenous heparin use, angiotensin-converting enzyme (ACE) inhibitor use, and calcium channel blocker (CCB) use. The incidence of vasoplegia associated with preoperative administration of these medications is as follows: (a) 44% for ACE inhibitors; (b) 47% for CCB; and (c) over 55% for intravenous heparin use [15, 19, 20]. While the actual incidence of VS among patients with these predisposing factors may vary, it is important to be aware and recognize these agents as potentially associated with perioperative VS [2].

With anaphylactic shock, refractory vasoplegia is encountered in relatively few cases [21]. In the setting of septic shock, although the primary source of shock is usually known, the vasoplegic response may or may not depend on adequate source control, depending on how advanced is the overall pathophysiologic process. Evidence also indicates that the hypotension associated with hemodialysis may be due to NO-dependent mechanisms [22, 23].

While the search continues for precise determination of risk factors for VS among patients with septic shock, anaphylactic shock, and those undergoing cardiopulmonary bypass, significant evidence exists with regards to the nature of a common effector pathway behind VS.

PATHOPHYSIOLOGY OF VASOPLEGIC SYNDROME AND ASSOCIATED EFFECTOR PATHWAYS

Physiologic response to an injurious stimulus is usually self-limited and commensurate to the initial stimulus. In more severe cases, the response may inappropriately persist, leading to severe systemic inflammatory response syndrome (SIRS), multiorgan dysfunction (MOD), multiorgan failure (MOF) and ultimately, death [16]. One of the most important manifestations of severe SIRS is circulatory failure with low systemic vascular resistance (SVR), low mean arterial pressure, systemic hypoperfusion and tissue malperfusion [1]. The physiologic response of SIRS appears to be mediated by: (a) various neurotransmitters (including acetylcholine, adenosine triphosphate (ATP) and substance P); (b) factors responsible for hemostasis (including adenosine diphosphate (ADP), serotonin, bradykinin and thrombin); as well as (c) biologic amines (including norepinephrine and histamine) [1]. These mediators, in turn, induce the synthesis of two endothelial autocoids – endothelium derived relaxing factor (EDRF or nitric oxide) and prostacyclin (PGI2) – contributing to vasoplegia [1].

Regardless of the initial etiology, VS appears to represent a dysregulation of NO synthesis/release and vascular smooth muscle cell GC activation. Nitric oxide is produced by two types of NO synthase (NOS) relevant to this review, a constitutive endothelial (eNOS) type and an inducible (iNOS) type [2]. Upregulation of iNOS and increases in NO production lead to generation of cyclic guanosine 3'-5' monophosphate (cGMP). This leads to myocardial depression, reduced contractile response to vasoconstrictor agents, increased vascular permeability, and circulatory collapse (Fig. **2**) [5, 18, 24, 25]. Nitric oxide-dependent pathways also participate in the pathophysiology of vasoplegia following hemorrhagic shock and post-cardiac arrest states [26, 27].

Vasoplegic syndrome has been attributed to a combination of endothelial injury, arginine-vasopressin system dysfunction, and the release of other vasodilatory inflammatory mediators [28, 29]. The most prominent inflammatory mediators known to increase NO production by iNOS include tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) (Fig. **3**) [30, 31]. These mediators, in turn, lead to activation of endothelial and vascular smooth muscle iNOS, resulting in drastic increase in NO and cGMP [2, 5]. Despite different initial stimuli, vasoplegias due to the various disease states appear to share the final effector pathway – activation of the soluble intracellular enzyme GC by numerous mediators (including NO), with subsequent production of cGMP [15, 18].

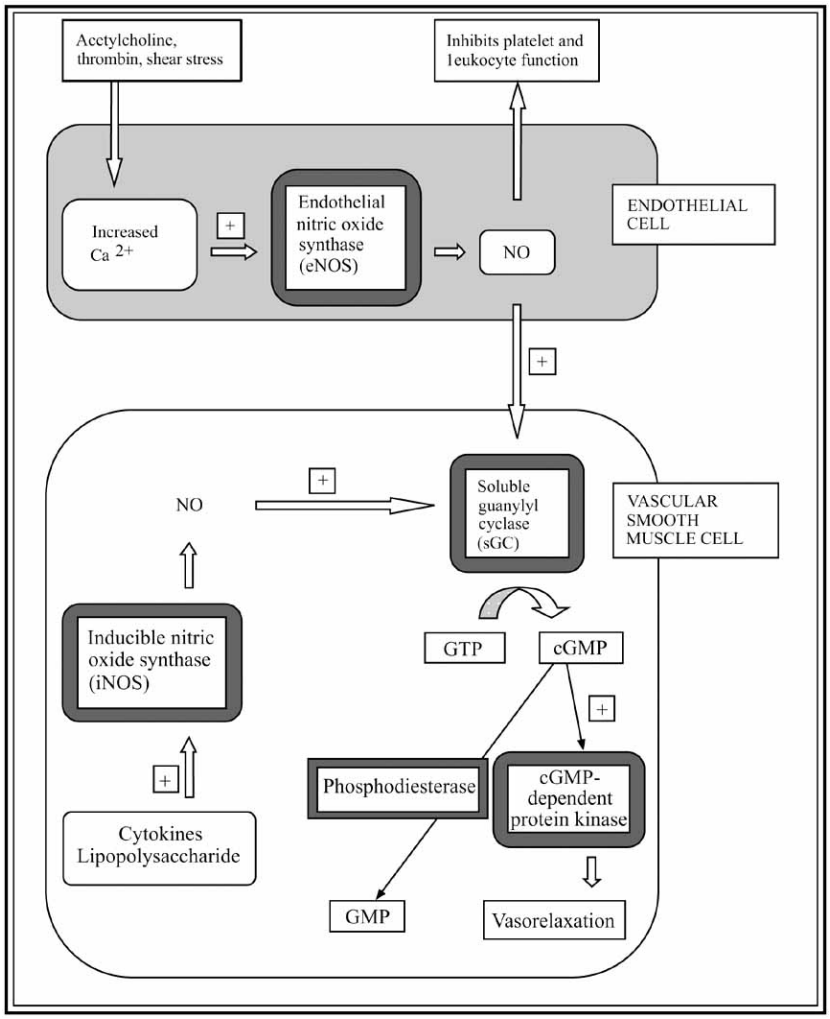

Fig. (2). Schematic representation of the NO-sGC-cGMP cycle, demonstrating the physiologic continuum between the endothelial cell and the vascular smooth muscle cell.

In addition to the traditionally accepted NO-mediated mechanisms, there is also evidence that vasoplegia may also be due to non-NO/cGMP-dependent pathways [32, 33]. It would not be suprising to find that more than one pathway is involved in the pathophysiology of VS. Regardless of the inciting mechanism, cGMP appears to be the most prominent mediator of vasodilation and decreased myocyte contractility in vascular smooth muscle [31, 34].

Vasoplegia due to non-septic mechanisms can be thought of as a type of "pure" form of SIRS. Vasoplegia following CPB is an example of such a state, and is characterized by increased atrial natriuretic peptide (ANP) levels, leading to increases in intracellular cGMP [2]. Although other factors, including endothelial injury, arginine-vasopressin system dysfunction, release of various inflammatory mediators (TNF-α and other interleukins) and free radicals have all been implicated in the development of post-cardiac surgery vasoplegia, it is thought that the vasodilatory state is based on the activation of the GC [28, 35-37].

In anaphylaxis, severe cardiovascular dysfunction can also lead to profound hypotension that, if untreated, may lead to systemic hypoperfusion and death [38]. Vasoplegia can be seen in the setting of acute, severe, and potentially fatal anaphylactic allergic reactions, where cardiovascular collapse may become refractory to the traditional treatment with intravenous fluids, epinephrine, corticosteroids, and supplemental oxygen [4, 8, 9, 38]. The cascade that leads to vasoplegia in anaphylaxis seems to be mediated by the platelet-activating factor (PAF), a biologically active phospholipid that is also known to contribute to hypotension and cardiac dysfunction during hemorrhagic, traumatic, and septic shock [38, 39]. While the molecular pathways downstream PAF-R triggering are still poorly defined, the final pathway of anaphylaxis-associated vasoplegia appears to be NO-dependent (Fig. **4**) [39].

