



Role of nitric oxide in myocardial dysfunction after combined burn and smoke inhalation injury

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Abstract

This study tested the hypothesis that nitric oxide (NO) synthesized from inducible NO synthase (iNOS) is responsible for the cardiac dysfunction observed after burn and smoke inhalation injury. Twelve sheep received 40% third-degree burn and smoke inhalation under halothane anesthesia. The animals were divided into two groups: a MEG group [iNOS was inhibited with mercaptoethylguanidine (MEG), a selective inhibitor of iNOS, $n = 6$] and a control group ($n = 6$). The control group showed a significant increase in $\text{NO}_2^-/\text{NO}_3^-$ (NO_x) concentration, metabolite of NO, in plasma after 24 h, whereas the MEG group did not. In the control group, cardiac depression was observed immediately after injury associated with hemoconcentration. Cardiac function returned to a normal level within 6 h following injury. In the control group cardiac dysfunction was observed again after 24 h although the hemoconcentration peaked at 24 h after injury and then began to resolve. In the MEG group, cardiac depression and hemoconcentration were not observed. The present data suggest that cardiac depression seen with this combination injury consists of two phases and that the later phase is mediated by iNOS–NO. © 2001 Elsevier Science Ltd and ISBI. All rights reserved.

Keywords: Inducible nitric oxide synthase; Inflammatory cytokine; Vascular hyperpermeability; Mercaptoethylguanidine; Peroxynitrite

1. Introduction

Patients with massive cutaneous burns and/or smoke inhalation injury suffer cardiopulmonary dysfunction, which can be a serious complication, especially in the early postburn period [1–6]. It is characterized by a decrease in cardiac output due to myocardial contractile depression [3,4,7]. However, there is still controversy on the mechanism of this myocardial dysfunction.

In cases of extensive cutaneous burns in which the burned area exceeds 25–30% of total body surface area (TBSA), capillary hyperpermeability occurs not only at the injured site but also in regions distant from the injury [8,9]. This systemic vascular hyperpermeability leads to

the loss of a large amount of fluid from the circulation, which results in hypovolemic shock unless rapid and adequate fluid resuscitation is administered [10,11]. Some investigators suggested that the decreased pre-load result of vascular fluid loss is responsible for the depressed cardiovascular function in burn shock [2,12].

On the other hand, other investigators have suggested the existence of specific myocardial depressant factors released from burned tissue [13–15]. Baxter et al. have demonstrated that serum from burned dogs created cardiac depression in normal dogs [14]. Recently, proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin (IL)- 1β have been shown as circulating myocardial depressant substances during inflammatory condition such as septic shock, ischemia-reperfusion injury or burn [15–17]. Giroir et al. have demonstrated that TNF is a critical mediator of postburn cardiac dysfunction [15].

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Nitric oxide (NO) is an endogenous vasodilator synthesized from L-arginine by the enzyme NO synthase. There are two types of the synthase, constitutive NO synthase (endothelial (eNOS) and neuronal (bNOS)) and inducible NO synthase (iNOS) in mammals. Under physiologic conditions, NO from eNOS is generally believed to be an important mediator to maintain vascular homeostasis [18,19]. However, under pathological condition iNOS may be formed in the vascular wall by endotoxin or inflammatory cytokines [20,21]. This results in the overproduction of NO, which can have harmful effects on vascular regulation [22]. Recent *in vitro* studies have suggested that NO is implicated in myocardial dysfunction induced by inflammatory cytokines [16,17,23–25]. Schussheim et al. have demonstrated that NO synthesized from iNOS attenuates myocyte contractile function using cultured ventricular myocyte from rat [26].

This study tests the hypothesis that induction of iNOS and subsequent increased production of NO are responsible for cardiac dysfunction in conscious sheep subjected to a combined injury of 40% TBSA third-degree burn and smoke inhalation. To test this hypothesis, iNOS was inhibited with mercaptoethylguanidine (MEG), a selective inhibitor of iNOS and also a scavenger of peroxynitrite that is an oxidative metabolite of NO [27–29].

2. Materials and methods

This study was approved by the Animal Care and Use Committee of the University of Texas Medical Branch and conducted in compliance with the guidelines of the National Institute of Health and the American Physiological Society for the care and use of laboratory animals.

