Intensive Care Med (2000) 26: 117–124

EXPERIMENTAL

C. Mitaka
Y. Hirata
Y. Masaki
T. Takei
K. Yokoyama
T. Imai

S-Methylisothiourea sulfate improves renal, but not hepatic dysfunction in canine endotoxic shock model

Received: 16 May 1999
Final revision received: 13 September 1999
Accepted: 15 November 1999

C. Mitaka (✉) · T. Imai
Department of Emergency and Critical Care Medicine,
Tokyo Medical and Dental University,
1–5-45, Yushima, Bunkyo-ku, Tokyo, 113–8519, Japan
e-mail: c.mitaka.icu@med.tmd.ac.jp
Tel.: + 81-3-5803 5652
Fax: + 81-3-5803 0168

Y. Hirata
Department of Internal Medicine II,
Tokyo Medical and Dental University,
1–5-45, Yushima, Bunkyo-ku, Tokyo, 113–8519, Japan

Y. Masaki · T. Takei · K. Yokoyama
Department of Anesthesiology,
Tokyo Medical and Dental University,
1–5-45, Yushima, Bunkyo-ku, Tokyo, 113–8519, Japan

Abstract *Objective*: Excess production of nitric oxide (NO) by inducible NO synthase (iNOS) has been implicated in the pathophysiology of septic shock. This study was designed to see whether S-methylisothiourea sulfate (SMT), a selective inhibitor for iNOS, prevents cardiovascular changes and multiple organ damage in the canine endotoxic shock model.
Design: Prospective, comparable, experimental study.
Setting: Laboratory at a university hospital.
Subjects: Twenty male mongrel dogs were studied under pentobarbital anesthesia.
Interventions: Dogs were divided into three groups: bacterial lipopolysaccharide (LPS) group ($n = 7$) receiving continuous infusion of LPS (2 mg/kg/h for 1 h); LPS plus SMT group ($n = 7$) receiving LPS and SMT (1 mg/kg, bolus i.v., followed by continuous infusion of 1 mg/kg/h for 1 h); and vehicle plus SMT group ($n = 6$).
Measurements and results: Hemodynamics, blood gas parameters, and urine output were measured during 6 h observation periods. Serum levels of lactate, transaminases, and bilirubin were measured at baseline, 1 and 6 h. Creatinine and free water clearance, urine sodium excretion and fractional excretion of sodium were calculated. LPS caused a profound hypotension associated with decreases in cardiac output and oxygen delivery, lactic acidosis, renal and liver dysfunction, and thrombocytopenia. SMT prevented the LPS-induced hypotension and renal dysfunction, whereas it did not affect the LPS-induced decreases in cardiac output or oxygen delivery, hyperlactatemia, liver dysfunction, or thrombocytopenia. SMT alone had no appreciable effects on hemodynamics, blood gases, liver or renal functions.
Conclusions: These findings show that SMT improves renal, but not hepatic dysfunction, in dogs with endotoxic shock, suggesting that iNOS-derived NO plays differential roles in sepsis-associated multiple organ dysfunction.

Key words Endotoxic shock · Nitric oxide synthase inhibitor · S-Methylisothiourea · Hemodynamic parameters · Renal dysfunction · Hepatic dysfunction

Introduction

Nitric oxide (NO) is produced from L-arginine by three isozymes of NO synthase (NOS); neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). NO production at the site of acute inflammation may be one of the defense mechanisms against microbial activity [1, 2]. However, in severe infections such as sepsis, excess production of NO contributes to hypotension and vascular hyporeactivity to

vasopressor agents in septic shock [3, 4, 5]. It has been reported that nonselective NOS inhibitors, such as N^G-monomethyl-L-arginine, N^G-nitro-L-arginine methyl ester, and N^G-nitro-L-arginine are used for septic shock in human and animal models [6, 7, 8]. However, these NOS inhibitors have been shown to decrease cardiac output and oxygen delivery [8] and deteriorate liver damage [9] due to total inhibition of all three NOS isozymes [1]. Therefore, inhibitors selective for iNOS are expected to be therapeutically useful for septic shock. In our previous study [10] we have shown that aminoguanidine, a relatively selective iNOS inhibitor, prevented hypotension without improvement of metabolic acidosis in dogs with endotoxic shock, a stable animal model manifesting many features common to sepsis in man.

It has been reported that S-methylisothiourea sulfate (SMT), a more potent inhibitor for iNOS than aminoguanidine, increases survival rate in rats with endotoxic shock [11] and bacterial peritonitis [12]. However, no detailed information regarding its beneficial effect on endotoxic shock animal models other than rodents has been available to date. Therefore, this study was designed to elucidate whether SMT prevents the hemodynamic and blood gas changes, organ damage, and hematologic abnormalities in dogs with endotoxic shock.

Materials and methods

Animal preparation

This study was approved by the Institutional Review Board for the care of animal subjects. Twenty male mongrel dogs (13.6 ± 0.6 kg) were used in this experiment. After intramuscular injection of ketamine (15 mg/kg), the dogs were placed in the supine position, and anesthetized with pentobarbital administered as an initial slow bolus (20 mg/kg, i.v.), followed by a constant infusion (4 mg/kg/h). They were intubated and connected to a ventilator (EV-A, Dräger, Lübeck, Germany). Inspired oxygen fraction was maintained at 0.3, and the ventilator rate and tidal volume were adjusted to obtain an arterial carbon dioxide tension ($PaCO_2$, 4.7 ± 0.1 kPa, mean ± SEM) by pH/blood gas analyzer (IL 1306A, Instrumentation Laboratory, Lexington, Mass., USA), and maintained throughout the experiment. A catheter was inserted into the femoral vein and lactated Ringer's solution (10 ml/kg/h) was administered throughout the study period. Muscle paralysis was obtained by the administration of pancuronium bromide with initial dose of 0.16 mg/kg and subsequent doses of 0.08 mg/kg/h. A pulmonary arterial catheter (93A-141–7F, Baxter Edwards Critical-Care, Irvine, Calif., USA) was inserted through the jugular vein. Another catheter was cannulated into the femoral artery for continuous measurements of systemic arterial pressure and for intermittent arterial blood sampling. Heart rate (HR) was monitored by electrocardiogram. Blood pressures were recorded on an eight-channel recorder (Polygraph 142–8, SAN-EI Instrument, Tokyo, Japan). After surgical preparation, the dogs were allowed to stabilize for 1 h.