Nitric oxide plays an important role in the physiologic mechanisms responsible for the hypotension of endotoxemia and septic shock [40]. Here, the pathophysiologic changes behind VS are thought to be due to actions induced by nitric oxide (NO) production from L-arginine *via de novo* synthesis of iNOS in various organs (the heart, the lungs, the vascular smooth muscle cells), under the influence of proinflam-

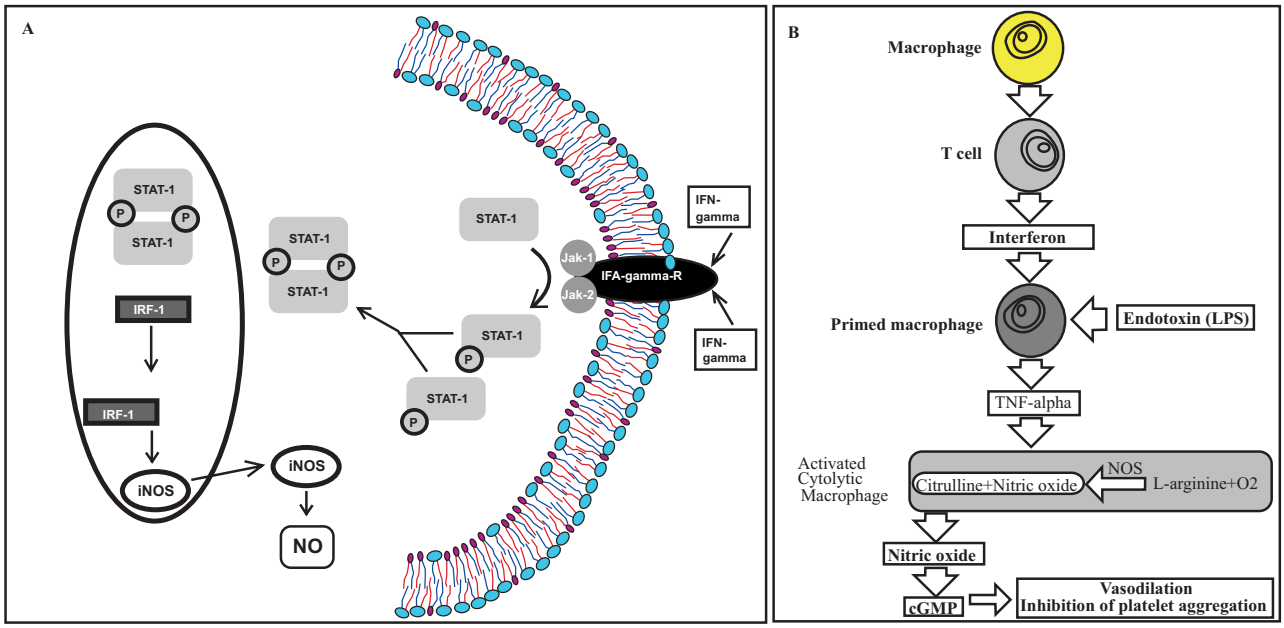

Fig. (3). (A) *Inducible nitric oxide synthase (iNOS) generation.* Nitric oxide (NO) is generated by inducible nitric oxide synthase (iNOS) following cellular exposure to cytokines (i.e., interferon-gamma or IFN-gamma). The IFN-gamma receptor (IFN-gamma-R) signals through the Janus kinase (JAK) family and signal transducer and activators of transcription (STAT) proteins. Receptor activation and dimerization induces the phosphorylation of STAT proteins. Activated STAT proteins, in turn, dimerize and translocate into the nucleus, where they induce expression of the transcription factor, IRF-1. IRF-1 then binds to specific DNA elements in the iNOS gene promoter region and increases iNOS gene expression. iNOS is a soluble enzyme that does not require elevated intracellular calcium levels for activation. **(B)** *The role of tumor necrosis factor alpha (TNF-alpha) in NO/cGMP pathway*. This example shows the progression of physiologic signaling in response to sepsis/endotoxin production. An interaction between the macrophage and the T cell leads to increased interferon production. This, in conjunction with LPS, leads to macrophage priming. The primed macrophage then produces TNF-alpha, resulting in the activation of NOS and production of NO, cGMP, and their 'downstream' physiologic effects.

matory cytokines and/or endotoxin [2, 24, 40, 41]. Subsequently, NO stimulates soluble guanylate cyclase (sGC) which, in turn, generates cyclic guanosine 3'-5' monophosphate (cGMP), leading to cGMP-mediated vasodilation and decreased myocyte contractility [31, 34]. Additional harmful effects of iNOS-produced NO in sepsis include impaired gas exchange, vascular leakage, and multi-organ failure [18, 40-44]. In contrast, the constitutive eNOS produces minute amounts of NO, maintaining basal regional vascular tone and blood flow [41].

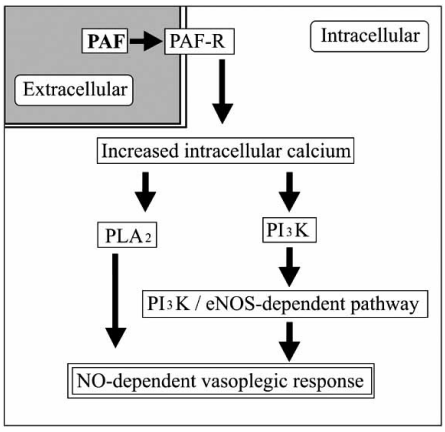

Fig. (4). *Schematic representation of the proposed mechanism of anaphylaxis-associated vasoplegia.* Platelet-activating factor interacts with a unique G protein-coupled 7 transmembrane receptor (PAF-R) and activates a number of signaling pathways. These pathways elevate intracellular calcium concentrations and activate phospholipase A2 (PLA2) and PI3K. The pathway that leads to vasoplegia appears to be NO-dependent. There is also some evidence that the vasoplegic response may be due to PI3K / eNOS-derived NO-based mechanism. Based on references 38 and 39.

METHYLENE BLUE: THE MOLECULE

Methylene blue USP (3,7-dipropan-2-ylphenothiazine chloride and 3,7-dipropan-2-ylphenothiazine chloride trihydrate, Fig. **1**) has a molecular weight of 371.923 and consists of dark green crystals that turn to deep blue color when dissolved in water or alcohol. The molecular formula of MB is $C_{16}H_{18}Cl\ N_3S{\cdot}3H_20$. Methylene blue (MB) is a soluble guanylyl cyclase (sGC) inhibitor that blocks GC action in vascular smooth muscle, scavenges NO and inhibits NO synthesis [21, 45-48]. By binding to the iron heme-moiety of sGC and blocking sGC action in vascular smooth muscle, MB decreases the levels of cGMP and alleviates the vasorelaxant effect seen in VS [6, 21, 45, 46]. Methylene blue also has the ability to scavenge NO as well as to inhibit NO synthesis [46, 47]. Having no sulfonic acid groups, MB does not bind to plasma proteins [49].

Methylene blue readily enters the erythrocyte, and at low concentrations is reduced to leucomethylene blue (Fig. **5**).

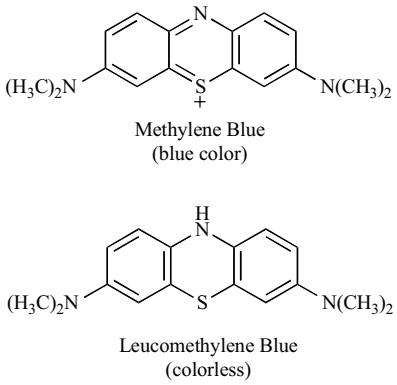

Fig. (5). Conversion of Methylene Blue to colorless Leucomethylene Blue.

MB is eliminated in bile, feces, and urine as leucomethylene blue [50, 51]. At higher doses, MB itself becomes an oxidant, and in the presence of excess MB or a defect in the methemoglobin reduction mechanisms, the dye accumulates and functions as a hemoglobin oxidizing agent, resulting in hemolysis, methemoglobinemia, and hyperbilirubinemia [50].

METHYLENE BLUE: INDICATIONS, CONTRAINDICATIONS, ADMINISTRATION, DRUG INTERATIONS AND SIDE EFFECTS

The literature reports numerous uses of MB, both orally and intravenously [52]. Perhaps the most common use of MB is as a dye in clinical investigations of the gastrointestinal or urinary systems [50, 52]. Methylene blue has also been used in oncology for sentinel lymph node detection [53]. However, this specific use of MB is decreasing and other dyes (Evan's blue, fluorescein, and indigo carmine) are being used more often [50]. A less common but well-established use of MB is the treatment of methemoglobinemia complicating topical benzocaine use, where MB hastens the conversion of methemoglobin to hemoglobin (Fig. **6**) [13, 52, 54]. Methylene blue has also been utilized in patients with congenital methemoglobinemia, priapism, neonatal hypotension, as an anti-malarial agent, hemolysis, and in clinical situations pertinent to this review – vasoplegias of cardiac surgery, sepsis, anaphylaxis, and hemodialysis [2, 4, 9-10, 22-23, 52, 55-59]. Methylene blue (MB) is available as a solution (10 mg/mL) and is usually administered enterally or intravenously. Oral absorption of MB ranges from 53% to 97% [2]. The onset of hemodynamic effects of MB is rapid [2]. Methylene blue has been used in patients of all ages [13, 14]. The recommended safe dose of MB appears to be between 1-2 mg/kg and 3-4 mg/kg, depending on source [50, 54]. Of interest, intratracheal MB administration has also been described experimentally [60].

A single dose of intravenous MB (2 mg/kg, 20-minute infusion time) has been used as a 'rescue' treatment in the setting of severe vasoplegia [15, 61]. A similar dose (1.5-2.0 mg/kg of 4% MB) has reportedly been effective in the setting of anaphylaxis-related VS [62]. In infants, MB has also been used as adjunctive medical therapy in severe sepsis and to treat acquired methemoglobinemia, with recommended doses of 1-2 mg/kg for a full-term infant [50].

