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Effects of methylene blue on oxygen availability and regional blood flow during endotoxic shock

[Laboratory Investigation]

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Outline

Graphics

Abstract

Objective: We hypothesized that methylene blue, by inhibiting the activation of soluble guanylate cyclase mediated by nitric oxide, may reverse systemic hypotension, enhance myocardial function, and improve peripheral distribution of blood flow during endotoxic shock.

Design: Randomized, controlled, acute intervention study.

Setting: University intensive care laboratory.

Subjects: Twenty-one healthy, anesthetized, mongrel dogs, weighing 26 plus minus 4 kg.

Interventions: Groups 1 (n equals 7) and 2 (n equals 7) received endotoxin (2 mg/kg iv) alone combined with increasing doses of 2.5, 5, 10, and 20 mg/kg iv of methylene blue. Each dose was administrated for 30 mins with a free interval of 30 mins. Group 3 (n equals 7) served as a control group, receiving the same doses of methylene blue in the absence of endotoxin. All animals were given normal saline to keep cardiac filling pressures constant. Blood flow probes were placed around the superior mesenteric, renal, and femoral arteries to measure regional blood flow by ultrasonic technique. Data were collected every 30 mins during the study.

Measurements and Main Results: After endotoxemia, methylene blue increased systemic and pulmonary arterial pressure and vascular resistances in a dose-dependent manner up to 10 mg/kg, but had no effect on cardiac index. At the highest dose, methylene blue decreased arterial pressure and systemic vascular resistance. At doses of methylene blue of less than equals 10 mg/kg, mesenteric and femoral blood artery flow increased. At the highest dose of 20 mg/kg, femoral artery blood flow further increased, but mesenteric blood flow decreased. Renal artery blood flow was unaffected by methylene blue. In the absence of endotoxin, methylene blue at doses of 2.5 or 5 mg/kg did not alter mean arterial pressure, but reduced cardiac index, indicating an increase in systemic vascular resistance. In contrast, the higher doses of 10 or 20 mg/kg of methylene blue decreased mean arterial pressure and systemic vascular resistance. However, pulmonary arterial pressure and pulmonary vascular resistance increased in a dose-dependent manner. Mesenteric and renal artery blood flow decreased but femoral blood flow increased. As in the presence of endotoxin, methylene blue induced doserelated increases in oxygen uptake and oxygen extraction ratio, but did not alter oxygen delivery. Methylene blue largely attenuated the endotoxin-induced increase in plasma nitrite concentrations.

Conclusions: Low and moderate doses of methylene blue can significantly increase arterial blood pressure but not cardiac index during endotoxic shock. Methylene blue infusion may selectively increase mesenteric blood flow. High doses of methylene blue can worsen systemic hypotension, myocardial depression, and pulmonary hypertension after endotoxemia.

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KEY WORDS: oxygen consumption; cardiac index; hypotension; systemic vascular resistance; nitric oxide; guanylate cyclase; guanosine-cyclic 3 prime,5 prime; nitrite; free radicals; sepsis; endothelium

Nitric oxide, an endothelium-derived relaxing factor, plays an important role in the regulation of blood pressure [1] and organ blood flow distribution under normal conditions [2]. This physiologic role of nitric oxide is controlled by a constitutive, calcium-dependent, nitric oxide synthase. Another nitric oxide synthase, which is inducible and calcium-independent, is expressed in various cells, including macrophages [3], endothelial cells [4] and vascular smooth muscle cells [5] in response to endotoxin and several cytokines [3,5,6]. Recent studies have focused on an increased release of nitric oxide in the profound vasodilation characterizing septic shock. Even the early (less than 5 to 30 mins) development of hypotension in response to endotoxin and tumor necrosis factor in vitro and in vivo may be mediated by an enhanced formation of nitric oxide before the inducible nitric oxide synthase can be expressed [7,8].