2.1. Surgical preparation

Twelve female sheep were surgically prepared for chronic study under halothane anaesthesia. The right femoral artery and vein were cannulated with Silastic catheters (Intracath[®], 16GA, 24IN, Becton Dickinson Vascular Access, Sandy, UT). A thermomodulation catheter (Swan Ganz[®], model 131F7, Baxter, Edwards Critical-Care Division, Irvine, CA) was introduced through the right external jugular vein into the pulmonary artery. Through the fifth intercostal space, a catheter (Durastic Silicone Tubing[®]DT08, 0.062 in. ID, 0.125 in. OD; AlliedBiomedical, Paso Robles, CA) was positioned in the left atrium. The animals were given 5–7 days to recover from the surgical procedure with free access to food and water.

2.2. Burn and smoke inhalation injury

Before the injury, a tracheostomy was performed under ketamine anesthesia (Ketaset[®], Fort Dodge Animal Health, Fort Dodge, IA) and a cuffed tracheostomy tube (10 mm diameter, Shiley, Irvine, CA) was inserted. Then anesthesia was continued with halothane. All animals then received a combined injury with a 40% TBSA third-degree burn and 48 breaths of cotton smoke inhalation. After shaving the wool, a 20% TBSA third-degree flame burn was made on one side of the flank using a Bunsen burner. Thereafter, inhalation injury was induced with a modified bee smoker. The bee smoker was filled with 40 g of burning cotton toweling and attached to the tracheostomy tube via a modified endotracheal tube containing an indwelling thermistor from the Swan Ganz[®] catheter. Four sets of 12 breaths of smoke (total 48) were delivered and the carboxyhemoglobin level was determined immediately after smoke inhalation. The temperature of the smoke was not allowed to exceed 40 °C during the smoking procedure. After smoke insufflation, another 20% TBSA third-degree burn was made on the remainder of the flank.

2.3. Measured variables

Vascular pressures, mean arterial pressure (MAP, mmHg), pulmonary arterial pressure (PAP, mmHg), left atrial pressure (LAP, mmHg) and central venous pressure (CVP, mmHg) were measured using pressure transducers (model PX-1800, Baxter, Edwards Critical-Care Division, Irvine, CA) which were adapted with a continuous flushing device. The transducers were connected to a hemodynamic monitor (model 78304A, Hewlett Packard, Santa Clara, CA). All hemodynamic measurements were made in the standing position on animals that were awake. Zero calibrations were taken at the level of the olecranon joints on the front leg of the animals. Cardiac output was measured by the thermodilution technique using a cardiac output computer (COM-1[™], Baxter, Edward Critical-Care Division, Irvine, CA). A 5% dextrose solution was used as the indicator. For evaluation of cardiac function, cardiac index (CI, $l\ min^{-1}\ m^{-2}$), stroke volume index (SVI, $ml\ beat^{-1}\ m^{-2}$), left ventricular stroke work index (LVSWI, $g\ m\ m^{-2}$) and systemic vascular resistance index (SVRI, $dyn\ s\ cm^{-5}\ m^{-2}$) were calculated using standard equations. Blood gases and acid/base balance was measured using a blood gas analyzer (model IL 1600, Instrumentation Laboratory, Lexington, MA). The blood gas results were corrected for the body temperature of the sheep. Oxyhemoglobin saturation and carboxyhemoglobin concentration were analyzed with a co-oximeter (model IL 482, Instrumentation Laboratory, Lexington, MA). Hematocrit (Ht) was

measured in heparinized micro-hematocrit capillary tubes (Fisherbrand®, Pittsburgh, PA). Concentrations of $\text{NO}_2^-/\text{NO}_3^-$ (NO_x , $\mu\text{mol l}^{-1}$) intermediate and end products of NO oxidation, in plasma, were measured by a chemiluminescence assay using a nitric oxide analyzer (ANTEK Model 745, Antek Inst. Inc., Houston, TX).