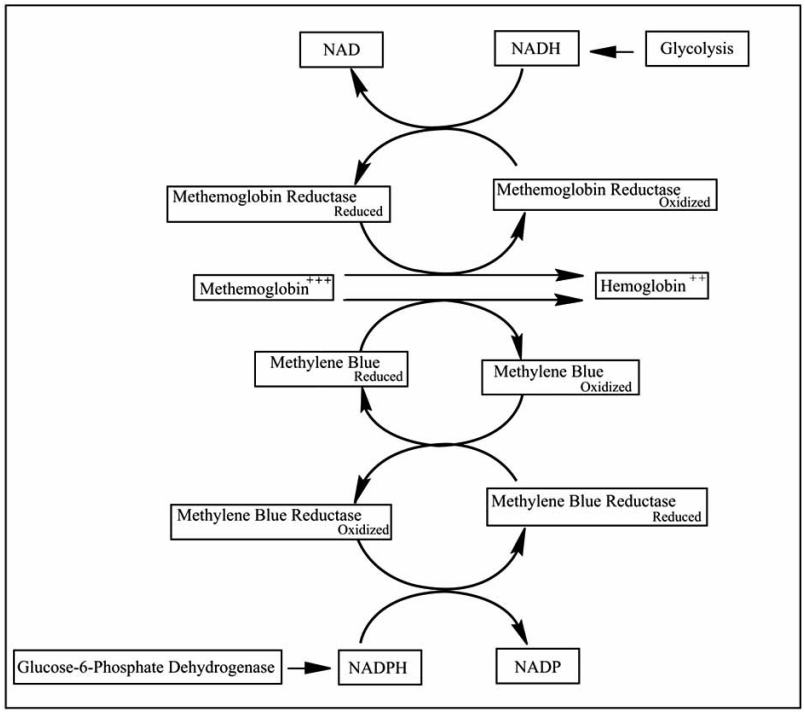

Fig. (6). *Schematic representation of the spontaneous (NADH) and dormant (NADPH) methemoglobin reductase systems.* Methemoglobin reductase is active in intact cells in the presence of substrates that can provide for NAD reduction. NAD then enzymatically reduces Methemoglobin to Hemoglobin. The NADPH system requires intact erythrocytes, glucose or its metabolic equivalent, a functioning pentose phosphate shunt (*via* Glucose-6-Phosphate Dehydrogenase), and Methylene Blue. Methylene Blue Reductase reduces Methylene Blue, which in turn nonenzymatically reduces Methemoglobin to Hemoglobin.

Continuous MB infusion is an option for patients who do not respond to a single dose of MB, and has been administered for variable lengths of time (120 mg of MB diluted in dextrose 5% in water, given continuously from 1 to 6 hours) [2, 62]. In the setting of hemodialysis-associated hypotension, MB has been shown to be effective in preventing hypotension when given as a bolus of 1 mg/kg body weight followed by an infusion at 0.1 mg/kg for 210 minutes on dialysis days and as a bolus-only on non-dialysis days [22]. Escalating doses of MB infusion have also been used [63].

Several reports describe administration of MB intraoperatively during cardio-pulmonary bypass (CPB) to treat profound vasodilation [25, 64]. In one report, MB was added to the CPB pump prime as treatment for septic endocarditis-associated vasoplegia during a valve operation, and then postoperatively as an infusion [25]. In another report, CPB had to be re-instituted due to severe protamine reaction refractory to norepinephrine, with the vasoplegia alleviated after MB infusion [64].

Inhibition of excessive production and activity of NO and cGMP may be of therapeutic value in treatment of vasodilatory shock [23, 29]. As stated previously, there is evidence that NO contributes to endotoxin- and TNF-induced vasodilation and decreased vasopressor sensitivity [30, 65]. Inhibition of NOS-related pathways by MB represents a potential treatment of vasodilatory shock-associated VS [5].

Methylene blue should not be used is patients with documented hypersensitivity to MB. Although contraindicated in severe renal insufficiency, MB has been used safely in hemodialysis-dependent patients [2, 22]. Methylene blue should be used cautiously in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency because of low levels of endogenous NADPH in this population (NADPH is essential to the production of leucomethylene) (Fig. **5** and Fig. **6**). This group of patients can develop a hemolytic anemia characterized by formation of Heinz bodies [66]. Methylene blue can also exacerbate dapsone-induced hemolytic anemia because of the formation of dapsone-reactive metabolite hydroxylamine, which oxidizes hemoglobin [67, 68]. The use of NO-cGMP pathway inhibitors may also be contraindicated in patients with hepatopulmonary syndrome and right to left intrapulmonary shunting, where it has been reported to result in reproducible and reversible worsening of hypoxemia despite substantial improvements in vascular tone and the hyperdynamic circulation [69].

Although rare, adverse reactions to intravenous MB may include cardiac arrhythmias (transient nodal rhythm and ventricular ectopy), coronary vasoconstriction, angina/precordial pain, decreased cardiac output, decreased renal blood flow, reduced mesenteric blood flow, increases in pulmonary vascular resistance and worsening gas exchange [17, 47, 64, 69]. Arrhythmias and angina associated with MB administration are usually transient and self-limiting [2].

Adverse reactions have been reported after oral administration of MB [50]. In the neonatal and pediatric population, enteric administration of more than 2 mg/kg of MB has been reported to result in severe methemoglobinemia, hemolytic anemia, Heinz body anemia, and hyperbilirubinemia [50, 70]. Heinz body hemolytic anemia and hyperbilirubinemia have also been seen after intraamnionic injections of MB [54, 70]. Methylene blue is reduced in the erythrocyte to leucomethylene blue, and is excreted primarily in the urine as leucomethylene blue and MB [71]. Anemia associated with MB usually manifests itself within 24 hours of administration, and can be seen up to 12 days after MB use [54]. The timing of anemia and hyperbilirubinemia complicating MB administration are similar, with the bilirubin peak at approximately 4 days after use and the anemia peak at approximately 5 days [54].

Most side effects of MB appear to be dose-dependent and do not occur with doses <2 mg/kg [72-74]. Most studies report normal renal function and pulmonary gas exchange (expressed as the ratio of PaO_2 to inspired oxygen fraction) following MB administration. Transient and self-limiting elevations in serum aspartate aminotransferase and alanine aminotransferase have been reported in one study, although it is not clear whether these elevations were due to MB, concurrent administration of norepinephrine, or both [17]. In one series, five patients who received preoperative intravenous MB infusion (3-5 mg/kg MB in 500 mL of normal saline) for localization of parathyroid adenomas, developed clinically significant but self-limiting postoperative encephalopathy [74]. It was postulated that the common factors in all five cases of encephalopathy were female gender of the patients and preoperative use of serotonin-metabolism modifying agents [74].

Methylene blue is well known to turn urine greenish-blue [17, 75]. This benign discoloration can be alarming to patients and their relatives, although it is self-limiting and disappears within a few days of discontinuing the drug. Mild skin discoloration can also be observed in most patients, but is self-limiting, and can be treated with administration of dilute hypochlorite solution [51].

Other side effects associated with MB include confusion, headache, fever, nausea, vomiting, abdominal pain and diaphoresis [2]. Subcutaneous injection of MB has been reported to cause necrotic abscesses [2]. Likewise, intradermal injections of MB have been noted to cause severe necrosis [76]. A rare but devastating photosensitivity epithelial desquamation has been reported in infants undergoing phototherapy after receiving MB [77, 78]. This serious reaction has been attributed to breakdown of lysosomal membranes after interacting with light in the presence of the photosensitizing MB [77, 78]. Anaphylactic reactions have been known to occur, although rarely, following MB administration [79].

Practitioners caring for patients who receive MB infusions have to be aware of the fact that MB interferes with the pulse oximeter's light emission (wavelength of approximately 660 nm), resulting in falsely depressed oxygen saturation readings [80]. The summary of literature describing adverse reactions to methylene blue can be found in Table **2** [50, 54, 69, 70, 74, 77, 79].

