BRIEF REPORT

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Short-term beneficial effects of methylene blue on kidney damage in septic shock patients

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Abstract *Objective:* We previously
demonstrated that upregulation of
renal inducible nitric oxide synthase
(iNOS) is associated with proximal
tubule injury during systemic inflam-
mation in humans. In this study we
investigated the short-term effect of
methylene blue (MB), an inhibitor of
the NO pathway, on kidney damage
and function in septic shock patients.
Design and setting: A prospective
clinical study conducted in an in-
tensive care unit. *Patients:* Nine
patients (four men, five women, mean
age 71 ± 3 years) with confirmed or
suspected bacterial infection and with
refractory septic shock defined as
a mean arterial pressure ≤ 70 mmHg
despite norepinephrine infusion
$\geq 0.2\,\mu g/kg$ per minute. *Interven-
tions:* A 4 h continuous intravenous
infusion of 1 mg/kg MB per hour.
Measurements and results: The
urinary excretion of NO metabolites
decreased with median 90% (range
75–95%) from baseline to 6 h after
MB administration. The first 24 h
creatinine clearance improved by 51%
(18–173%) after MB treatment but
was still strongly impaired. During
the first 6 h after the start of MB
treatment both the urinary excretion
of cytosolic glutathione *S*-transferase
A1-1 and P1-1, markers for proximal
and distal tubule damage, respectively,
decreased by 45% (10–70%) and 70%
(40–85) vs. baseline. After termi-
nation of the MB infusion the NO
metabolites and markers of tubular
injury returned to pretreatment levels.
Conclusions: In septic patients with
refractory shock short-term infusion
of MB is associated with a decrease in
NO production and an attenuation of
the urinary excretion of renal tubular
injury markers.

Keywords Inducible nitric oxide
synthase expression · Acute kidney
injury · Glutathione *S*-Transferase ·
Nitric oxide metabolites

Introduction

Refractory septic shock remains the major cause of death in noncoronary intensive care units, especially when accompanied by multiple organ failure, with an estimated mortality rate of 50–60% [1]. The incidence of acute renal failure in refractory septic shock is approx. 40–50% [2]. Nitric oxide has emerged as an important contributory factor to the pathogenesis of septic shock. We previously demonstrated that induction of renal NO is associated with proximal tubule injury during systemic inflammation in humans [3]. NO stimulates soluble guanylate cyclase (sGC) by binding to its heme moiety, which generates cyclic guanosine monophosphate (cGMP) [4]. In the kidney NO and cGMP production are associated with lipopolysaccharide-induced renal proximal tubular cell toxicity [5]. Selective sGC inhibition during septic shock in rats resulted in an attenuation of renal dysfunction [6], indicating that blocking sGC may be a potential therapeutic strategy to treat septic shock-associated renal failure.

Methylene blue (MB) binds to sGC, blocks cGMP production and has the ability to scavenge NO and to inhibit NO synthases [7, 8]. Although several controlled and uncontrolled clinical studies showed beneficial effects of MB on the hemodynamic instability during septic shock (reviewed in [9]), there are no studies on the putative protective renal effects. We examined the short-term effects of continuous infusion of low-dose MB (1 mg/kg per hour) in patients with septic shock on urinary excretion of the acute kidney injury markers, cytosolic glutathione S-transferases (GSTs) present in proximal tubule (GSTA1-1) and distal tubule (GSTP1-1) [10].

Material and methods

Patients

Nine patients received a 4 h continuous infusion of 1 mg/kg per hour MB (1% w/v) provided by the VieCuri Medical Center pharmacy (for inclusion criteria see Electronic Supplementary Material, ESM). Arterial blood and catheterized urine were collected at several time points during the first 24 h. Clinical parameters, the severity of illness using Acute Physiology and Chronic Health Evaluation II (APACHE II), and Sepsis-Related Organ Failure Assessment (SOFA) were recorded.

Chemical assays

Biochemical parameters were determined by routine clinical chemistry. Hemoglobin, methemoglobin, and bilirubin were measured to assess possible side effects of MB, such as hemolytic anemia and methemoglobine-