2.4. Experimental protocol

Twenty-four hours prior to the experiment, vascular catheters were connected to the monitoring devices and maintenance fluid administration (Ringer's lactate, 2 ml kg^{-1}) via femoral vein was started. After baseline measurements and sample collections were completed, all animals received burn and smoke inhalation-combined injury, as described above. A silicone Foley catheter (Dover®, 14Fr., 5 ml, Sherwood Medical, St. Louis, MO) was placed in the urinary bladder to determine urine output. Immediately after injury, anesthesia was discontinued and the animals were allowed to awaken but were maintained on mechanical ventilation (Servo Ventilator®900C, Seimens–Elema, Sweden) throughout the 48-h experimental period. Ventilation was performed with a positive end-expiratory pressure (PEEP) of 5 cm H_2O and a tidal volume of 15 ml kg^{-1} . During the first 3 h following injury, the inspiratory O_2 concentration was maintained at 100% and the respiratory rate was kept at 30 per minute to induce rapid clearance of carboxyhemoglobin after smoke inhalation. Then ventilation was adjusted according to blood gas analysis to maintain the arterial O_2 saturation above 90% and the PCO_2 between 25 and 30 mmHg. Fluid resuscitation during the experiment was performed with Ringer's lactate solution following the Parkland formula (4 ml/% burned surface area/kg body weight for the first 24 h and 2 ml/% burned surface area/kg body weight for the next 24 h). One-half of the volume for the first day was infused in the initial 8 h, and the remainder was infused in the next 16 h. Fluid balance was determined by urine output every 3 h subtracted from total fluid volume infused. Net fluid balance accumulation was calculated and represented as $\text{ml kg}^{-1} \text{hr}^{-1}$. During this experiment, the animals were allowed free access to food, but not to water so as to accurately measure fluid intake.

The animals were randomized into two groups: a MEG group (30 mg kg^{-1} of MEG was given 1 h after injury and then every 8 h for 41 h, $n = 6$) and a control group (0.9% NaCl was given in same manner, $n = 6$). MEG was prepared by Inotek Corporation as described previously [28]. MEG was dissolved in normal saline and 15 min before administration its pH was adjusted to 4.0. The infusion rate was 150 ml h^{-1} . The lymph and blood samples for determination of total protein concentration, colloid osmotic pressure and NO_x were

collected at 3, 6, 12, 18, 24, 36 and 48 h following injury in both groups. Hemodynamic variables and blood gases were obtained at 3, 6, 12, 18, 24, 30, 36, 42 and 48 h post-injury in both groups.

2.5. Statistical methods

Summary statistics of data are expressed as mean \pm standard error of the mean. Data were analyzed using analysis of variance for a two-factor experiment with repeated measures over time. Measurements at various time periods were tested at the 0.05 level of significance. Fisher's least-significant difference procedure was used for multiple comparisons (or post hoc analysis).

3. Results

All animals survived after the combined injury with 40% burn and smoke inhalation during the 48-h experimental period. Fluid resuscitation strictly followed the Parkland formula. The arterial carboxyhemoglobin levels immediately after the smoke exposure were 62.2 ± 9.08 in the control group and 65.5 ± 7.34 in the MEG group. There was no significant difference between these values.

The NO_x concentrations in plasma (PNO_x) significantly increased from the baseline value in the control group, whereas in the MEG group NO_x was not increased significantly throughout the experiment (Fig. 1). Notably, a significant difference between the groups was observed beginning 24 h after injury.

The control group showed severe hemoconcentration evident from the significant increase in Ht immediately after injury ($123.1 \pm 6.3\%$ of the baseline value, $P < 0.05$) (Fig. 2). It peaked at 24 h following injury and then returned toward the baseline value. In the MEG group, the Ht was maintained in normal range during

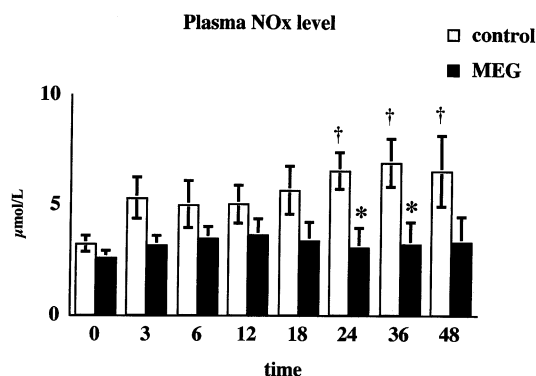


Fig. 1. Concentration of $\text{NO}_2^-/\text{NO}_3^-$ (NO_x , $\mu\text{mol l}^{-1}$) in plasma. The control group showed significant increase in NO_x beginning 24 h following injury. †: Significant difference from baseline value, $P < 0.05$. *: Significant difference from the control group, $P < 0.05$.