USE OF METHYLENE BLUE IN VASOPLEGIA: CLINICAL AND PRECLINICAL STUDIES

In the subsequent sections of this review, we will discuss the various areas of MB application in the setting of

Table 2. Summary of Selected Literature Reports Describing Adverse Events Associated with Methylene Blue Administration

Author (Reference) and Year	Type of Report (Sample Size) and Mode of MB Administration	Major Findings	Comment
Sills *et al.* [70] 1994	Case series. (N = 2) One neonate with trisomy 21 received MB as an intraoperative diagnostic marker. Another neonate received MB for type II glutaric acidemia. Adverse effects became apparent within hours of MB exposure.	Within hours of MB exposure, the infants voided green-blue urine, followed by hyperbilirubinemia, recurrent anemia requiring transfusions, and red blood cell dysmorphology. Red blood cell dysmorphology included the appearance of blister cells and Heinz bodies visible in both Wright's and supravital-stained peripheral blood smears. After the initiation of phototherapy, both infants exhibited cutaneous bullae followed by desquamation.	This report highlights the photosensitizing property of methylene blue, resulting in a desquamative response.
Porat *et al.* [76] 1996	Case report. (N = 1) Photosensitivity reaction following methylene blue administration in the setting of phototherapy for hyperbilirubinemia. Adverse reaction started with hours of exposure to phototherapy.	The patient's skin was initially deep blue. Within hours of exposure to phototherapy, redness developed on all exposed areas of the patient's skin. This was followed by bullae and desquamation of about 35% of the total skin surface area. The desquamation of erythematous areas continued even after discontinuation of phototherapy.	Complete re-epithelialization was achieved within 3 weeks of age.
Albert *et al.* [54] 2003	Case report. (N = 1) Hemolytic anemia, hyperbilirubinemia, and acute renal failure in an infant following enteric MB administration to confirm the integrity of the gastrostomy tube (6 mg/kg). Immediately following the procedure, the infant was noted to have bluish green urine and his nose was noted to be bluish, as expected. However, other adverse effects followed over the subsequent few days.	Three days after MB administration, the infant developed hyperbilirubinemia, hemolytic anemia, hemoglobinuria, and acute renal failure. Bilirubin peaked at 14.4 mg/dL, and hematocrit level dropped from 45% postoperatively to 18% on day 5. The blood urea nitrogen peaked at 74 mg/dL, and creatinine peaked at 1.3 mg/dL postoperatively on day 3. Hemolytic anemia on peripheral smear, with Heinz bodies and "bite" cells.	The patient had mild peripheral cyanosis with oxygen saturations in the high 80s despite normal arterial blood gas. Methemoglobin levels were elevated to 5.2 mg/dL (normal, 0 to 1.3 mg/dL) Recovery without long-term sequelae.
Allegaert *et al.* [50] 2004	Case report. (N = 1) Methemoglobinemia and hemolysis after enteral administration of MB in a preterm 1,135 gram infant with suspected tracheoesophageal fistula. MB given as 1 mL (10 mg/mL) diluted in 2 mL of normal saline.	The infant developed a discrepancy between oxygen saturation measurements and arterial oxygen tension, and was found to have signs of active hemolysis. Hyperbilirubinemia to 15 mg/dL was accompanied by a drop in hematocrit level from 44% to 34%. Concurrently, lactate dehydrogenase increased to 3,164 IU/L. Blood smear demonstrated fragmentocytes and signs of active erythropoiesis (increased normoblasts).	Treatment was supportive with hyperhydration, phototherapy, and a single blood transfusion. No signs of cutaneous phototoxicity were noted during phototherapy.
Dewachter *et al.* [78] 2005	Case report. (N = 1) Severe anaphylactic shock after intrauterine 1% MB instillation for verification of tubal permeability. Reaction occurred 2 minutes after MB administration.	Decreased O_2 saturation, $EtCO_2$ of 18 mmHg, hypotension (60/26 mmHg), tachycardia (140 beats per minute), severe bronchospasm. Following hemodynamic stabilization, generalized urticaria appeared. Treated with titrated epinephrine injections (total 1.8 mg), intravenous fluid bolus, salbutamol spray, and methylprednisolone 40 mg.	Patient continued to require epinephrine for 18 hours but was discharged to home after a few days without sequelae. Plasma histamine was noted to be markedly increased to 700 nmol/L (normal <10).

(Table 2. Contd….)

Author (Reference) and Year	Type of Report (Sample Size) and Mode of MB Administration	Major Findings	Comment
Almeida *et al.* [69] 2007	Case report. (N = 1) Report of worsening pulmonary function following MB administration in a patient with hepatopulmonary syndrome.	Administration of MB in the setting of hepatopulmonary syndrome with a large right to left intrapulmonary shunt was reproducibly and reversibly associated with worsening hypoxemia.	This report suggests that the NO-cGMP pathway inhibition should be avoided in the clinical setting of hepatopulmonary syndrome.
Sweet *et al.* [74] 2007	A report of MB-related postoperative encephalopathy in five patients who received MB for intraoperative parathyroid localization. (N=5) An institutional review of 132 patients who underwent MB infusion for parathyroidectomy, with 3.8% (5/132) incidence of encephalopathy. Patients received MB (3-5 mg/kg) in 500 mL of normal saline, given over 45 minutes and beginning approximately 30 minutes before the operating room time.	All five patients who developed encephalopathy were women between the ages 34 and 73 years. All patients in this group received MB doses between 3-5 mg/kg. All patients in the encephalopathic group used some kind of serotonin metabolism-modifying drug. Two patients used fluoxetine, one in combination with bupropion. Two were taking venlafaxine, one in combination with mirtazipine and quetiapine. Another patient was taking escitalopram. All patients were initially extubated in the operating room. Approximately 1-3 hours following extubation, a slowly progressive encephalopathy developed, which required prolonged hospitalization in all five patients. In two patients, the progression of symptoms occurred over approximately 5 hours and necessitated intubation. Common symptoms included confusion, lethargy, disorientation, difficulty with speech and ambulation, and a varying amount of agitation.	A common 'theme' connecting all five patients with postoperative encephalopathy was the history of depression and preoperative use of serotonin metabolism-altering drugs. Of note, the high dose of MB administered in this group (3-5 mg/kg) has previously been associated with increased side effect profile. By discharge, all patients returned to their baseline mental status.

vasoplegia. We will begin with the use of MB in cardiac surgical-related applications (including reaction to heparin reversal with protamine), followed by MB use in anaphylactic shock. A discussion of MB use in septic shock will follow. A section describing studies of other selective and non-selective NOS inhibitors will be presented last. Collected literature evidence pertaining to MB use in the various clinical settings has also been tabulated.

STUDIES OF METHYLENE BLUE IN THE SETTINGS OF CARDIAC SURGERY AND PROTAMINE REACTION

Severe vasoplegia after cardiopulmonary bypass (CPB) can have devastating consequences. In the setting of such vasoplegic response, a single dose of 2 mg/kg MB as a bolus has been reported to be effective in normalizing peripheral vascular resistance as a 'rescue' therapy, often leading to discontinuation of vasoactive drugs in patients with otherwise refractory VS [61, 81]. Continuous MB infusion is an option for patients not responding to a single dose of MB, and has been reported at doses up to 2 mg/kg/hour [2, 5].

Several reports describe preoperative administration of MB in patients at high risk for VS during cardiac surgery [17]. In that setting, preoperative MB administration reduced the incidence of VS in high-risk patients (0% in MB group versus 26% in non-MB group) and resulted in significantly shorter ICU and average hospital stays [17]. In another study, 54 patients with norepinephrine-refractory vasoplegia after CPB were treated with 2 mg/kg intravenous infusion of MB administered over a 20-minute period, with no apparent adverse effects [15]. A clinically relevant increase in systemic vascular resistance and a decrease in norepinephrine dosage was noted in 51/54 (94%) patients within 1 hour after MB use. There was only one case of mortality (1.9%) in this group [15]. Four patients (7.4%) did not respond to MB, two of whom died [15].

A recent randomized trial of MB administration in post-cardiac surgery vasoplegia showed a statistically significant and clinically relevant reduction in mortality (0% in MB group versus 21.4% in controls) [19]. In addition, the incidence of renal failure, respiratory failure, neuropathy, myopathy, supraventricular arrhythmia, sepsis, and multi-organ dysfunction, were significantly lower in the group receiving MB [19].

Vasoplegic response was also reported following a thoracotomy performed for a hemothorax resulting from thoracen-

tesis for a pleural effusion following coronary bypass and valve replacement [1]. In that case, a norepinephrine-refractory vasoplegia was successfully treated with MB administration, resulting in a rapid and long-lasting hemodynamic improvement [1]. It is unclear whether the initial cardiac surgery constituted a 'priming' signal for the development of vasoplegia or whether the VS was due to the thoracic procedure itself [1]. Table **3** summarizes the clinical use of MB in the setting of post-cardiac surgery vasoplegia [1, 14-15, 17, 19, 61, 81-83]. Table **4** summarizes experimental studies of MB in various settings [11, 26, 60, 84-90].

Experimental evidence points to the NO/cGMP-dependent nature of protamine induced vasoplegia (Fig. **7**) [91]. However, no clinical studies offer compelling evidence that MB is a valuable adjunct in the setting of protamine-associated vasoplegia, and only second-hand and extrapolated information has been quoted in support of this contention [91, 92]. Some insight into the utility of MB in the setting of post-CPB protamine induced vasoplegia may be gained by examining the available evidence on MB use in the setting of anaphylaxis (see next section). Table **4** summarizes experimental studies of MB in the setting of protamine reaction [11, 26, 60, 84-90]. Table **5** summarizes clinical studies of MB use in the setting of protamine reaction [4, 21-22, 62, 69, 93].

METHYLENE BLUE USE IN THE SETTING OF ANAPHYLAXIS

Although MB is able to induce an anaphylactic response itself, the role of MB in treatment of anaphylaxis and anaphylactic shock has been described [4, 79]. A recent report suggests that anaphylactic shock depends at least in part on PI3K and eNOS-derived NO-dependent pathways (Fig. **4**) [38]. Experimental evidence suggests that NOS inhibitors attenuate hypotension, although they do not improve cardiac depression in the setting of anaphylaxis [62]. On the other hand, there is some evidence that NO production may reduce some pathophysiologic changes associated with anaphylaxis with the exception of vasodilation [94]. Given the above observation, as well as the multiple physiologic pathways involved, one might speculate that the hemodynamic sequelae of anaphylaxis represent an imbalance between the vasoconstrictive and vasodilatory mechanisms. Unlike the settings of post-CBG and sepsis-related vasoplegia, the literature describing MB use in anaphylaxis is scarce and limited to experimental studies, case reports, and small case series.