Experimental protocol

Dogs were divided into three experimental groups; lipopolysaccharide (LPS) group ($n = 7$), LPS plus SMT group ($n = 7$), and vehicle plus SMT group ($n = 6$). In LPS group, bacterial LPS (*Escherichia coli* endotoxin, 0127:B8, Difco, Detroit, Mich.) was infused intravenously at a rate of 2 mg/kg/h for 1 h. This method of LPS administration has been shown to reproduce the hemodynamic effects seen in human septic shock [13]. In LPS plus SMT group, both LPS (2 mg/kg/h, for 1 h) and SMT (Alexis, Laufelfingen, Switzerland; 1 mg/kg, bolus i.v., followed by continuous infusion of 1 mg/kg/h for 1 h) were simultaneously administered. The doses of SMT were chosen on the basis of pilot experiments and previously published data in rodent model [11]. In vehicle plus SMT group, vehicle and SMT in the same dose were infused. During the initial 1 h period, hydroxyethyl-starch (20 ml/kg/h) instead of lactated Ringer's solution was infused in all three groups.

Measurements of hemodynamic and blood gas parameters

HR, mean arterial pressure (MAP), mean pulmonary arterial pressure, pulmonary capillary wedge pressure, and central venous pressure, and arterial and mixed venous blood gases were measured at one-hour intervals for 6 h. Cardiac output was measured by injecting 5 ml of iced 5% dextrose solution using the thermodilution technique with a cardiac output computer (9520A, Baxter Edwards Critical-Care, Irvine, Calif., USA), and the mean value of three measurements were calculated; body-surface area (m^2) was calculated by the formula [body weight $(kg)^{2/3} \times 0.11$]. Arterial and mixed venous blood samples were obtained simultaneously from femoral and pulmonary artery, respectively. Systemic vascular resistance index, pulmonary vascular resistance index, oxygen delivery index, oxygen consumption index, and oxygen extraction ratio were calculated by standard formulas.

Additional arterial blood samples were obtained at baseline, 1, and 6 h for measurements of lactate by an automatic analyzer (aca SX, Dade Behring, Deerfield, Ill., USA), aspartate and alanine aminotransferase, bilirubin, sodium and creatinine by an automatic analyzer (7170, Hitachi, Tokyo, Japan). Urine volume was measured hourly and urinary concentrations of sodium and creatinine were also measured at baseline, 1, and 6 h. Creatinine clearance, urine sodium excretion (UNaV), fractional excretion of sodium (FENa), and free water clearance were calculated. Osmolality of serum and urine was measured by cryoscopy using an osmometer (MARK3, Fiske Associates, Norwood, Mass., USA). Differential blood cell counts and hematocrit were measured by Celltak α (MEK-6108, Nihon Kohden, Tokyo, Japan).

Statistical analysis

All data were presented as mean ± SEM. Parameters were compared over the time course using an analysis of variances for repeated measures. When appropriate, multiple comparisons were made with Bonferroni adjustments for the effects of treatment at specific times, and for the effect of time in specific group with baseline as a control. $P < 0.05$ was considered statistically significant.

Table 1 Hemodynamic and gas exchange parameters in anesthetized dogs after administration of bacterial lipopolysaccharide and/or S-methylisothiourea sulfate

Parameters	Treatment	0 h	1 h	2 h	3 h	4 h	5 h	6 h
HR	LPS	165 ± 11	160 ± 9	156 ± 9	152 ± 9	153 ± 8	159 ± 10	163 ± 11
(beats/min)	LPS + SMT	188 ± 9	177 ± 7	173 ± 7	166 ± 9	167 ± 9	167 ± 10	174 ± 12
	Vehicle + SMT	177 ± 3	160 ± 7	150 ± 9	150 ± 7	157 ± 5	164 ± 4	166 ± 6
PCWP	LPS	10 ± 1	9 ±1	9 ± 2	10 ± 2	10 ± 2	11 ± 2	13 ± 2
(mm Hg)	LPS + SMT	12 ± 1	11 ± 2	11 ± 2	12 ± 1	13 ± 2	13 ± 1	14 ± 2
	Vehicle + SMT	11 ± 1	12 ± 1	12 ± 1	11 ± 1	12 ± 1	12 ± 1	12 ± 2
CVP	LPS	4 ± 1	4 ± 1	4 ± 1	5 ± 1	6 ± 1	6 ± 1	7 ± 1
(mm Hg)	LPS + SMT	5 ± 1	5 ± 1	5 ± 1	5 ± 1	6 ± 1	6 ± 1	7 ± 1
	Vehicle + SMT	5 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1
VO_2I	LPS	139 ± 13	119 ± 14	115 ± 12	120 ± 9	122 ± 13	122 ± 14	123 ± 13
(ml/min/m^2)	LPS + SMT	120 ± 4	122 ± 10	117 ± 8	123 ± 9	123 ± 11	112 ± 9	121 ± 10
	Vehicle + SMT	134 ± 12	146 ± 8	137 ± 9	134 ± 7	140 ± 13	150 ± 12	148 ± 13
O_2EXT	LPS	0.22 ± 0.04	0.20 ± 0.02	0.22 ± 0.02	0.25 ± 0.03	0.26 ± 0.03	0.28 ± 0.04	0.32 ± 0.04
	LPS + SMT	0.16 ± 0.01	0.20 ± 0.03	0.20 ± 0.01	0.22 ± 0.02	0.24 ± 0.02	0.22 ± 0.02	0.22 ± 0.02
	Vehicle + SMT	0.18 ± 0.01	0.18 ± 0.01	0.21 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.24 ± 0.03