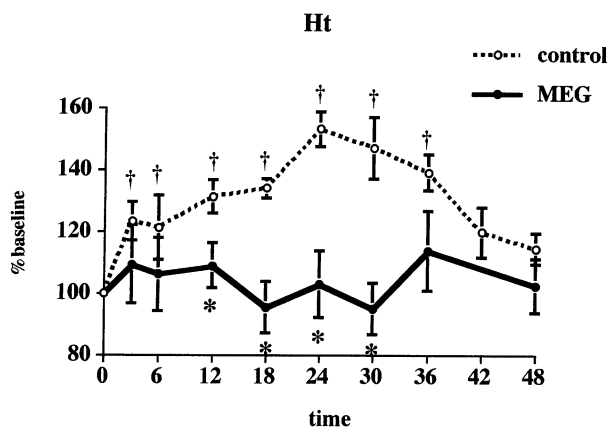


Fig. 2. Changes in hematocrit (Ht). The control group showed significant increase in Ht. It peaked at 24 h after injury, then it returned to the baseline. In the MEG group, there was no change in the Ht during experiment. †: Significant difference from baseline value, $P < 0.05$. *: Significant difference from the control group, $P < 0.05$.

the study. Net fluid accumulation was significantly lower in the MEG group than in the control group (Fig. 3).

Despite a large amount of fluid administration, the control group showed a significant decrease in CI immediately after injury ($72.7 \pm 3.7\%$ of the baseline value at 3 h after injury, $P < 0.05$) (Fig. 4) associated with hemoconcentration. In the MEG group, these changes were not observed. This fall in CI in the control group returned to a non-significant level within 6 h, but remained depressed more than 24 h and reached a significant level again during 24–30 h after injury, and then gradually returned toward baseline values. There was also a significant difference in the left ventricular stroke work index (LVSWI) between groups (Fig. 5). In the control group, the LVSWI significantly decreased

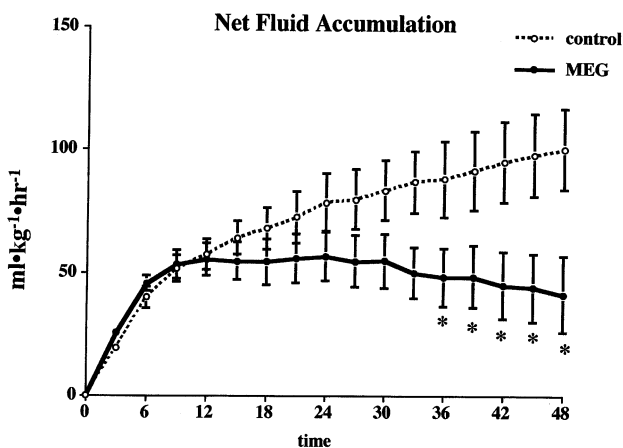


Fig. 3. Net fluid accumulation: the fluid requirement was significantly less in the MEG group than control group. †: Significant difference from baseline value, $P < 0.05$. *: Significant difference from the control group, $P < 0.05$.

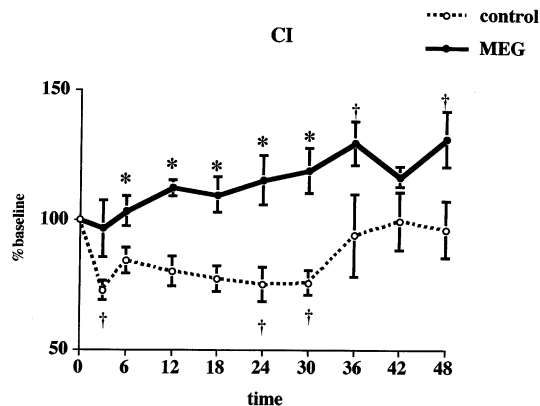


Fig. 4. Changes in cardiac index (CI): the control group showed significant decrease in CI immediately after injury and 24–30 h following injury. The MEG group did not show decrease in CI. †: Significant difference from baseline value, $P < 0.05$. *: Significant difference from the control group, $P < 0.05$.