Inhibitors of nitric oxide synthase, such as N^G-monomethyl-L-arginine and N^G-nitro-L-arginine methyl ester, have been shown to reverse the decrease in vascular tone induced by endotoxin or tumor necrosis factor [6,9,10] or to reverse the sepsis-induced myocardial depression [11,12]. However, an increased survival rate, or even an improvement in cardiovascular status, has not been consistently observed following these interventions [9,13,14]. Moreover, nitric oxide inhibitors have been reported to potentiate the tissue damage in endotoxic shock [14,15], so that the administration of nitric oxide synthase inhibitors may not always be beneficial in severe sepsis [13,14].

Nitric oxide activates a soluble guanylate cyclase, which leads to increased concentrations of guanosine 3 prime,5 prime-cyclic monophosphate in the endothelial and smooth muscle cells, resulting in vasorelaxation. Methylene blue is an inhibitor of the soluble guanylate cyclase activity that counteracts the effects of nitric oxide and other nitrovasodilators in endothelium and vascular smooth muscle [16]. Methylene blue prevents the endotoxin- or interleukin-1-induced vasorelaxation by abolishing guanosine 3 prime,5 prime-cyclic monophosphate production [17] and reverses endotoxin- or cytokines-induced hypotension and myocardial depression [17,18]. Keaney et al. [19] recently showed that 1-, 5- and 10-mg/kg doses of methylene blue, after endotoxic shock in rabbits, produced a prompt dose-dependent increase in mean arterial pressure (MAP) in association with normalization of plasma nitric oxide concentration. In pilot studies [20,21] in patients with septic shock, methylene blue administration was shown to increase arterial pressure and systemic vascular resistance.

Differences in mechanisms of action might result in different effects of methylene blue and nitric oxide inhibitors. We hypothesized that by inhibiting the excessive nitric oxide-mediated guanylate cyclase activity but not nitric oxide production, methylene blue may have different effects on blood flow and oxygen availability to the organs during endotoxic shock. The present study was therefore undertaken to examine whether various doses of methylene blue could influence regional blood flow and tissue oxygen availability to the mesenteric, renal, and femoral vascular beds following endotoxemia.

MATERIALS AND METHODS

Experimental Preparation.

This study was approved by our Institutional Review Board. Care and handling of the animals were in accord with National Institutes of Health guidelines. Twenty-one mongrel dogs of either sex (weight 26 plus minus 4 kg) were anesthetized with sodium pentobarbital, which was administered as a slow intravenous bolus of 30 mg/kg, followed by a constant infusion of 4 mg/kg/hr, using an infusion pump (Infusomat Trademark II, Braun, Melsungen AG, FRG). After endotracheal intubation, each dog was mechanically ventilated with room air (Servo ventilator 900B, Siemens-Elema, Solna, Sweden). Muscle paralysis was obtained by the administration of pancuronium bromide, administered at an initial dose of 0.15 mg/kg and subsequent doses of 0.075 mg/kg/hr. Respiratory rate was 12 breaths/min, and tidal volume was adapted to keep end-tidal PCO_2 (47210A Capnometer, Hewlett-Packard, Waltham, MA) between 28 and 38 torr (3.7 and 5.1 kPa). The left forepaw vein was used for the intravenous administration of sodium pentobarbital and pancuronium bromide. The right forepaw vein was catheterized for intravenous infusion of fluids and methylene blue. The right femoral artery was catheterized for monitoring of arterial blood pressure and withdrawal of arterial blood samples. Through the right external jugular vein, a balloon-tip pulmonary artery catheter (93A-131-7F, Baxter Edwards Critical-Care, Irvine, CA) was placed under guidance of pressure waves (Sirecust Trademark monitor 302A, Siemens). A splenectomy was performed through a midline laparotomy to prevent blood autotransfusion during methylene blue infusion. Ultrasonic flow probes of an ultrasonic volume flowmeter (T208, Transonic Systems, Ithaca, NY) were placed around the superior mesenteric, left renal, and left femoral arteries, for simultaneous measurement of blood flow of these vessels.

Experimental Protocol.