Values are mean ± SEM. LPS, lipopolysaccharide; SMT, S-methylisothiourea sulfate; HR, heart rate; PCWP, pulmonary capillary wedge pressure; CVP, central venous pressure; VO_2I, oxygen consumption index; O_2EXT, oxygen extraction ratio

Table 2 Blood gas parameters in anesthetized dogs after administration of bacterial lipopolysaccharide and/or S-methylisothiourea sulfate

Parameters	Treatment	0 h	1 h	2 h	3 h	4 h	5 h	6 h
pH	LPS	7.42 ± 0.01	7.32 ± 0.02[a]	7.30 ± 0.02[b]	7.32 ± 0.02[a]	7.33 ± 0.02	7.32 ± 0.02[a]	7.31 ± 0.02[b]
	LPS + SMT	7.44 ± 0.02	7.36 ± 0.02	7.36 ± 0.02	7.36 ± 0.02	7.36 ± 0.02	7.35 ± 0.02	7.35 ± 0.03
	Vehicle + SMT	7.43 ± 0.02	7.40 ± 0.02	7.39 ± 0.02	7.38 ± 0.01	7.37 ± 0.01	7.37 ± 0.01	7.36 ± 0.01
PaO_2	LPS	18.5 ± 1.1	14.1 ± 1.5	15.4 ± 1.6	15.7 ± 1.7	15.7 ± 2.0	15.3 ± 2.1	15.3 ± 2.4
(kPa)	LPS + SMT	19.4 ± 0.5	17.4 ± 0.9	17.3 ± 1.3	17.4 ± 1.3	18.2 ± 0.8	17.4 ± 1.2	16.6 ± 1.1
	Vehicle + SMT	18.6 ± 1.1	19.0 ± 0.9	18.9 ± 0.8	19.0 ± 0.8	18.6 ± 0.9	18.9 ± 0.8	18.2 ± 1.1
$PaCO_2$	LPS	4.7 ± 1.3	4.9 ± 0.3	4.8 ± 1.3	4.7 ± 0.3	4.8 ± 0.3	4.7 ± 0.3	4.5 ± 0.3
(kPa)	LPS + SMT	4.5 ± 1.3	4.7 ± 0.3	4.7 ± 1.3	4.7 ± 0.3	4.7 ± 1.3	4.7 ± 0.3	4.5 ± 0.3
	Vehicle + SMT	4.7 ± 1.3	4.8 ± 1.3	4.7 ± 1.3	4.7 ± 1.3	4.7 ± 1.3	4.7 ± 1.3	4.7 ± 1.3
HCO_3^-	LPS	22.7 ± 0.4	19.1 ± 0.4[b,c]	18.3 ± 0.5[b,d]	18.7 ± 0.7[b,d]	19.4 ± 0.8[b]	18.4 ± 0.7[b,c]	17.5 ± 0.9[b,c]
(mEq/l)	LPS + SMT	25.1 ± 1.8	19.6 ± 0.8[b,d]	19.8 ± 0.7[b,d]	20.0 ± 0.5[b]	20.2 ± 0.6[b]	19.4 ± 0.5[b]	19.1 ± 0.6[b]
	Vehicle + SMT	24.3 ± 0.3	22.8 ± 0.1	22.5 ± 0.1	22.0 ± 0.1	21.2 ± 0.3	20.9 ± 0.3	20.4 ± 0.2
Base excess	LPS	–0.6 ± 0.4	–5.6 ± 0.5[b,d]	–6.5 ± 0.7[b,d]	–5.7 ± 0.9[b,d]	–5.0 ± 0.8[b,c]	–6.0 ± 0.9[b,c]	–6.9 ± 1.1[b,c]
(mEq/l)	LPS + SMT	0.7 ± 1.1	–4.4 ± 0.8[b,d]	–4.3 ± 0.9[b,c]	–3.9 ± 0.9[a]	–3.9 ± 0.8[a]	–4.7 ± 0.8[b]	–4.9 ± 1.0[b]
	Vehicle + SMT	0.4 ± 0.2	–0.6 ± 0.3	–0.9 ± 0.2	–1.4 ± 0.3	–2.0 ± 0.4	–2.5 ± 0.5	–2.7 ± 0.6

Values are mean ± SEM. LPS, lipopolysaccharide; SMT, S-methylisothiourea sulfate. [a] $P < 0.05$, [b] $P < 0.01$ vs 0 h, [c] $P < 0.05$, [d] $P < 0.01$ vs Vehicle + SMT group

Results

Changes in hemodynamic and blood gas parameters

The changes in hemodynamic and blood gas parameters following intravenous administration of LPS and/or SMT are presented in Fig. 1 and Tables 1 and 2. Administration of LPS caused a gradual decrease in MAP over the first 15 min and a significant ($P < 0.05$) decrease in MAP as early as 1 h which persisted during a 6-h period. Decreases in cardiac index (4–6 h) and oxygen delivery index (6 h) with concomitant increase in pulmonary vascular resistance index (6 h) were observed (Fig. 1). Co-administration of SMT prevented the initial (15 min–1 h) and sustained (1–6 h) hypotension after LPS administration, but did not affect increase in pulmonary vascular resistance index or decreases in cardiac index and oxygen delivery index. Neither did HR, pulmonary capillary wedge pressure, central venous pressure, oxygen consumption index, or oxygen extraction ratio change after LPS administration (Table 1). SMT administered alone tended to produce an increase in MAP, although this did not reach statistical significance ($P > 0.05$); SMT alone did not signifi-