Fig. 1 NO metabolites in plasma and urine and the urinary excretion of tubular injury markers glutathione S-transferase (*GST*) A1-1 and P1-1. NO metabolite levels in plasma (**a**, $n = 9$) and urine (**b**, $n = 8$) and levels of GSTA1-1 (**c**, proximal tubule, $n = 8$) and GSTP1-1 (**d**, distal tubule, $n = 8$) were measured in urine at various times after MB administration in septic shock patients. The urinary excretion of NO metabolites and GSTs was corrected for creatinine excretion. Data are expressed as median with 25–75% range and analyzed by analysis of variance with repeated measures over the two time periods; * $p < 0.05$ vs. baseline, # $p < 0.05$ vs. 6 h after MB treatment
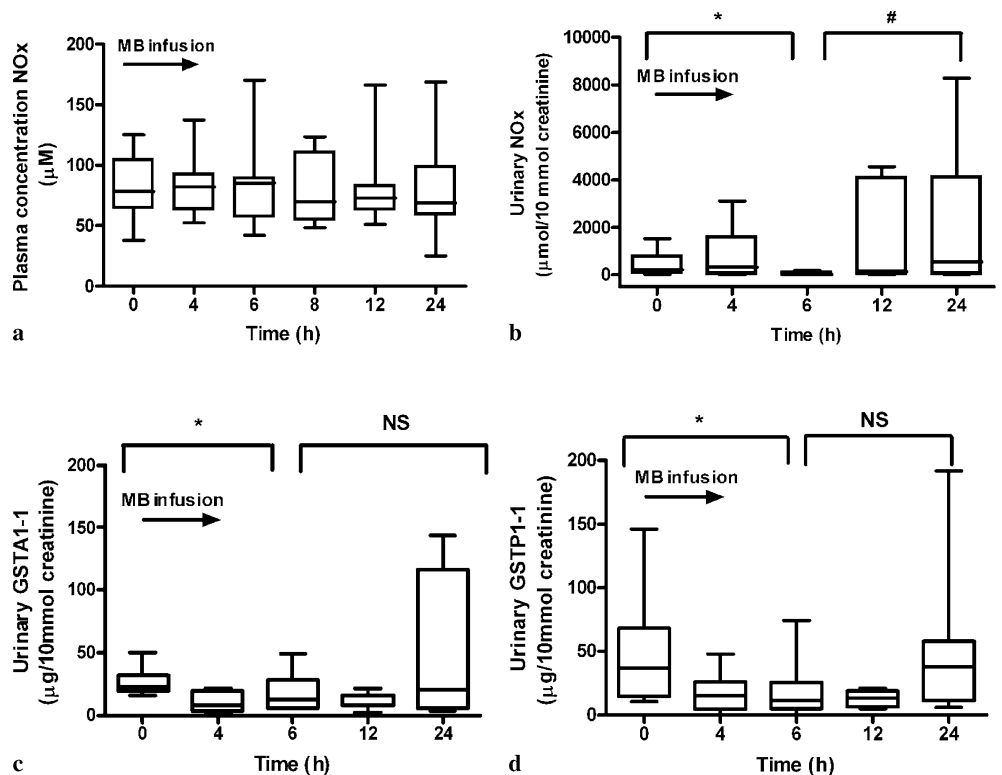
mia [11–13]. Total amount of the stable NO metabolites, nitrate and nitrite, measure of NO radicals production, and the amounts of GSTA1-1 and GSTP1-1 in urine were assayed as described [3]. The blue color in urine due to MB excretion did not affect the chemical assays.

Statistical analysis

Values are given as mean ± S.E. or as median (25–75% range) depending on their distribution. Differences between experimental groups were tested by analysis of variances for repeated measures. A two-tailed p-value less than 0.05 was considered statistically significant.

Results

Patients

Seven patients ultimately died in the intensive care unit, one of refractory shock (within 12 h) and six patients because of multiple organ failure. In the latter group two died within 7 days and the remaining four within 28 days after intervention. The mean calculated predicted mortality rate was 61%, and all patients had at least three organ failures, reflecting a mean SOFA score of 11.1 ± 0.9. The median stay at the intensive care unit was 16 days (range 7–24); the two survivors stayed 89 days (52–121) in hospital. Pathogenic organisms isolated by culture and site of infection are illustrated in the ESM. Median C-reactive protein was 178 mg/l (118–189); all patients had lactic acidemia (median 2.7 mmol/l, range 2.1–3.7) and thrombocytopenia (68×10^9/l, 50–104). The median MAP increased slightly by 5 mmHg (2–11) from 69 (65–70) at baseline to 74 (68–82) 3 h after the start of

Table 1 Kidney function parameters of septic shock patients ($n = 8$) treated with MB

	Median	Range
Total urine volume, 0–24 h (ml)	495	169–885
Protein excretion, 0–24 h (mg/day)	342	245–434
Creatinine clearance (ml/min)		
Baseline	8.2	4.2–17.4
24 h	10.6*	9.6–14.8
Blood urea nitrogen		
Baseline	17.8	10.8–20.0
24 h	17.3	10.4–22.5
Fractional excretion of sodium (%)		
> 2%		
Baseline ($n = 0$)	–	–
24 h ($n = 3$)	3.1	2.6–3.6
< 1%		
Baseline ($n = 8$)	0.6	0.3–0.7
24 h ($n = 5$)	0.4	0.2–0.5

$p < 0.05$ vs. baseline

MB ($p < 0.05$) with no change in norepinephrine infusion rate. Methemoglobinemia or hemolytic anemia did not develop after MB (data not shown). All MB-treated patients showed blue-coloring of urine and skin.