After surgical preparation, the dog was allowed to stabilize its condition for 30 mins before baseline measurements (baseline 1) were made. The dogs were randomly divided into three groups. In group 1 (endotoxin alone, n equals 7) and group 2 (endotoxin plus methylene blue, n equals 7), each dog received Escherichia coli endotoxin (lipopolysaccharide E. coli 055:B5, No. 3120-10-7, Difco, Detroit, MI) as a slow intravenous bolus of 2 mg/kg over 2 mins. Group 3 (methylene blue alone, n equals 7) served as time-matched controls without endotoxin challenge. Thirty minutes later, a second set of measurements (baseline 2) was obtained. At that time, the dogs in groups 2 and 3 received methylene blue (Sterop, Brussels, Belgium) at successive doses of 2.5, 5, 10, and 20 mg/kg. Each dose of methylene blue was infused as a 10-mg/mL solution for 30 mins. Systemic and pulmonary pressures were found to stabilize approximate 5 mins after starting each dose, so that a 30-min infusion was long enough to reach a steady state during measurements. A 30-min pause was permitted between two successive methylene blue infusions, and measurements were repeated every 30 mins throughout the experiments. These measurements included arterial pressure, pulmonary arterial pressure, pulmonary artery occlusion pressure, right atrial pressure, cardiac output, regional blood flow, expired oxygen fraction, end-tidal PCO_2, minute volume, blood gases, hematocrit, blood lactate concentration, and plasma nitrite concentration. In all dogs, a saline infusion was started and titrated 30 mins after the second baseline measurements were made to keep the pulmonary artery occlusion pressure constant. A heating blanket and warming lamps were used to maintain a constant body temperature.

Measurements of Pressures, Cardiac Output, and Regional Blood Flow.

Pressures from femoral arterial and pulmonary arterial catheters were monitored continuously, using pressure transducers (966020-01, Baxter Edwards Critical-Care) with amplifiers (Servomed, Hellige, Freiburg, FRG) and a pen recorder (2600S, Gould, Instruments Division, Cleveland, OH). All pressures were determined at end-expiration. Cardiac index (L/min/kg) was measured by the thermodilution technique (COM-2, Baxter Edwards Critical-Care), using three to five 5-mL bolus injections of cold 5% dextrose in ice water at end-inspiration. Left ventricular stroke work index was calculated as: stroke index times (MAP minus pulmonary artery occlusion pressure) times 0.0136.

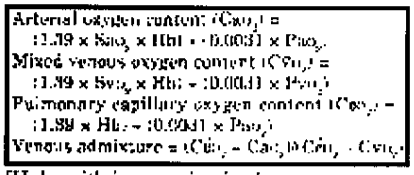
Blood flow of the superior mesenteric, left renal, and left femoral arteries was simultaneously measured by ultrasound volume flowmeter (T208, Transonic Systems; calibrated by the manufacturer).

Measurements of Expired Gases, Blood Gases, Hematocrit, and Blood Lactate.

Exhaled gases were directed through a mixing chamber for sampling of expired oxygen fractions (P.K. Morgan Ltd, Chatham, Kent, UK). Expired minute volume was measured with a spirometer (Haloscale Respirometer, Wright, Edronton, UK) over a 2-

min period. Arterial and mixed venous blood samples were simultaneously withdrawn for immediate determinations of blood gases (analyzer Stat Profile 7, NOVA Biomedical, Waltham, MA), oxygen saturations (arterial and mixed venous), and total hemoglobin concentration (Hb) (OSM 3 Trademark Hemoximeter, Radiometer, Copenhagen, Denmark; calibrated for dog blood). Hematocrit was determined by capillary method (Hettich Haematokrit, Tuttlingen, FRG).

The following formulas were used for calculations: Equation 1 where SaO_2 is arterial oxygen saturation, SvO_2 is venous oxygen saturation, and PVO_2 is mixed venous oxygen tension. Oxygen uptake (VO_2) was determined from the expired gases, as previously described [22]. Oxygen delivery (DO_2) was calculated by the product of cardiac index and arterial oxygen content. The oxygen extraction ratio was derived from the ratio of VO_2/DO_2. Blood lactate concentration was determined by a glucose/lactate analyzer (2300 Stat Plus, Yellow Springs Instrument Laboratory, Yellow Springs, OH).