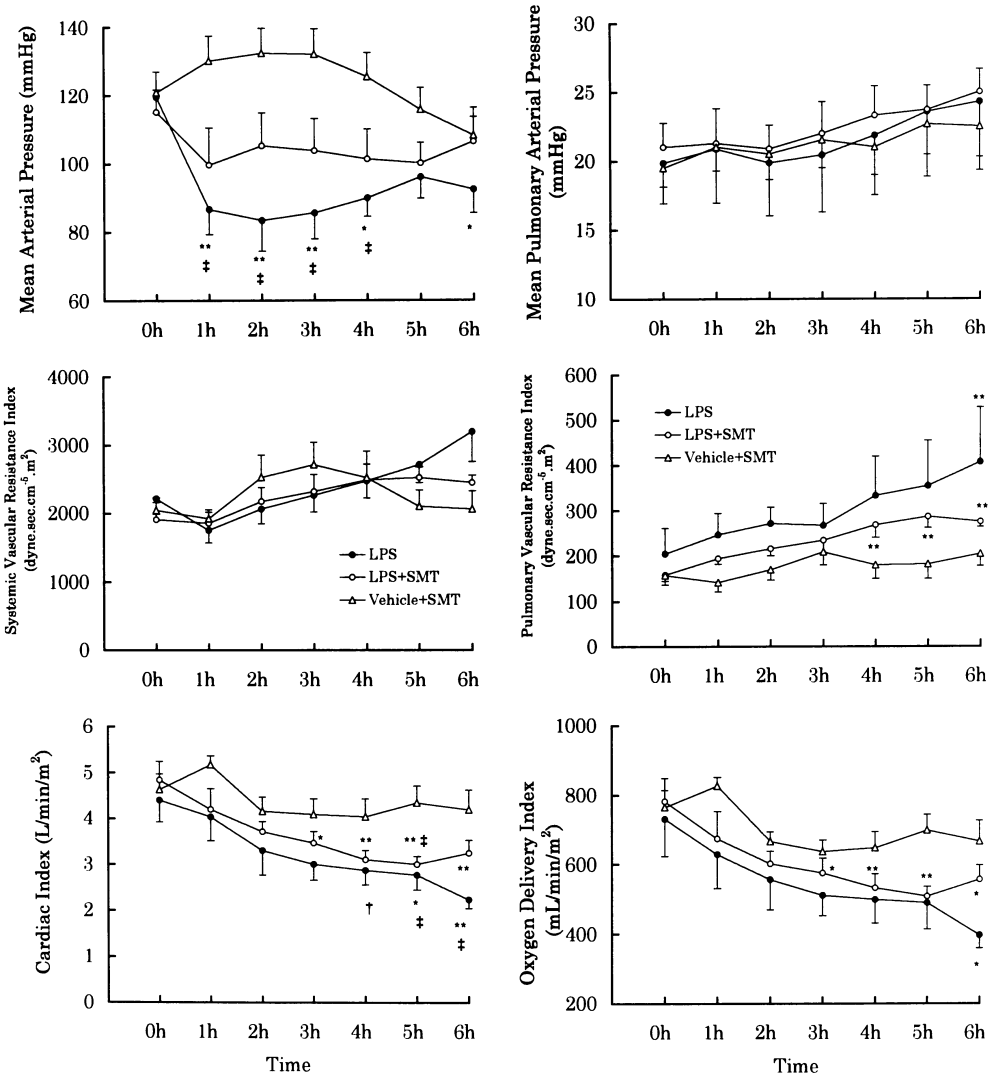

Fig. 1 Changes in mean arterial pressure, mean pulmonary arterial pressure, systemic vascular resistance index, pulmonary vascular resistance index, cardiac index, and oxygen delivery index after administration of bacterial lipopolysaccharide (LPS, 2 mg/kg/h, for 1 h) and/or S-methylisothiourea sulfate (1 mg/kg, i.v. plus 1 mg/kg/h, for 1 h). Values are means ± SEM. LPS group ($n = 7$, *closed circles*), LPS + SMT group ($n = 7$, *open circles*), and vehicle + SMT group ($n = 6$, *open triangles*). *$P < 0.05$, **$P < 0.01$ vs. 0 h, †$P < 0.05$, ‡$P < 0.01$ vs vehicle + SMT group

cantly affect other hemodynamic parameters during a 6-h period

Administration of LPS induced decreases in arterial pH, bicarbonate, and base excess consistent with metabolic acidosis whose effects were not significantly affected by co-administration of SMT (Table 2). PaO_2 and $PaCO_2$ did not change during a 6-h period after LPS administration. SMT administered alone did not significantly affect blood gas parameters during a 6-h period.

Changes in liver and renal functions

The changes in serum concentrations of aspartate aminotransferase, alanine aminotransferase, bilirubin, and lactate, following intravenous administration of LPS and/or SMT are shown in Fig. 2. Administration of LPS caused significant increases in serum concentrations of aspartate aminotransferase, alanine aminotransferase, bilirubin (6 h) and lactate (1 h) with or without co-administration of SMT. SMT administered alone had no appreciable effects on liver function.

The changes of renal function after LPS administration and/or SMT are shown in Table 3. Intravenous administration of LPS caused a significant ($P < 0.05$) decrease in creatinine clearance at 6 h, and co-administration of SMT improved LPS-induced decrease in creatinine clearance without changes in urine volume. Administration of LPS also significantly ($P < 0.05$) decreased urine osmolality and increased free water clearance without changes in UNaV or FENa. Co-administration of SMT prevented the LPS-induced decrease in creatinine clearance and increase in free water clearance. SMT with or without LPS administration increased FENa, but not UNaV, after 6 h.