The largest series describing MB use in anaphylaxis includes six patients, one of whom had an anaphylactic reaction to penicillin and five had anaphylaxis following administration of iodinated radiographic contrast [47]. In that series, the traditional therapeutic interventions (high-dose epinephrine and corticosteroids) failed to reverse the circulatory collapse [47]. Four patients in that study received a single infusion of 1.5 mg/kg of MB, followed by a continuous infusion of 120 mg MB diluted in Dextrose 5% in water. Two patients received between 30 and 40 minutes of continuous MB infusion only [47]. The anaphylactic manifestations were reportedly reversed within 10 to 15 minutes in this group, but one patient developed self-limited nodal cardiac rhythm and another patient reported angina pectoris-like symptoms during MB infusion [47]. Table **4** summarizes experimental studies of MB in various settings, including anaphylaxis [11, 26, 60, 84-90]. Table **5** summarizes clinical studies of MB use in the setting of anaphylaxis [4, 21-22, 62, 69, 93].

STUDIES OF METHYLENE BLUE IN SEPSIS

Refractory hypotension is present in approximately half of the patients who succumb to sepsis and is the number one cause of death in the first week after the initial diagnosis of sepsis [95]. This is in contrast to delayed deaths attributed to sepsis which result from multi-organ failure secondary to prolonged hypotension and maldistribution of systemic blood flow days to weeks later [95]. The investigations of MB use in septic shock are partly based on the reports of improved mortality and morbidity seen in cardiac surgery patients treated with MB as well as isolated case reports and animal models of endotoxemia that describe promising observations but no conclusive evidence of the benefits of MB in sepsis [5, 8, 24, 36].

The literature describing the use of MB in the setting of septic shock-related vasoplegia is scarce, although enough is known to generate important new research questions. The cumulative experience with MB in the setting of sepsis is dominated by retrospective case series and reports, and there are only two randomized, controlled trials investigating MB administration in vasoplegia related to sepsis [5]. Both of these trials suffer from small patient size and certain methodologic deficiencies. It is noteworthy that all of the studies demonstrate an increase in systemic vascular resistance, evidenced by either increased mean arterial pressure and/or decreased vasopressor requirement following MB administration [5].

The first study, by Kirov and colleagues, involved 20 patients who were randomized either to 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/hr, each continued for 1 hour [63]. That study examined patient hemodynamics over 24 hours, and showed increased mean arterial pressures among patients who received MB [63]. Of interest, stroke volume and left ventricular stroke work indices were maintained despite MB use [63]. In addition, oxygen delivery was unchanged in the MB group but it decreased in the control group [63]. Patients who received MB demonstrated reduced requirements for norepinephrine, epinephrine, and dopamine by 87%, 81%, and 40%, respectively [63]. Mortality at 28 days was 50% in the MB group and 70% in the isotonic saline group, with no adverse events of MB administration noted [63].

The second major study of MB in the setting of sepsis was conducted by Memis, *et al.* [96]. In this prospective, controlled trial, 30 patients were randomized into two groups – those who received isotonic saline (n = 15) and those who received an infusion of MB (0.5 mg/kg/hr) for 6 hours (n = 15). Patients in the MB group had significantly increased mean arterial pressure compared to the control group, without adverse events [96].

Several other, smaller reports of MB use in sepsis were published, limited mainly to case series and case reports.

Table 3. Clinical Evidence of Methylene Blue Efficacy in the Setting of Vasoplegia Associated with Cardiac and Thoracic Surgery

Author (Ref.) and Year	Clinical Setting	Major Results/Findings	Comment
Friedrich *et al.* [1] 2002	Case report of MB administration in severe systemic inflammatory response syndrome (SIRS) with vasoplegia after thoracic surgery. (N = 1) Methylene blue (2 mg/kg) administered for refractory hypotension associated with escalating doses of norepinephrine after a thoracotomy for evacuation of hemopneumothorax following cardiac surgery.	Hemodynamic stabilization occurred shortly after MB injection. The effect was permanent, allowing for continued reduction of norepinephrine dose.	It is unclear whether the initial cardiac procedure 'primed' the patient for a vasoplegic response following the subsequent thoracotomy. No adverse effects to MB were noted.
Dagenais *et al.* [61] 2003	Case report of unresponsive vasoplegia following cardiopulmonary bypass. Methylene blue (2 mg/kg) was administered intravenously to a patient with refractory vasoplegia three days after cardiopulmonary bypass procedure.	A single MB bolus resulted in normalization of the peripheral resistance.	
Leyh *et al.* [15] 2003	Case series of 54 patients with norepinephrine-refractory vasoplegia after cardiopulmonary bypass. A single dose of MB (2 mg/kg) was administered intravenously over a period of 20 minutes.	A clinically relevant increase in systemic vascular resistance and a decrease in norepinephrine dose was observed in 51/54 patients within 1 hour after MB infusion. Four patients showed no response to MB. No significant change in mean pulmonary artery pressure was noted following MB administration. The study noted a decrease in cardiac output following MB administration at 1, 6, and 12 hours post-MB infusion.	No adverse effects related to MB were observed.
Levin *et al.* [19] 2004	A study of 638 cardiac surgery patients identified 56 patients (8.8%) who met criteria for vasoplegia. Patients with vasoplegia were randomized into two groups: (a) those who received 1.5 mg/kg of MB; and (b) those who received placebo.	Patients with vasoplegia had higher mortality than the overall cardiac surgical group (10.7% *vs* 3.6%). Among patients who had vasoplegia, those who received MB had significantly lower mortality (0%) than those who did not receive MB (21.4%). The duration of vasoplegic syndrome was significantly shorter in the MB group (all patients <6 hours) than in the control group (>48 hours in 8 patients). In addition, the incidence of renal failure, respiratory failure, sepsis, multi-organ dysfunction, and supraventricular arrhythmia was significantly lower in the MB group.	
Ozal *et al.* [17] 2005	Clinical trial of preoperative MB administration in cardiac surgical patients at high risk for vasoplegic syndrome. (N = 100) Fifty patients assigned to control group (no MB given). Fifty patients were assigned to MB (2 mg/kg of 1% solution) administration group. MB was given 1 hour prior to surgery over a time period of 30 minutes.	The MB group was noted to have no vasoplegia, while the incidence of vasoplegia was 26% in non-MB group. The MB group had shorter ICU stays (1.2 *vs* 2.1 days) and average hospital stays (6.1 *vs* 8.4 days). There was also a significant decrease in inotrope and vasopressor requirement in the MB group.	In terms of mortality, 2/50 patients died in non-MB group, while no mortality was noted in the MB group. No adverse effects of MB were noted at the 2 mg/kg dose used in this study.
Taylor *et al.* [14] 2005	A case report of a pediatric patient with bacterial endocarditis complicated by cerebral infarction/hemorrhage who required emergency mitral valve replacement. The patient developed severe hypotension. The patient received 2 mg/kg of MB pre-anesthesia. An additional 2 mg/kg MB after the commencement of cardiopulmonary bypass followed by an infusion of 1 mg/kg was administered.	No significant immediate improvement was noted following MB administration. The patient was noted to have a relatively benign postoperative course, and was reported to be off vasopressors by postoperative day 2.	Despite the lack of an immediate response to MB, it is unclear whether MB administration contributed to the relatively benign postoperative course.

(Table 3. Contd….)

Author (Ref.) and Year	Clinical Setting	Major Results/Findings	Comment
Evora *et al.* [81] 2006	A letter describing MB use in a patient with history of drug addiction who underwent a placement of bileaflet aortic valve prosthesis for native aortic valve endocarditis. The patient required high-dose norepinephrine infusion intraoperatively and remained hypotensive after weaning from cardiopulmonary bypass. He experienced persistent increase in cardiac output, low systemic vascular resistance, and pulmonary edema associated with hypoxemia. Methylene blue was started as a continuous infusion, followed by a bolus of 3 mg/kg twice daily.	Despite lack of increase in mean arterial pressure, even with norepinephrine, the cardiac output gradually decreased and the systemic vascular resistance increased following MB administration. The patient also experienced rapid resolution of pulmonary edema and improvement in oxygenation after MB was given.	The authors cite the ability of MB to reduce vascular permeability.
Mora-Ordonez *et al.* [80] 2006	Case report of a patient who developed refractory vasoplegia and shock following myocardial revascularization. (N = 1) Methylene blue (2 mg/kg) was administered intravenously in the setting of continued requirement for vasoactive drug administration.	A single dose of MB resulted in resolution of hemodynamic instability and facilitated total discontinuation of vasoactive agents.	No adverse effects were noted with MB use.
Riha *et al.* [82] 2006	Case report of methylene blue use in the setting of vasoplegic syndrome after cardiac surgical procedure.		