Equation 1

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Measurement of Plasma Nitrite.

Nitric oxide release was determined spectrophotometrically by measuring the accumulation of both nitrite and nitrate (the latter chemical is reduced to nitrite) in plasma and was reported by the percentage of changes in nitrite from the baseline. Nitrate was stoichiometrically reduced to nitrite by incubation of the sample (100 micro Liter of plasma) for 2 hrs at 37 degrees C, in the presence of 0.1 U/mL of nitrate reductase (NAD[P]H: nitrate oxidoreductase, EC 1.6.6.2; Aspergillus species; Sigma Chemical, St. Louis, MO), 120 micro Meter of NADPH, and 5 micro Meter of flavin adenine dinucleotide (Sigma Chemical) in a final volume of 103 micro Liter. After nitrate had been reduced to nitrite, NADPH (which interfered with the subsequent nitrite determination) was oxidized with 10 U/mL of L-lactic dehydrogenase (EC 1.1.1.27; type XI; from rabbit muscle; Sigma Chemical) and 10 mM of sodium pyruvate for 30 mins at 37 degrees C in a final volume of 114 micro Liter. Sodium nitrate was used as a standard. Nitrite concentration in plasma was assayed by a standard Griess reaction [23]. Briefly, 100 micro Liter of plasma was incubated with an equal volume of Griess reagent (1% sulfanilamide/0.1% N-(1-naphthyl)ethylenediamine dihydrochloride/2.5% H_3PO_4) at room temperature for 10 mins. The absorbance of the chromophore formed was determined at 540 nm using a microtiter plate reader (Molecular Devices, Menlo Park, CA). Sodium nitrite was used as a standard, with control baseline plasma as a control reference.

Statistics.

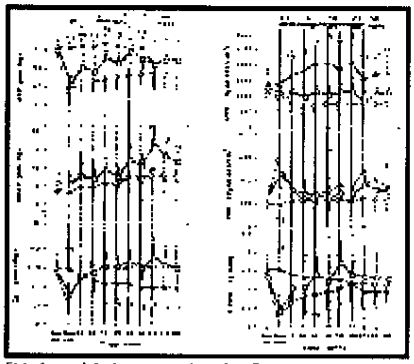
An analysis of variance for repeated measurements, followed by Dunnett's test, was used. A p less than .05 was considered statistically significant. All values are expressed as mean plus minus SD.

RESULTS

Endotoxin Experiments.

In all animals, endotoxin administration was followed by decreases in MAP, cardiac index, and left ventricular stroke work index, and an increase in pulmonary vascular resistance Figure 1. The reduction in blood flow was similar in the superior mesenteric artery, the renal artery, and the femoral artery Figure 2. DO_2 decreased but VO_2 was preserved by an increase in oxygen extraction ratio. Both PaO_2 and mixed venous PO_2 decreased Figure 3. Blood lactate concentration increased Table 1.

Figure 1. Time course of mean arterial pressure (MAP), mean pulmonary arterial pressure (MPAP), cardiac index (CI), systemic vascular resistance (SVR), pulmonary vascular resistance (PVR), and left ventricular stroke work index (LVSWI) in the animals treated with endotoxin alone (circles, interrupted line), endotoxin and methylene blue (squares, continuous line), and methylene blue alone (triangles, interrupted line). *p less than .05 vs. endotoxin alone; paragraph p less than .05 vs. endotoxin and methylene blue; dagger p less than .05 vs. methylene alone. MB, methylene blue. Data are expressed as mean plus minus SD.



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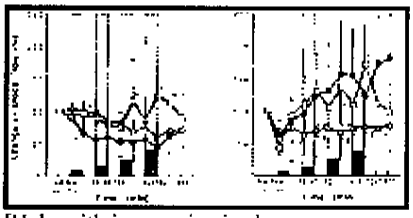

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Figure 2. Changes in blood flow in the superior mesenteric artery (circles), the left renal artery (triangles), and the left femoral artery (squares) after methylene blue administration in the control (left panel) and the endotoxic (right panel) groups. *p less than .05 vs. superior mesenteric artery; dagger p less than .05 vs. left femoral artery; paragraph p less than .05 vs. superior mesenteric and left renal arteries. Data are expressed as mean plus minus SD.