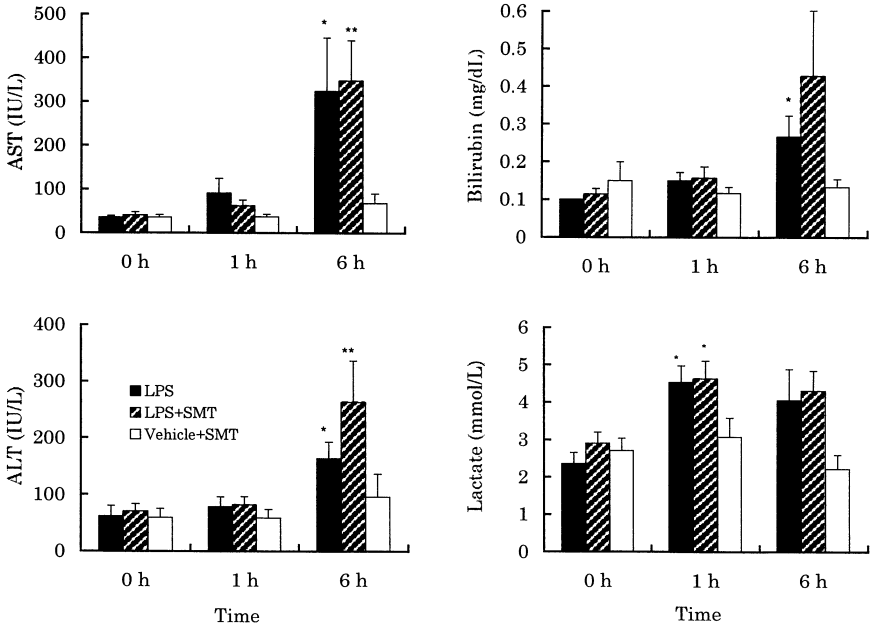

Fig. 2 Changes in serum concentrations of aspartate (AST) and alanine aminotransferase (ALT), bilirubin, and lactate after administration of bacterial lipopolysaccharide (LPS, 2 mg/kg/h, for 1 h) and/or S-methylisothiourea sulfate (1 mg/kg, i.v. plus 1 mg/kg/h, for 1 h). Values are means ± SEM. LPS group ($n = 7$, *solid bars*), LPS + SMT group ($n = 7$, *hatched bars*), and vehicle + SMT group ($n = 6$, *open bars*). *$P < 0.05$, **$P < 0.01$ vs 0 h

Table 3 Renal function parameters in anesthetized dogs after administration of bacterial lipopolysaccharide and/or S-methylisothiourea sulfate

Parameters	Treatment	0 h	1 h	6 h
UV (ml/h)	LPS	37 ± 9	38 ± 8	30 ± 11
	LPS + SMT	37 ± 12	44 ± 13	65 ± 22
	Vehicle + SMT	38 ± 4	66 ± 10	71 ± 26
Ccr (ml/min)	LPS	146 ± 48	83 ± 22	27 ± 9[a]
	LPS + SMT	153 ± 36	105 ± 22	91 ± 35
	Vehicle + SMT	144 ± 14	133 ± 13	72 ± 12
UNaV (microEq/min)	LPS	38 ± 8	48 ± 9	35 ± 8
	LPS + SMT	49 ± 15	77 ± 22	114 ± 43
	Vehicle + SMT	55 ± 5	104 ± 9	123 ± 39
FENa (%)	LPS	0.3 ± 0.1	0.3 ± 0.1	0.8 ± 0.2
	LPS + SMT	0.2 ± 0.1	0.6 ± 0.1	1.1 ± 0.3[b]
	Vehicle + SMT	0.3 ± 0.1	0.6 ± 0.1	1.6 ± 0.4[b]
Uosm (mmol/kg)	LPS	903 ± 134	474 ± 56[a]	360 ± 69[b]
	LPS + SMT	839 ± 158	569 ± 107	467 ± 49
	Vehicle + SMT	892 ± 208	570 ± 62	530 ± 58
C_{H_2O} (ml/min)	LPS	–1.3 ± 0.3	–0.4 ± 0.1[a]	–0.1 ± 0.1[b,c]
	LPS + SMT	–1.2 ± 0.3	–0.7 ± 0.3	–0.7 ± 0.2
	Vehicle + SMT	–1.3 ± 0.5	–1.1 ± 0.2	–0.9 ± 0.2

Values are mean ± SEM. LPS, lipopolysaccharide; SMT, S-methylisothiourea sulfate; UV, urine volume, Ccr, creatinine clearance; UNaV, Urine sodium excretion; FENa, Fractional excretion of sodium; Uosm, Urine osmolality; C_{H_2O}, Free water clearance; [a] $P < 0.05$, [b] $P < 0.01$ vs 0 h, [c] $P < 0.05$ vs Vehicle + SMT group

Hematologic changes

The changes in numbers of leukocyte and platelet, and hematocrit following intravenous administration of LPS with or without SMT are shown in Table 4. Leukocytosis occurred after 6 h in all three groups. A transient thrombocytopenia occurred at 1 h after administration of LPS with or without co-administration of SMT. Hematocrit did not change in any groups.

Discussion

The present study showed that administration of SMT reversed hypotension and renal dysfunction in dogs with endotoxic shock, although our canine model with increased HR and creatinine clearance under basal conditions is somewhat unphysiological, possibly resulting from animal preparation. As excess production of NO has been suspected to be one of the major causes of endotoxic shock [1], NOS inhibitors have been expected to improve endotoxic shock [4, 5]. It has been reported that several derivatives of isothiourea are found to be

Table 4 Leukocyte, platelet, and hematocrit in anesthetized dogs after administration of bacterial lipopolysaccharide and/or S-methylisothiourea sulfate