MB attenuates NO formation

The concentration NO metabolites in plasma was higher in septic shock patients than in to healthy volunteers [3] but did not change after MB administration (Fig. 1a). In contrast, MB significantly attenuated the urinary excretion of NO metabolites by a median 90% (75–95%, $p < 0.05$) from baseline (233 μmol/mmol creatinine, 112–536) to 6 h (37, 10–87) after the start of MB (Fig. 1b). At 4h the MB infusion was stopped, after which the median excretion of urinary NO metabolites increased by 135% (65–795%, $p < 0.05$) within 6–24 h after MB treatment (Fig. 1b).

MB attenuates kidney damage

All patients showed impaired renal function with oliguria and mild proteinuria (Table 1). One patient suffered from anuria during the first day. Six patients required continuous venovenous hemofiltration renal replacement therapy with a flow of 35 ml/kg (for median 8.5 days, 4–13), of which 3 during the MB infusion. The creatinine clearance improved by median 51% (18–173%, $p < 0.05$) during the first 24 h after MB but was still strongly impaired (Table 1). The urinary excretion of both GSTA1-1 and GSTP1-1 was elevated in all septic shock patients, indicating both proximal and distal renal tubule damage. During the first 6 h of MB urinary excretion of GSTA1-1 and GSTP1-1 was attenuated by median 45% (10–70%) and 70% (40–85%) vs. baseline (Fig. 1c, d, $p < 0.05$). After ending MB infusion urinary excretion of GSTA1-1 and GSTP1-1 increased again, although not significantly (Fig. 1c, d).

Discussion

Several clinical studies in septic shock patients have investigated the effects of MB on the heart, vascular wall, and lungs [9]. This is the first report demonstrating that MB attenuates kidney damage in human septic shock. To determine the effect of NO pathway inhibition on renal damage we examined the urinary excretion of early tubular injury markers and found that MB inhibited the NO pathway and preserved the integrity of renal tubules. After termination of MB infusion these parameters returned to their elevated pretreatment levels. Both plasma concentrations (more than twofold) and urinary NO metabolites levels in our septic shock patients were much higher than in healthy volunteers, as demonstrated earlier [3]. Hydrocortisone may have inhibited iNOS

activation, however, the patients received a continuous infusion of hydrocortisone that started before MB infusion and continued during the 24 h period. Therefore the observation that urinary NO metabolite excretion was attenuated only in the first 6 h after the start of MB suggests that this effect is not related to steroids. Although urinary NO metabolite excretion was attenuated, we did not find a reduction in plasma NO metabolites. This is in contrast with an earlier report, in which patients received a bolus injection of MB prior to the continuous infusion [14].

Renal failure in septic shock patients is a complex and multifactorial disease process. During septic shock the systemic vasodilation increases renal sympathetic activity and angiotensin concentration which results in intrarenal vasoconstriction with sodium and water retention and decreased glomerular filtration rate [2]. We previously demonstrated that induction of renal iNOS, constitutively expressed in the kidney [15], is associated with proximal tubule injury during systemic inflammation in humans [3]. As a result of its active secretory transport function and role in urine concentration, the proximal tubule is a susceptible target and often the first site of damage [16]. Therefore inhibition of peroxynitrite formation from excessively produced NO and superoxide [17] by MB may be beneficial for the kidney during septic shock, possibly explained by the local accumulation of MB in renal proximal tubules [18]. Global hemodynamic variables can influence renal function, however, only a small but statistically significant increase was found for mean arterial pressure, whereas other global hemodynamic parameters did not change during MB treatment.

Since the detailed nature of our investigation, obviously these methods are not feasible in a large-scale clinical intervention study. The most elegant way to examine the effects of MB would be in a randomized, placebo-controlled cross-over study. However, with such critically ill patients this design may be considered unethical. We deliberately chose a subgroup of severe septic shock patients with a high chance of sepsis-induced renal damage for two reasons: first, this is the group of patients in which MB is used as a "last resort therapy" and, second, to demonstrate the putative beneficial effects of MB on renal damage. With an estimated standard deviation of 36% in urinary GST excretion, 80 patients would be needed to demonstrate with 80% power a 10% reduction in renal injury. This number of patients was not feasible for our investigations. Therefore we decided to determine the parameters before, during and after MB infusion during a 24 h period, which allows each patient to serve as own control. Because of the observational nature and limited size of the present study and the heterogeneity of the patient population, our findings warrant conformation on hard endpoints by a larger clinical trial. However, in our view, first a long-term study is necessary to assess the safety of chronic MB administration in septic patients with refractory shock. Promising effects of MB were found in a trial with vasoplegic patients after cardiac surgery treated with MB [19], in which a reduction in both mortality and incidence of renal failure was observed.

In conclusion, short-term infusion of MB in septic patients with refractory shock, is associated with a decrease in NO production and an attenuation of the urinary excretion of renal tubular injury markers.

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