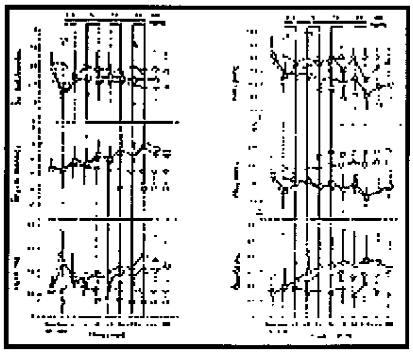

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Figure 3. Time course of oxygen delivery (DO_2), oxygen consumption (VO_2), oxygen extraction ratio (O_2 ER), PaO_2, mixed venous oxygen tension (PvO_2), and venous admixture (Qsp/Qt) in the animals treated with endotoxin alone (circles, interrupted line), endotoxin and methylene blue (squares, continuous line), and methylene blue alone (triangles, interrupted line). p less than .05 vs. endotoxin alone; paragraph p less than .05 vs. endotoxin and methylene blue; dagger p less than .05 vs. methylene alone. MB, methylene blue. Data are expressed as mean plus minus SD.

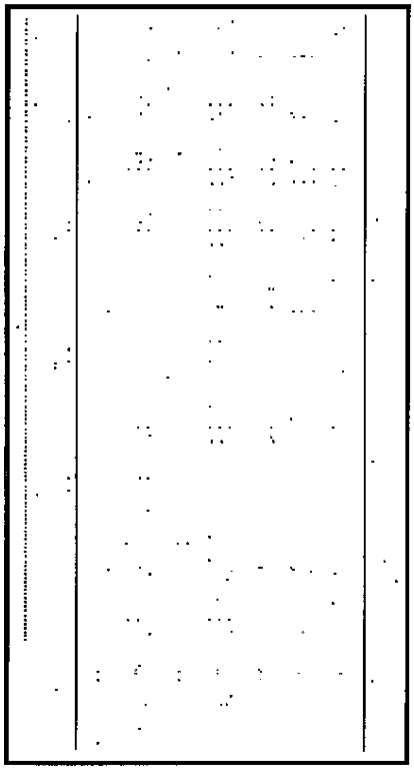

Table 1. Selected hemodynamic and blood gas variables (mean plus minus SD)

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Effects of Fluids Alone.

After endotoxin administration, fluid infusion partly reversed these changes. However, arterial pressure, left ventricular stroke work index, and systemic vascular resistance remained lower than baseline Figure 1. Mesenteric, renal, and femoral arterial blood flows increased, but only femoral artery blood flow returned to baseline values Figure 2. Blood lactate concentration progressively decreased Table 1. Plasma nitrite concentration progressively increased and reached a peak value 3 hrs after endotoxin administration Figure 4.

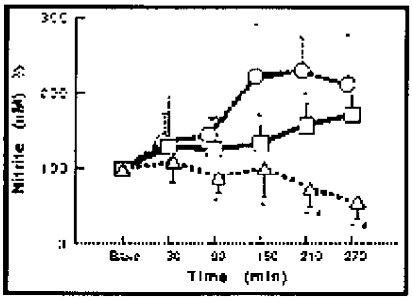

Figure 4. Time course of plasma nitrite concentration in the animals treated with endotoxin alone (circles, interrupted line), endotoxin and methylene blue (squares, continuous line), and methylene blue alone (triangles, interrupted line). *p less than .05 vs. endotoxin alone; paragraph p less than .05 vs. endotoxin and methylene blue. Data are expressed as mean plus minus SD.

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Effects of Fluids and Methylene Blue.