Parameters	Treatment	0 h	1 h	2 h	3 h	4 h	5 h	6 h
Leukocyte (cells/μl)	LPS	2586 ± 485	1843 ± 409	2657 ± 460	3843 ± 767	6214 ± 1740	9300 ± 3108	11 400 ± 3681[a]
	LPS + SMT	2417 ± 515	1200 ± 113	2050 ± 361	4367 ± 1280	7567 ± 2411	10 183 ± 3161	13 516 ± 4088[b]
	Vehicle + SMT	3317 ± 936	4283 ± 1374	5883 ± 1568	8733 ± 1903	10 666 ± 2560	12 416 ± 2860	13 300 ± 2318[b]
Platelet (10^4 cells/μl)	LPS	15.6 ± 4.5	7.0 ± 0.9[a,c]	10.7 ± 1.2[c]	12.0 ± 1.7	11.2 ± 1.6	11.7 ± 1.5	11.1 ± 1.3
	LPS + SMT	14.2 ± 2.1	6.3 ± 0.8[b,c]	11.5 ± 0.8	11.9 ± 0.5	12.3 ± 1.1	10.7 ± 1.0	11.1 ± 0.7
	Vehicle + SMT	16.2 ± 1.3	14.5 ± 0.9	15.2 ± 1.1	15.8 ± 1.2	15.2 ± 1.0	15.0 ± 1.1	14.6 ± 0.9
Hematocrit (%)	LPS	35.2 ± 1.4	35.8 ± 2.3	37.9 ± 2.1	38.0 ± 2.0	38.2 ± 1.7	39.1 ± 1.9	40.8 ± 2.0
	LPS + SMT	35.6 ± 2.0	35.8 ± 2.2	36.5 ± 2.0	37.6 ± 1.6	39.1 ± 1.6	38.2 ± 1.0	38.7 ± 1.2
	Vehicle + SMT	37.7 ± 4.8	35.1 ± 3.5	36.1 ± 3.8	36.3 ± 3.8	36.4 ± 3.9	36.3 ± 3.5	35.3 ± 4.6

Values are mean ± SEM. LPS, lipopolysaccharide; SMT, S-methylisothiourea sulfate. [a] $P < 0.05$, [b] $P < 0.01$ vs 0 h, [c] $P < 0.01$ vs Vehicle + SMT group

NOS inhibitors [14] among which SMT was shown to be tenfold more potent than N^G-monomethyl-L-arginine to inhibit iNOS. Furthermore, it has been reported that administration of SMT reversed hypotension and improved survival rate and organ functions in rodent model of endotoxic shock [11]. Since eNOS is involved in the regulation of basal vascular tone [1], usage of the drugs that selectively inhibit iNOS activity while preserving eNOS activity should have more appropriate therapeutic potential for endotoxic shock than nonselective NOS inhibitors.

However, recent observations have suggested that activation of eNOS is involved in a marked hypotension during early phase (≤ 5 to 60 min) following administration of endotoxin [5, 15]. The present study has shown that co-administration SMT prevented the initial and sustained hypotension following LPS administration. Thus, the early reversal of MAP by SMT is likely due to its partial inhibition of eNOS activation.

Reduced cardiac output and hypotension observed in this study are characteristic of endotoxic shock, and some investigators [16, 17] insist that myocardial depression may be a major cause of decreased hemodynamic state in endotoxic shock. It has been suggested that myocardial depression during sepsis is largely mediated by cytokine-stimulated NO production [18, 19]. If that is the case, selective inhibition of iNOS should prevent LPS-induced myocardial depression. However, co-administration of SMT did not affect LPS-induced decrease in cardiac index in the present study. These data suggest that inhibition of iNOS by SMT is insufficient to block negative inotropic effect by excess NO production. Alternatively, NO may not be responsible for LPS-induced myocardial contractile dysfunction. In fact, it has been reported that administration of LPS failed to induce iNOS in the myocardium of guinea pig [20]. Therefore, it remains unknown whether prevention of hypotension by SMT is related to improved myocardial function.

In this study, administration of LPS caused lactic acidosis and decreased oxygen delivery that reduced organ blood flow and induced subsequent tissue hypoxia. Co-administration of SMT with LPS maintained pH > 7.35 during a 6-h period, although it did not correct hyperlactatemia. Elevation of blood lactate concentrations, a sign of tissue hypoxia, would represent preferential anaerobiosis for energy metabolism to aerobiosis. This is most likely due to decreased tissue blood flow and an impaired cellular oxygen utilization [21]. However, lactate is not solely derived from tissue hypoxia, but endotoxin per se may also increase lactate concentrations because endotoxin directly inactivates pyruvate dehydrogenase [22]. In addition, hyperlactatemia can result not only from its overproduction, but also by its impaired metabolism. In fact, Levraut et al. [23] have recently shown that a mild hyperlactatemia in the stable septic patients is mainly due to a defect in lactate utilization rather than its overproduction. Therefore, hyperlactatemia in the present endotoxic shock dogs may be, in part, a consequence of disturbances of the lactate metabolism other than tissue hypoxia. Namely, SMT in endotoxic shock did not further aggravate hyperlactatemia without exacerbation of tissue perfusion.

Excess production of NO in endotoxemia appears to be a double-edged sword, being protective [9, 24] and deleterious [11] on the liver. NO allows maintaining liver perfusion via hepatic arterial vasodilation and attenuation of increased portal resistance during endotoxic shock [24]. It has been reported that nonselective NOS inhibitors (N^G-monomethyl-L-arginine, N^G-nitro-L-arginine methyl ester) deteriorated LPS-induced liver damage [9, 25]. In the present study, administration of LPS caused mild liver damage based on the elevation of serum concentrations of aspartate and alanine aminotransferase, although co-administration of SMT failed to affect the LPS-induced elevation of liver enzymes. Our result is consistent with those of previous studies [25, 26] showing the failure of SMT (12.5 mg/kg [25], 0.1 mg/kg/h or 1 mg/kg/h for 4 h [26]) to prevent the LPS-induced liver damage in rats. However, others showed that SMT (5 mg/kg, i.p.) improved LPS-induced

liver damage in rats [11]. This discrepancy may be due to the different doses of SMT used and/or species difference. Because there have been no data available about the appropriate doses of SMT in dog models thus far reported, we used the dose of SMT (1 mg/kg, bolus i.v., followed by continuous infusion of 1 mg/kg/h for 1 h) based on the data obtained from our preliminary dog experiments and rodent study [11]. However, the concentration of SMT chosen might have been so high as to inhibit eNOS as well, because co-administration of SMT reversed LPS-induced hypotension at 1 h. Therefore, the potential effect of SMT on liver function in the present study may be masked by the inhibition of eNOS. Recently, it has been shown that iNOS gene knockout mice showed LPS-induced liver damage similar to that of wild type [27], suggesting the involvement of both NO-dependent and independent pathways in the development of LPS-induced liver damage.