Table 4. Experimental Evidence of Methylene Blue Efficacy in Various Clinical Settings Associated with Vasoplegia

Author (Ref.) and Year	Setting & Animal/Experimental Model	Major Results/Findings	Comment
Raikar *et al.* [83] 1996	Investigation of the effect of nitric oxide (NO) inhibition on systemic hypotension produced by protamine. Part I: Protamine sulfate (50 mcg/mL) was added to perfusate of eight isolated rabbit heart preparations. In six other preparations, a similar concentration of protamine was added to heparinized (5 units/mL) Krebs perfusate. Part II: Study of systemic effects of protamine included administration of protamine (1.5 mg/kg over 30 seconds) to 12 heparinized dogs. This was followed by a recovery phase, after which MB and/or N-(G)-monomethyl-L-arginine were given prior to another infusion of protamine.	Part I: Left ventricular developed pressure, maximum rate of pressure rise, and heart rate all declined significantly in hearts exposed to protamine only. Protamine added to heparinized perfusate caused little change in developed pressure, maximum rate of pressure rise, and heart rate. Part II: Mean blood pressure decrease of 46%, cardiac output decrease by 38%, and systemic vascular resistance decrease by 14% during initial protamine infusion. Second protamine infusion caused no significant change in blood pressure or cardiac output.	Protamine-heparin complex does not appear to cause direct myocardial depression but does lead to severe hypotension *in vivo*. Protamine-related hypotension can be blocked by inhibitors of the NO pathway, confirming previous *in vitro* studies indicating that the effects of protamine are mediated, at least partly, by the vascular endothelium.

(Table 4. Contd….)

Author (Ref.) and Year	Setting & Animal/Experimental Model	Major Results/Findings	Comment
Cheng *et al.* [84] 1998	A study of MB effects on mean arterial pressure, cardiac output, total peripheral resistance, mesenteric blood flow, and renal blood flow in a rat model. Following lipopolysaccharide (LPS) injection, the animals were divided into four groups: (a) MB at 1 mg/kg/hr; (b) MB at 3 mg/kg/hr; (c) MB at 10 mg/kg/hr; (d) administration of vehicle. Two other groups received saline in lieu of LPS, followed by MB or vehicle. Methylene blue was given at 2.5 hours post-LPS administration in all treated animals.	MB alone had no effect on measured variables in control animals. In LPS-treated animals, MB elevated total peripheral resistance at all doses and attenuated the fall in mean arterial pressure at the two low doses. Cardiac output was unaltered by low doses of MB but reduced by the high dose. Mesenteric blood flow was unaltered, but renal blood flow was increased by the lowest dose and decreased by the highest dose of MB.	Overall, in the rat endotoxemia model, a low dose of MB increases mean arterial pressure and total peripheral resistance but does not alter cardiac output. High dose MB does not raise mean arterial pressure but increases total peripheral resistance and reduces cardiac output.
Evgenov *et al.* [24] 2001	Study of the effects of MB on the early cardiopulmonary response to endotoxin in awake sheep. (N = 21) Eighteen animals randomly received either an intravenous injection of 10 mg/kg of MB or isotonic saline. Thirty minutes later, the animals were injected with Escherichia coli endotoxin over a 20 minute period and either an intravenous infusion of MB (2.5 mg/kg) or isotonic saline, respectively for five hours. Three animals were exposed to the same dose of MB alone.	MB reduced the early endotoxin-induced declines in stroke volume, left ventricular stroke work and cardiac indices, prevented decline in systolic BP. MB appeared to alleviate the increases in pulmonary arterial pressure and pulmonary vascular resistance index, prevented the rises in body temperature and plasma nitrites and nitrates. MB reduced the increments in venous admixture and $AaPO_2$, decreased the decline in PaO_2, SaO_2, and oxygen delivery, and maintained O_2 consumption.	MB administration delayed the elevation of plasma lactate in the endotoxin group. When administered alone, MB transiently reduced plasma lactate and PaO_2, and increased $AaPO_2$.
Viaro *et al.* [85] 2002	Review article from the University of Sao Paulo, Brazil, summarizing evidence from four experimental studies from Mayo Clinic, Rochester, MN, USA, demonstrating effectiveness of MB and nitric oxide synthase blockers in neutralization of the protamine vasodilatory effects.	Summary of experimental studies: (a) The first study reported *in vitro* systemic and coronary vasodilation after protamine infusion; (b) The second *in vitro* study suggested extensive protamine-mediated involvement of the pulmonary endothelium; (c) The third study of MB and nitric oxide synthase blockers in anesthetized dogs reported neutralization of the protamine vasodilatory effects; and (d) The fourth study suggested that protamine also causes endothelium-dependent vasodilation in cardiac microvessels and conductance arteries.	Viaro *et al.* argue that a growing body of evidence is emerging that MB may be useful in treatment of distributive shock.
Kirov *et al.* [86] 2003	Acute lung injury model after endotoxin administration in sheep. (N = 24) Animal groups included: (a) control group receiving endotoxin and saline; (b) inhaled NO group received endotoxin, saline, and inhaled NO; and (c) combined MB/NO groups receiving endotoxin, saline inhaled NO, and MB (3 mg/kg bolus followed by infusion at 3 mg/kg/min).	MB/NO combination reduced the increments in pulmonary vascular pressure, pulmonary vascular resistance index, and pulmonary microvascular pressure by 60% compared with controls in early phase (0-2 hours). In the late phase, all the above parameters returned close to baseline in the MB/NO group, but remained elevated in controls. Inhaled NO/MB reduced the increase in extravascular lung water by 80%.	There was no change in blood gases in the MB/NO group.

(Table 4. Contd….)

Author (Ref.) and Year	Setting & Animal/Experimental Model	Major Results/Findings	Comment
Ghiassi *et al.* [26] 2004	A canine model of resuscitation after refractory hemorrhagic shock. (N = 19) Animal groups included: (a) no treatment (control); (b) MB bolus (c) limited-volume lactated Ringer's solution; and (d) combined MB/lactated Ringer's solution therapies.	Methylene blue/lactated Ringer's significantly improved prehospital survival, mean arterial pressure and cardiac output, vital end-organ blood flow and oxygen delivery, and decreased serum lactate levels as compared with MB and lactated Ringer's single therapies.	Hemodynamic parameters were collected at baseline, during shock, during refractory hemorrhagic shock, and 30, 60, 90, and 120 minutes after treatment. Radiolabeled microspheres were used to assess end-organ perfusion/O_2 delivery.
Weinbroum [60] 2004	Study of MB effect on lung injury following superior mesenteric artery clamping/unclamping in anesthetized adult male Wistar rats. (N = 144) Ten minutes before unclamping, methylene blue or its vehicle was administered intratracheally or intravenously.	Intratracheal methylene blue was found to mitigate lung reperfusion injury following superior mesenteric artery clamping/unclamping at a similar magnitude as an intravenous regimen.	Methylene blue was effective when administered intratracheally, potentially introducing a novel method of *in vivo* administration.

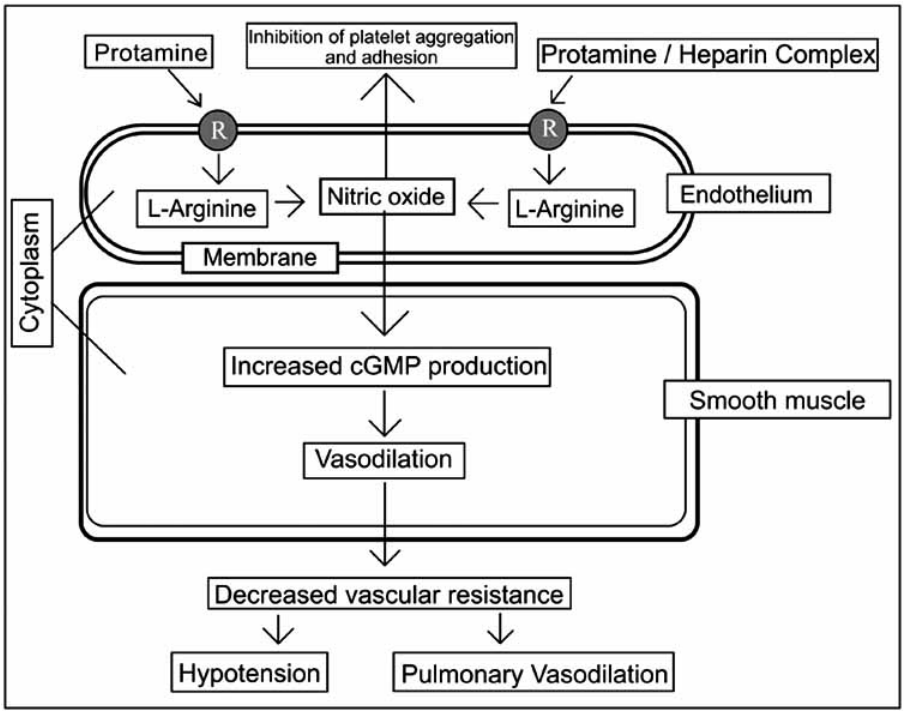

Fig. (7). *Schematic representation of the proposed mechanism of protamine-induced hypotension in the systemic and pulmonary circulations.* Protamine (free or complexed with heparin) binds to a proposed endothelial cell receptor that mediates the conversion of L-arginine to NO. The release of NO causes activation of soluble guanylyl cyclase in vascular smooth muscle cells, causing cGMP-mediated vasodilation. This results in decreased peripheral vascular resistance, hypotension, and pulmonary vasodilation. In addition, luminally released NO can induce thrombolysis and inhibit platelet adhesion. Modified from Reference 12.