In the endotoxin-treated dogs, the addition of methylene blue to fluids had different effects on the systemic and the pulmonary circulations, and these effects varied with the doses administered. MAP and systemic vascular resistance increased with methylene blue at low-to-moderate doses, but decreased at the highest dose. Pulmonary arterial pressure and pulmonary vascular resistance also increased, and these changes became significant at a higher dose. Cardiac index and heart rate did not change significantly Table 1; Figure 1. Left ventricular stroke work index increased, except at the highest dose of methylene blue Figure 1. At low-to-moderate doses of methylene blue, mesenteric and femoral artery blood flows increased. At the highest dose, femoral blood flow further increased, but mesenteric blood flow decreased. Renal artery blood flow was unaffected by methylene blue Figure 2. Fractional mesenteric blood flow progressively increased at low-to-moderate doses of methylene blue Figure 5.

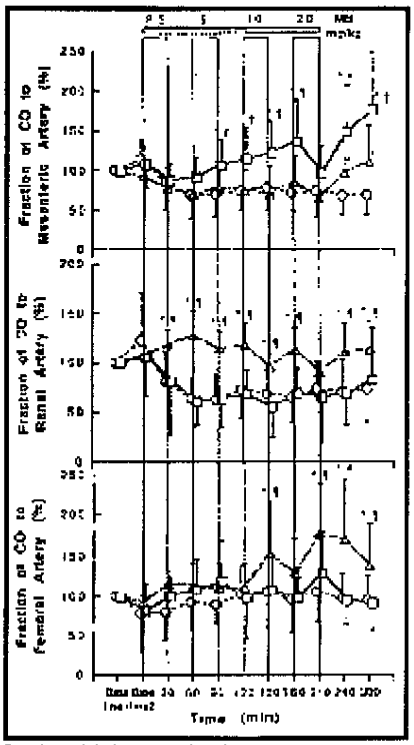

Figure 5. Changes in blood flow in the superior mesenteric, the left renal, and the left femoral vascular beds, in proportion to cardiac output (CO), before and after administration of methylene blue, in the animals treated with endotoxin alone (circles, interrupted line), endotoxin and methylene blue (squares, continuous line), and methylene blue alone (triangles, interrupted line). *p less than .05 vs. endotoxin alone; paragraph p less than .05 vs. endotoxin and methylene blue; dagger p less than .05 vs. methylene alone. MB, methylene blue. Data are expressed as mean plus minus SD.

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DO_2 was unchanged, but VO_2 increased in a dose-dependent manner by an increase in the oxygen extraction ratio Figure 3. Both PaO_2 and mixed venous PO_2 decreased during each administration of methylene blue, but venous admixture was not significantly influenced Figure 3.

The endotoxin-induced increase in plasma nitrite was largely inhibited by methylene blue Figure 4.

The administration of methylene blue had no significant effect on the fluid requirements of the animals and the hematocrit values were also unaffected Table 1.

Effects of Methylene Blue in Control Experiments.

In the absence of endotoxin, low doses of methylene blue did not alter MAP but reduced cardiac index, indicating an increase in systemic vascular resistance. In contrast, higher doses of methylene blue decreased MAP and systemic vascular resistance. Left ventricular stroke work index tended to decrease at the highest dose. However, pulmonary artery pressure and pulmonary vascular resistance increased in a dose-dependent manner Figure 1. Mesenteric and renal artery blood flow progressively decreased from baseline, but femoral blood flow did not significantly change Figure 2. Fractional femoral blood flow increased at higher doses of methylene blue Figure 5.

As in the presence of endotoxin, methylene blue induced dose-related reductions in PaO_2 and mixed venous PO_2, and induced dose-related increases in VO_2 and oxygen extraction ratio. Venous admixture was not significantly altered Figure 3.

There was a progressive decrease in plasma nitrite concentration as a consequence of methylene blue Figure 4.