Histologic assessment of the vital organs affected could definitely reveal the degree of tissue damage in a short-term as well as a longer-term endotoxic model. Unfortunately, we have not performed histologic examination in this study. It has been reported that liver damage as assessed by increases in serum concentrations of aspartate and alanine aminotransferase 6 h after LPS administration in rats is accompanied by the marked histologic changes in the liver; accumulation of many inflammatory cells and sporadic single cell acidophilic necrotic hepatocytes (Councilman's bodies) within the parenchyma [25]. Thus, the assessment of biochemical liver function even in short-term endotoxic model may reflect the degree of LPS-induced hepatic injury. Although it is not clear whether LPS induced histologic changes in the liver of our dog model similar to those of rat model, transient increases in liver enzymes at 6 h after LPS administration in our dog model may reflect mild hepatic injury.

Despite the limited assessment of renal function during a 6 h-observation period, the present study showed that LPS-induced decrease in creatinine clearance was prevented by co-administration of SMT without significant changes in urine volume. Our data are consistent with those of previous studies [11, 26]; treatment with SMT increased renal perfusion pressure and blood flow in anesthetized rats [11]. Since we did not measure renal blood flow, it remains unknown whether renal perfusion was increased by SMT alone. In fact, SMT administered alone tended to produce an increase in arterial pressure, although this did not reach statistical significance. Therefore, the SMT-induced increase in renal perfusion per se may have contributed to the maintenance of renal creatinine clearance even after administration of LPS. Furthermore, SMT administered alone increased FENa, suggesting its possible action on tubular sodium reabsorption. Although the increase in sodium excretion may be largely due to elevations in renal perfusion pressure and/or sodium loading, the increased sodium excretion by SMT in the present study implies that endogenous NO may participate in tubular sodium handling.

The present study also showed that co-administration of SMT prevented the LPS-induced decrease in urine osmolality and increase in free water clearance (water diuresis) without change in urine volume. These observations are similar to those of our previous study using an endothelin receptor antagonist [28]. Therefore, it is possible to speculate that blockage of excessive NO production may lead to inhibition of enhanced water reabsorption at the collecting duct during endotoxemia, although its mechanism remains unknown.

It has been shown that excess NO production contributes to the development of tubular hypoxia/reoxygenation injury in rats [29]. NO interacts with iron/ sulfur cluster-containing enzymes, such as cis-aconitase, complex I and II, and inactivates them to inhibit mitochondrial respiration and citric acid cycle [2]. Previous clinical study suggested that excess NO production could be involved in the pathogenesis of renal dysfunction in patients with severe sepsis [30]. Although it is not clear whether the reversal of renal creatinine clearance by SMT in this study is due to its inhibition of NO production in the kidney, SMT may directly prevent NO-induced renal damage. In fact, it has been reported that the maintenance of blood pressure with norepinephrine similar to that with SMT did not influence renal function in endotoxic shock rats, whereas treatment with SMT attenuated renal dysfunction [26].

No data have been available thus far as to whether iNOS is induced in vital organs, particularly in liver and/ or kidney, in dogs with endotoxic shock. However, it has been reported that induction of iNOS mRNA and protein expression was observed in liver and kidney in rats 6 h after LPS administration [31]. Assuming that there were differences in the time and the degree of iNOS induction between liver and kidney in our dog model, this might explain the apparently differential effects of SMT on these two affected vital organs. Since there are few reports about histologic assessment of the vital organs in canine endotoxic shock model, detailed histologic examination with or without NOS inhibitors in this model will be another important subject for future research for the elucidation of the pathophysiological role of iNOS in endotoxic shock, and better selection of type, dose, and timing of administration of iNOS inhibitors.

In conclusion, SMT in the dose used in this study prevented hypotension and renal dysfunction in dogs with endotoxic shock without further deterioration of cardiac output, oxygen delivery, or liver damage. These findings suggest that NO in endotoxic shock plays differential roles in multiple organ dysfunction depending on organs affected, such as kidneys and liver. Thus, therapeutic trial of iNOS-selective inhibitors in sepsis should be considered with caution until the pathophysiologic role of iNOS-derived NO is elucidated in humans.