Table 5. Clinical Evidence of Methylene Blue Efficacy in the Setting of Anaphylaxis, Hemodialysis, and other Conditions

Author (Ref.) and Year	Clinical Setting	Major Results/Findings	Comment
Peer *et al.* [22] 2001	Investigational study of MB administration in hemodialysis patients. Study based on the fact that plasma NO levels have been found to be elevated in hemodialysis (HD) patients, potentially contributing to HD-associated hypotension. (N = 41, 18 HD patients with hypotensive episodes, 18 HD patients without hypotension, and 5 healthy controls). Methylene blue was given as a bolus of 1 mg/kg followed by a constant infusion of 0.1 mg/kg for 210 minutes until the end of HD session. On non-dialysis days, only the bolus dose was given.	In hypotension-prone patients, MB completely prevented the hypotension during dialysis and increased both systolic and diastolic blood pressure on non-dialysis days. In normotensive patients, MB increased blood pressure during the first hour of dialysis and for 90 minutes on the non-dialysis day. The blood pressure in healthy controls remained unchanged.	The generation of nitrates was significantly higher in the hypotensive group than in the normotensive group. No side effects were recorded.
Evora *et al.* [4] 2002	A letter describing three cases of contrast medium-induced anaphylaxis successfully treated with intravenous MB. Three patients developed anaphylactic shock following injection of radiocontrast media and were treated with intravenous bolus of methylene blue (2 mg/kg). MB given to two patients after hydrocortisone and adrenaline failed to provide adequate clinical response.	Injection of methylene blue was associated with prompt improvement in circulatory status.	Side effects included self-limited episode of nodal rhythm and chest pain in one patient each. Another patient experienced chest pain without EKG changes.
Oliveira Neto *et al.* [21] 2003	A series of three patients treated with MB for contrast medium-induced anaphylaxis. (N = 3) Patients were treated with 1.5-2.0 mg/kg methylene blue after developing radiographic contrast-induced anaphylaxis.	In two cases, 1.5 mg/kg methylene blue bolus resulted in normalization of hemodynamics within 20 minutes following the infusion. In one case, methylene blue (2 mg/kg bolus followed by a 2 mg/kg continuous drip given for 2 hours) resulted in significant improvement in mean systemic arterial pressure within 10 minutes of administration.	Transient cardiac rhythm changes and chest pain were observed with MB administration.
Van Der Horst *et al.* [92] 2003	Review of etiology, pathophysiology, and management of priapism.	The authors discuss intracavernous methylene blue administration (dose of 50 mg) as non-invasive treatment alternative of priapism.	The authors discuss the importance of the NO/cGMP mechanism as a mediator of the pathophysiologic response to priapism.
Almeida *et al.* [69] 2007	Case report of MB use in a patient with hepatopulmonary syndrome.	Administration of MB in the setting of hepatopulmonary syndrome with a large right to left intrapulmonary shunt resulted in significant improvements in vascular tone and the hyperdynamic circulation, but was reproducibly and reversibly associated with worsening hypoxemia.	This report suggests that NO-cGMP pathway inhibition should be avoided in the clinical setting of hepatopulmonary syndrome.
Rodrigues *et al.* [62] 2007	Case report of MB use (1.5 mg/kg or 120 mg of 4% MB intravenous bolus followed by infusion of an additional 120 mg for 1 hour) in a patient with severe anaphylaxis and impending respiratory failure. The patient failed conventional therapy with antihistamine and methylprednisolone.	Administration of MB resulted in complete resolution of angioedema, urticaria, vasodilation and upper respiratory dyspnea in less than 20 minutes.	This report identifies MB as a potential 'rescue' therapy for refractory anaphylaxis.

Universally, these reports describe increases in mean arterial pressure, systemic vascular resistance, and reduction in vasopressor requirements following MB administration. While most reports of MB use in sepsis do not note significant adverse effects, some authors note that there may be an association with MB use and undue mortality [97] as well as worsening oxygenation in the setting of acute respiratory distress syndrome (ARDS) [98]. The worsening arterial oxygenation following MB infusion in patients with acute lung injury (ALI)/ARDS may represent a dose-dependent effect, with studies reporting increments in pulmonary vascular pressure and resistance at the higher doses (i.e., 3 mg/kg), but not at the lower dosing range [3, 98, 99]. Table **6** summarizes clinical studies of MB in the setting of septic shock-associated vasoplegia [3, 14, 25, 59, 63, 72, 82, 96-98, 100-106]. Table **4** summarizes experimental studies of MB in various settings, including animal sepsis models [11, 26, 60, 84-90].

NON-MB NOS INHIBITORS

The use of non-MB NOS inhibitors in the clinical settings discussed above is still experimental. While NOS inhibitors may help normalize blood pressure, their use can be associated with a significant reduction in cardiac output [62]. In the setting of anaphylaxis, NO produced by the bronchial epithelium may be important in counteracting bronchospasm, and inhibition of its production may be harmful [62].

Table 6. Clinical Evidence of Methylene Blue Efficacy in the Setting of Vasoplegia Associated with Sepsis

Author (Ref.) and Year	Type of Study (Sample Size) and Mode of MB Administration	Major Results/Findings	Comment
Daemen-Gubbels *et al.* [99] 1995	Open-label, nonrandomized clinical trial of MB in the setting of sepsis. The trial involved consecutive patients with a pulmonary artery catheter in place. (N = 9) Methylene blue given as an intravenous bolus (2 mg/kg) over 20 minutes.	Increased mean arterial pressure and oxygen uptake, associated with a decrease in arterial compliance and increases in myocardial function and oxygen delivery.	Cardiac filling pressures did not change after MB administration.
Gachot *et al.* [97] 1995	A prospective, open, single-dose study of MB in sepsis. (N = 6) Methylene blue administered as a bolus (3 mg/kg) over 10 minutes.	Increased mean arterial pressure, increased systemic vascular resistance and peripheral vascular resistance, increased mean pulmonary artery pressure. Mortality reported in 5/6 patients in this series.	Worsening oxygenation may limit MB use in patients with ARDS.
Preiser *et al.* [105] 1995	Prospective clinical trial in a mixed medical-surgical ICU. (N = 14) Methylene blue given as a bolus (2 mg/kg) over 15 minutes. An additional dose of MB was administered to 6 patients due to the transient response to the initial dose.	Methylene blue administration was associated with increased mean arterial pressure and increased systemic vascular resistance. There was a significant drop in serum lactate following MB administration, which may be related to the reductor effect of MB rather than to improved tissue oxygenation.	Pulmonary arterial pressure, cardiac filling pressures, cardiac output, oxygen delivery, and oxygen consumption were not significantly affected.
Schneider [96] 1995	Observational study. (N = 2) Methylene blue given as boluses (1 mg/kg, 2 mg/kg, 3 mg/kg).	Increased mean arterial pressure, increased systemic vascular resistance, decreased norepinephrine requirement.	Study suggests that MB given in sepsis may be tied to undue mortality, as opposed to MB in other settings.
Andersen *et al.* [104] 1996	Observational study of septic patients with persistent hypotension requiring at least two vasoactive drugs. (N = 14) Methylene blue administered as an intravenous bolus of 1 mg/kg over 15 minutes.	Increased mean arterial pressure, increased systemic vascular resistance, increased mean pulmonary arterial pressure.	MB appears to have an acute pressor effect in septic shock.
Brown *et al.* [59] 1996	A case report. (N = 1) MB given as a bolus (1.5 mg/kg) followed by and infusion of 17 mg/hour.	Increased mean arterial pressure, decreased dopamine requirement, decreased norepinephrine requirement	

(Table 6. Contd….)