DISCUSSION

The present study investigated the effects of methylene blue during endotoxic shock in fluid-resuscitated dogs. As we [24] reported previously, the hemodynamic pattern of the control endotoxic group was characterized by marked hypotension, peripheral vasodilation, but a well-maintained cardiac index. Blood flow was reduced in the mesenteric and renal vasculatures but was unchanged in the femoral bed, as has been reported by others [25]. The induction of nitric oxide synthase activity in rats and guinea pigs that are subjected to the effects of endotoxin may last approximate 1 to 3 hrs [26,27]. Our finding that plasma nitrite concentrations increased more than 1 hr after endotoxin administration in the present study suggests that the same mechanism for induction of nitric oxide synthase activity may occur in dogs. However, recent studies [7,8] reported that the rapid (less than equals 5 to 30 mins) development of hypotension in response to endotoxin and tumor necrosis factor in vitro and in vivo may also be mediated by an enhanced release of nitric oxide. Thus, we started the methylene blue infusion 30 mins after endotoxin administration to limit the duration of the experiments. The onset of the hemodynamic effects of methylene blue is relatively rapid, but the duration of action or the half-life of the substance has not been well documented. Thus, we elected to administer methylene blue as short-term (30-min) infusions of increasing doses and to maintain a 30-min interval between the successive infusions. Although we did not measure the methylene blue concentrations, we found that each dose of methylene blue was followed by rapid hemodynamic changes that were well maintained during the course of infusion. Nevertheless, all variables did not return to baseline after each dose so that some accumulation of methylene blue may have occurred. Hence, the doses used should be considered in relative terms: low, moderate, and very high.

We observed that the effects of methylene blue differed according to the dose administered. Low-to-moderate doses of methylene blue increased arterial blood pressure and systemic vascular resistance. In contrast, the highest dose of methylene blue had a strong vasodilating effect, as characterized by decreases in arterial pressure and systemic vascular resistance. This effect, which has also been reported in rats [28], may be related to the release of oxygen free radicals in the presence of methylene blue, converting the phenothiazine group of methylene blue to a phenyl radical, which is known to activate rather than to block guanylate cyclase [29]. Chen and Gillis [29] showed that high doses of methylene blue enhanced photorelaxation in response to ultraviolet-mediated release of oxygen free radicals in vascular rings.

An important observation was that at the low and moderate doses, methylene blue increased left ventricular stroke work index, which was reduced in the presence of endotoxin. This effect was not observed in the control group. Since cardiac filling pressures were similar in the endotoxic animals, with and without methylene blue, this observation indicates an improved myocardial performance. Such a phenomenon is consistent with observations by Brady et al. [11], who showed that methylene blue increased cardiac contractility in vitro in endotoxemia. This cardiac effect of methylene blue may be associated with methylene blue's attenuation of nitric oxide, as shown in the present study and by others [19,30].

Unlike in the systemic circulation, methylene blue induced dose-dependent increases in pulmonary arterial pressure and vascular resistance that persisted at the highest dose of methylene blue. Another experimental study [31] indicated that methylene blue increases pulmonary arterial tone in a dose- and time-dependent manner, presumably by inhibiting the effects of nitric oxide.

Similar observations have been made with nitric oxide inhibitors in endotoxemia [10,32]. Moreover, nitric oxide inhalation attenuates sepsis-induced pulmonary hypertension [33]. By inhibiting prostacyclin synthesis, methylene blue could also enhance the effects of thromboxane, which is largely implicated in the chain of events leading to endotoxin-induced pulmonary hypertension [34,35]. In the absence of severe hypoxemia following endotoxin challenge, potentiation of the hypoxic pulmonary vasoconstriction by methylene blue did not play an important role in the present study.