References

1. Moncada S, Palmer RMJ, Higgs EA (1991) Nitric Oxide: Physiology, pathophysiology, and pharmacology. Pharmacol Rev 43: 109–142
2. Nathan C (1992) Nitric oxide as a secretory product of mammalian cells. FASEB J 6: 3051–3064
3. Kilbourn RG, Griffith OWP (1992) Overproduction of nitric oxide in cytokine-mediated and septic shock. J Natl Cancer Inst 84: 827–831
4. Thiemermann C, Vane JR (1990) Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharide in the rat in vivo. Eur J Pharmacol 182: 591–595
5. Szabó C, Mitchell JA, Thiemermann C, Vane JR (1993) Nitric oxide-mediated hyporeactivity to noradrenaline precedes the induction of nitric oxide synthase in endotoxin shock. Br J Pharmacol 108: 786–792
6. Cobb JP, Natanson C, Quezado ZMN, Hoffman WD, Koev CA, Banks S, Correa R, Levi R, Elin RJ, Hosseini JM, Danner RL (1995) Differential hemodynamic effects of L-NMMA in endotoxemic and normal dogs. Am J Physiol 268:H1634-H1642
7. Wright CE, Rees DD, Moncada S (1992) Protective and pathological roles of nitric oxide in endotoxin shock. Cardiovasc Res 26: 48–57
8. Mitaka C, Hirata Y, Ichikawa K, Uchida T, Yokoyama K, Nagura T, Tsunoda Y, Amaha K (1995) Effects of nitric oxide synthase inhibitor on hemodynamic change and O_2 delivery in septic dogs. Am J Physiol 268:H2017–2023
9. Harbrecht BG, Billiar TR, Stadler J, Demetris AJ, Ochoa JB, Curran RD, Simmons RL (1992) Nitric oxide synthesis serves to reduce hepatic damage during acute murine endotoxemia. Crit Care Med 20: 1568–1574
10. Mitaka C, Hirata Y, Ichikawa K, Uchida T, Yokoyama K, Amaha K (1996) Effect of aminoguanidine, a more selective inhibitor for inducible nitric oxide synthase, on cardiovascular changes in endotoxin shock dogs. Appl Cardiopulm Pathophysiol 6: 145–150
11. Szabó C, Southan GJ, Thiemermann C: (1994) Beneficial effects and improved survival in rodent models of septic shock with S-methylisothiourea sulfate, a potent and selective inhibitor of inducible nitric oxide synthase. Proc Natl Acad Sci USA 91: 12472–12476
12. Aranow JS, Zhuang J, Wang H, Larkin V, Smith M, Fink MP (1996) A selective inhibitor of inducible nitric oxide synthase prolongs survival in a rat model of bacterial peritonitis: comparison with two nonselective strategies. Shock 5: 116–121
13. Cain S, Curtis SE (1991) Experimental models of pathologic oxygen supply dependency. Crit Care Med 19: 603–612
14. Southan GJ, Szabó C, Thiemermann C (1995) Isothioureas: potent inhibitors of nitric oxide synthases with variable isoform selectivity. Br J Pharmacol 114: 510–516
15. Salvemini D, Korbut R, Anggard E (1990) Immediate release of nitric oxide-like factor from bovine aortic endothelial cells by E. coli lipopolysaccharide. Proc Natl Acad Sci USA 87: 2593–2597
16. Abel FL (1989) Myocardial function in sepsis and endotoxin shock. Am J Physiol 257:R1265-R1281
17. Hung J, Lew WY (1993) Cellular mechanisms of endotoxin-induced myocardial depression in rabbits. Circ Res 73: 125–134
18. Brady AJB, Poole-Wilson PA, Harding SE (1992) Nitric oxide production within cardiac myocytes reduces their contractility in endotoxemia. Am J Physiol 263:H1963-H1966
19. Finkel MS, Oddis CV, Jacob TD, Watkins SC, Hattler BG, Simmons RL (1992) Negative inotropic effects of cytokines on the heart mediated by nitric oxide. Science 257: 387–389
20. Decking UKM, Flesche CW, Gödecke A, Schrader J (1995) Endotoxin-induced contractile dysfunction in guinea pig hearts is not mediated by nitric oxide. Am J Physiol 268:H2460-H2465
21. Hayes MA, Timmins AC, Yau EH, Palazzo M, Watson D, Hinds CJ (1997) Oxygen transport patterns in patients with sepsis syndrome or septic shock:influence of treatment and relationship to outcome. Crit Care Med 25: 926–936
22. Kilpatrick-Smith L, Erecinska M (1983) Cellular effects of endotoxin in vitro. I. Effect of endotoxin on mitochondrial substrate metabolism and intracellular calcium. Circ Shock 11: 85–99
23. Levraut J, Ciebiera JP, Chave S, Rabary O, Jambou P, Carles M, Grimaud D (1998) Mild hyperlactatemia in stable septic patients is due to impaired lactate clearance rather than overproduction. Am J Respir Crit Care Med 157: 1021–1026
24. Ayuse T, Brienza N, Revelly JP, Boitnott JK, Robotham JL (1995) Role of nitric oxide in porcine liver circulation under normal and endotoxic conditions. J Appl Physiol 78: 1319–1329
25. Vos TA, Gouw ASH, Klock PA, Havinga R, van Goor H, Huitema S, Roelofsen H, Kuipers F, Jansen PLM (1997) Differential effects of nitric oxide synthase inhibitors on endotoxin-induced liver damage in rats. Gastroenterology 113: 1323–1333
26. Rosselet A, Feihl F, Markert M, Gnaegi A, Perret C, Liaudet L (1998) Selective iNOS inhibition is superior to norepinephrine in the treatment of rat of endotoxic shock. Am J Respir Crit Care Med 157: 162–170
27. MacMicking JD, Nathan C, Hom G, Chartrain N, Fletcher DS, Trumbauer M, Stevens K, Xie Q-W, Sokol K, Hutchinson N, Chen H, Mudgett JS (1995) Altered responses to bacterial infection and endotoxic shock in mice lacking inducible nitric oxide synthase. Cell 81: 641–650
28. Mitaka C, Hirata Y, Yokoyama K, Nagura T, Tsunoda Y, Amaha K (1999) Improvement of renal dysfunction in dogs with endotoxemia by a nonselective endothelin receptor antagonost. Crit Care Med 27: 146–153
29. Yu L, Gengaro PE, Niederberger M, Burke TJ, Schrier RW (1994) Nitric oxide: A mediator in rat tubular hypoxia/reoxygenation injury. Proc Natl Acad Sci USA 91: 1691–1695
30. Groeneveld PHP, Kwappenberg KMC, Langermans JAM, Nibbering PH, Curtis L (1996) Nitric oxide (NO) production correlates with renal insufficiency and multiple organ dysfunction syndrome in severe sepsis. Intensive Care Med 22: 1197–1202
31. Hom GJ, Grant SK, Wolfe G, Bach TJ, Macintyre DE, Hutchinson NI (1995) Lipopolysaccharide-induced hypotension and vascular hyporeactivity in the rat: tissue analysis of nitric oxide synthase mRNA and protein expression in the presence and absence of dexamethasone, N^G-monomethyl-L-arginine or indomethacin. J Pharmacol Exp Ther 272: 452–459