Author (Ref.) and Rear	Type of Study (Sample Size) and Mode of MB Administration	Major Results/Findings	Comment
Andersen *et al.* [72] 1998	Prospective observational study. (N = 10) Methylene blue given as bolus dose (1 mg/kg) over 15 minutes.	Increased mean arterial pressure, increased systemic vascular resistance, increased mean pulmonary artery pressure.	No adverse effects of MB on respiratory function were noted.
Weingartner *et al.* [103] 1999	Prospective, open, non-randomized study of MB in human septic shock. (N = 10) Methylene blue administered 4 mg/kg bolus over 1 hour.	Increased mean arterial pressure, increased systemic vascular resistance index, increased left ventricular stroke work index. MB administration was associated with decreases in serum lactate levels. Heart rate, cardiac filling pressures, cardiac output, oxygen delivery and consumption did not change following MB administration.	No adverse effects to MB were noted. Mixed venous oxygen saturation and PaO2/FiO2 ratio decreased slightly after MB.
Kirov *et al.* [63] 2001	Prospective, randomized, placebo-controlled study. (N = 20, control = 10, treatment = 10) Methylene blue administered as 2 mg/kg bolus over 15 minutes, with stepwise 1-hour infusions of 0.25, 0.5, 1.0, and 2.0 mg/kg/hr.	Increased mean arterial pressure, decreased epinephrine requirement (by 87%), decreased epinephrine requirement (by 81%), decreased dopamine requirement (by 40%).	Mean pulmonary arterial pressure remained stable. Oxygen delivery remained stable during MB infusion.
Memis *et al.* [95] 2002	Prospective, randomized, placebo-controlled study of MB in severe sepsis. (N = 30, control = 15, treatment = 15) Methylene blue infusion (0.5 mg/kg/hr) over 6 hours in treatment group. Isotonic saline administered in the control group.	Increased mean arterial pressure with MB administration. Methylene blue infusion did not affect cytokine levels (tumor necrosis factor alpha, interleukin-1, interleukin-2 receptor, interleukin-6, interleukin-8). There was no difference in mortality between the two groups.	Higher methemoglobin levels reported in the MB group.
Donati *et al.* [3] 2002	Prospective, open study of patients with septic shock. Patients with persistent hypotension despite conventional therapeutic measures. (N = 15) Methylene blue administered as an intravenous bolus (3 mg/kg over 10 minutes).	Methylene blue administration associated with increased mean arterial pressure, increased systemic vascular resistance, increased mean pulmonary arterial pressure. Blood lactate level transiently decreased after MB administration. The study used a method of determination of blood and extravascular volumes by the thermal-dye double indicator technique using indocyanine green.	Vasoconstrictive and positive inotropic effects of MB are not associated with changes in blood volume, myocardial diastolic function, or pulmonary vascular permeability.

There are several known NOS inhibitors, and detailed discussion of all of them is beyond the scope of this manuscript. In the setting of severe sepsis, a non-selective inhibitor of NO production N-omega-nitro-L-arginine methyl ester (L-NAME, Fig. **8**) was shown to increase blood pressure and vascular resistance, but produced only limited effects on outcome [107]. Another NOS inhibitor, N(G)-methyl-L-arginine hydrochloride (546C88, L-NMMA, Fig. **9**), studied in the setting of septic shock across ten intensive care units in the United States and Europe, was able to restore the vasomotor tone and maintain blood pressure with satisfactory safety profile in preliminary trials [108]. Despite the promising early results from the trial of 546C88 in sepsis [108], a subsequent multi-center, randomized, double-blind, placebo-controlled trial of 546C88 demonstrated that this nonselective NOS inhibitor contributed to increased mortality in patients with septic shock [109].

When examining the role of NOS inhibition in refractory cardiogenic shock, there appears to be more evidence of clinical benefits. In one prospective study of L-NAME administration for refractory cardiogenic shock, 30 patients were randomized into either L-NAME treatment group (1 mg/kg bolus followed by 1 mg/kg/hour continuous intravenous drip for 5 hours, n=15) or control group (n=15). The L-NAME group demonstrated lower mortality at 1 month (27% versus 67%), significantly higher unaugmented mean arterial

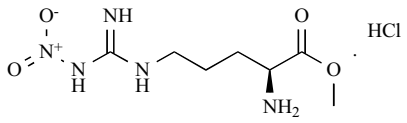

Fig. (8). Chemical structure of L-arginine methyl ester (L-NAME).

blood pressures at 24 hours post-randomization, improved urine output at 24 hours, and significantly shorter times on intra-aortic balloon pump and mechanical ventilation [110]. In another study of cardiogenic shock, a relatively non-selective inhibitor of all NOS isoforms, L-NMMA (Fig. **9**), was shown to produce significant increases in mean arterial pressure and urine output at 24 hours when given as an intravenous bolus of 1 mg/kg followed by a 1 mg/kg infusion over 5 hours [111]. Ten out of eleven patients were successfully weaned off mechanical ventilation and intra-aortic balloon pump assistance [111]. However, the cardiac output did not improve significantly at 24 hours after L-NMMA discontinuation [111].

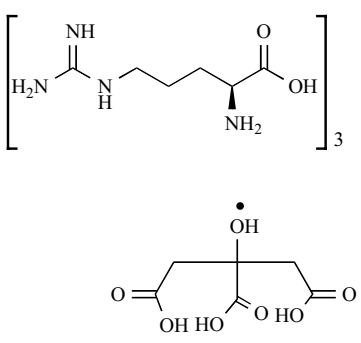

Fig. (9). (NG-Monomethyl-L-arginine monoacetate (L-NAME).

Of interest, it has also been noted that NO has antithrombotic properties that result from inhibition of platelet adhesion and aggregation, and that these properties can be influenced by inhibitors of NO synthesis [107]. Given the differences in mechanism of action, MB may offer some advantages over other NOS inhibitors by 'sparing' at least some of the various NOS-dependent physiologic effects.

NITRIC OXIDE SYNTHASE INHIBITION: STRUCTURAL AND FUNCTIONAL RELATIONSHIPS

Mammalian nitric oxide synthases are homodimeric flavocytochromes. Each NOS subunit consists of a unique heme/biopterin-binding oxygenase domain and a reductase domain (Fig. **10**). Nitric oxide inhibitors are known to interact with the NOS enzymes in a variety of ways – including several distinct mechanisms, sites, varying time- and substrate-dependence. Most NOS inhibitors identified to date are competitive with the substrate L-arginine [112]. Therefore, it has been inferred that many NOS inhibitors bind to the arginine-binding site [112]. This was subsequently confirmed experimentally, showing that such inhibitors bind to the active site and interact with the conserved glutamate (Glu^{363} of bovine eNOS and Glu^{371} of murine iNOS) which forms a close relationship with the guanidino group of L-arginine [113-115].

Many arginine-site NOS inhibitors involve mechanisms beyond simple competitive binding to L-arginine site. Some of these inhibitors require active enzyme and NADPH substrate to advance from the relatively weak initial enzyme binding to complete enzyme inhibition and inactivation [116-117]. Multiple pathways of covalent modification of the enzyme are likely involved [117]. Other mechanisms involving initial binding that proceeds to enzyme inhibition without incorporation of the inhibitor into the protein have also been described [117].

Certain NOS inhibitors act at the biopterin site, which is located adjacent to the arginine-binding region and the heme-cofactor [112]. Another group of NOS inhibitors interact directly with the heme site of the nitric oxide synthase enzyme [113]. Multiple questions remain with regards to the exact three-dimensional structure of the complete nitric oxide synthase, precise inhibitor-enzyme interactions and the basis for high isoform selectivity of NOS inhibitors, specific differences between the *in vivo* and *in vitro* enzyme, and whether the most therapeutic value can be derived from specific or non-specific NOS inhibitors [112]. Detailed discussion of the precise mechanisms of NOS inhibition and the multiple factors involved in these complex interactions are beyond the scope of this manuscript.

CONCLUSIONS

Although much has been learned about MB and its applications in the setting of vasoplegia, many questions remain. A prominent pattern emerges when examining clinical trials of both MB and non-MB NOS inhibitors. Universally, administration of MB/NOS inhibitors in patients with VS results in increases in blood pressure, systemic vascular resistance, and decreases in vasopressor administration requirements. While administration of MB and other NOS inhibi-

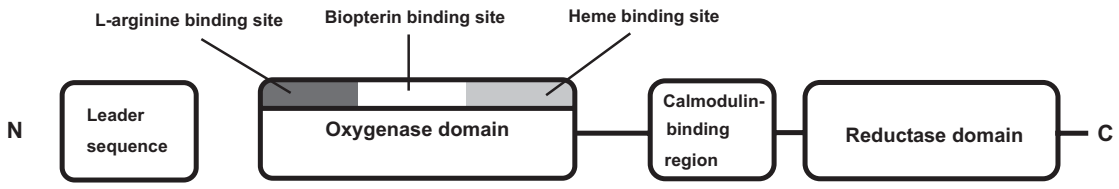

Fig. (10). Schematic representation of the nitric oxide synthase basic structure. Proceeding from the N-terminus, the leader sequence is followed by the oxygenase domain. The oxygenase domain has three major binding regions for L-arginine, Biopterin, and Heme. The calmodulin-binding region then follows, and the reductase domain is located closer to the C-terminus.

tors may be beneficial in the setting of cardiogenic shock and hemodialysis, many questions remain about its benefits in sepsis, anaphylaxis, and hemorrhagic shock.

Much more clinical and experimental investigation will be needed to determine the exact indications, contraindications, precise dosages and routes of administration, as well as the optimal timing of administration in relation to the temporal disease progression (early versus late or 'rescue therapy') for both MB and other NOS inhibitors. Future studies of these compounds in the various clinical settings described above will need to be conducted in multi-institutional, prospective, blinded, and randomized fashion, and will need to be designed to answer specific clinical questions with sufficient statistical power. Until such studies are available, the use of MB and the other NOS inhibitors in any clinical setting should be considered cautiously, with full knowledge of indications, contraindications, and potential side effects of this drug.

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