Methylene blue markedly influenced regional blood flow. In the absence of endotoxin, the vasoconstricting effects of methylene blue were associated with a progressive decrease in mesenteric and renal, but not in femoral artery blood flow. A fundamental observation in our study was that low-to-moderate doses of methylene blue selectively increased superior mesenteric artery blood flow after endotoxemia. Absolute blood flow increased in the mesenteric and the femoral beds, but not in the renal bed, and fractional blood flow increased more in the mesenteric bed than in the other beds. These unexpected observations can ascribed to several mechanisms. First, the higher arterial pressure induced by methylene blue may increase the perfusion pressure to the mesenteric circulation. Second, there may be some regional differences in the nitric oxide release [7,26,27,36] in various regional beds and in the guanylate cyclase activity [37,38], especially in the setting of sepsis. However, the effects of methylene blue on regional blood flow may be different from those effects of nitric oxide synthase inhibitors. Several investigators [2,14,15,28,39,40] reported the effects of nitric oxide inhibitors on systemic and regional hemodynamics, both under control and endotoxic conditions. In control anesthetized rats and rabbits, as in conscious rats, the intravenous administration of nitric oxide synthase inhibitors (i.e., N^G-monomethyl-L-arginine or N^G-nitro-L-arginine methyl ester) caused a dose-dependent reduction in blood flow in mesenteric, renal, and hindquarters vascular beds [2,28,39]. After endotoxic shock in rats and rabbits, Hutcheson et al. [15] and Wright et al. [14] observed that N^G-monomethyl-L-arginine pretreatment enhanced intestinal damage and the increases in vascular permeability, and decreased hepatic and hindquarter blood flow. However, their models were different from our canine model of hyperdynamic endotoxic shock. In sheep, N^G-nitro-L-arginine methyl ester has been shown to decrease blood flow rates to the brain, splanchnic, and renal beds after endotoxemia [40]. Hence, we do not think that alteration of the perfusion pressure and some regional differences in the nitric oxide release were implicated in the increased mesenteric blood flow under the influence of methylene blue.

Methylene blue may exert effects other than blocking guanylate cyclase [41]. Methylene blue may induce the generation of oxygen free radicals [19,36]. If methylene blue acted only as a guanylate cyclase inhibitor, one would expect nitrite concentrations to be unaffected by methylene blue after endotoxin administration. Instead, we observed in the endotoxic animals that the plasma nitrite concentration was lower after methylene blue administration, suggesting that methylene blue also inhibited the release of nitric oxide. These observations are consistent with observations from other recent studies reporting that methylene blue can directly inhibit nitric oxide synthase in vivo [19] and in vitro [30].

Methylene blue administration reduced mesenteric blood flow in the control group, so that the increase in mesenteric blood flow induced by methylene blue took place only in the presence of endotoxin. These effects may be due to a potentiating effect of methylene blue on the nitrovasodilator-induced, endothelium-independent relaxation of the mesenteric vasculature [36]. Thus, during endotoxic shock, methylene blue may have beneficial effects by diverting blood flow away from organs with a substantial oxygen extraction reserve (i.e., the femoral circulation) to organs that primarily depend on blood flow to maintain tissue oxygen availability (i.e., the splanchnic circulation) [42,43].

Despite the lack of significant increase in cardiac index and DO_2, methylene blue significantly increased VO_2 in a dose-dependent manner, probably by increasing the oxygen demand of the cells. Studies in vivo [44,45] and in vitro [46] have reported that methylene blue increases oxygen demand in a dose-dependent manner by altering cytochrome function [45]. Depletion of glutathione stores by methylene blue may also contribute to the higher oxygen demand associated with an increase of the inorganic phosphate/adenosine triphosphate ratio [47]. This increase in VO_2 was primarily obtained by an increase in oxygen extraction ratio, since oxygen supply was unaltered. During endotoxic shock in sheep, such changes may not be observed during N^G-nitro-L-arginine methyl ester administration [10]. An enhancement in cellular oxygen availability may be associated with an improvement in blood flow distribution, but one would expect this effect to be associated with a decrease in lactate concentrations [24], and that decrease was not observed here.

There was a progressive decrease in PaO_2 in methylene blue-treated animals in the presence and in the absence of endotoxin. Since venous admixture was not altered by methylene blue, the lower PaO_2 value was related to the higher oxygen extraction ratio, which resulted in a lower mixed venous PO_2.

In conclusion, our findings indicate that methylene blue exerts different effects corresponding to the absence or the presence of endotoxin. Our findings also demonstrate that the effects of methylene blue critically depend on the dose administered. Low-to-moderate doses of methylene blue can significantly increase arterial pressure during endotoxic shock, and low doses of methylene blue may also selectively increase mesenteric blood flow. However, higher doses of methylene blue may be potentially deleterious by further reducing vascular tone and also increasing pulmonary hypertension in endotoxic shock.

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