immediately ($53.3 \pm 2.2\%$ baseline, $P < 0.05$ at 3 h). The LVSWI showed slight improvement transiently, then significantly deteriorated again after 24 h and stayed low throughout the remainder of its experimental period. In the MEG group, the LVSWI did not fall and it was significantly higher than the control group. The relationship between pre-load and stroke work index as an index of myocardial contractile function showed a right shift. In the control group it significantly shifted downward and to the right side. It indicates that this cardiac dysfunction was due to myocardial contractile depression independent of the Starling mechanism (Fig. 6). The MAP was maintained in the normal range during the experiment. The mean PAP, the CVP, and the LAP were increased after injury

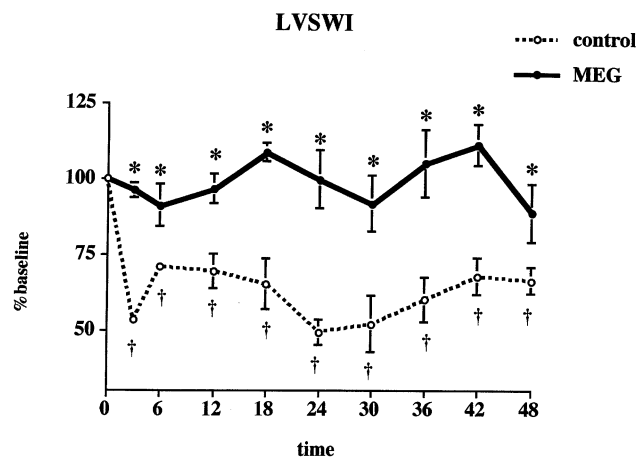


Fig. 5. Left ventricular stroke work index (LVSWI): in the control group, LVSWI significantly deteriorated in the initial phase. It showed improvement transiently and then significantly deteriorated again after 24 h and it sustained throughout the remainder of the experimental period. In the MEG group, it did not decrease. †: Significant difference from baseline value, $P < 0.05$. *: Significant difference from the control group, $P < 0.05$.

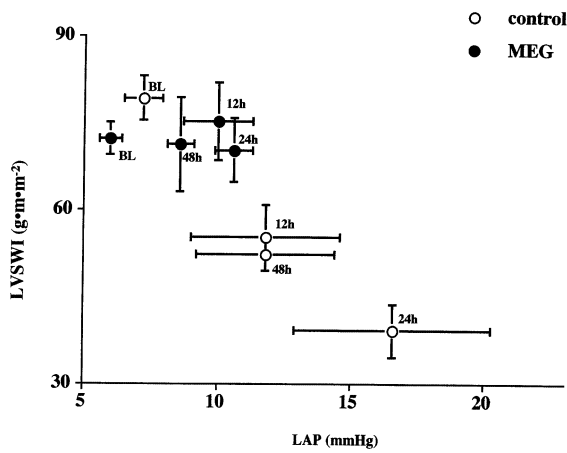


Fig. 6. The relationship between pre-load and stroke work index as an index of myocardial contractile function: in the control group it significantly shifted downward and to the right side. It indicates that this cardiac dysfunction was due to myocardial contractile depression independent of the Starling mechanism.

under mechanical ventilation with PEEP. There was no statistical difference in the MAP, PAP and CVP between groups, although the CVP tended to be higher in the control group. The LAP was significantly higher in the control group (Table 1).

4. Discussion

In the present study, the control group showed a typical hemodynamic response to combined injury with extensive cutaneous burn and smoke inhalation injury [30,31]. Despite receiving large amounts of fluid, significant low CI associated with hemoconcentration was observed. The hemoconcentration was evident from the significant increase in Ht. It peaked at 24 h following injury and then improved, which suggest that the hemoconcentration did not persist after 24 h. The cardiac dysfunction in the control group seemed to consist of two phases. In the initial phase, cardiac depression was observed within 3 h and seemed to correlate with hypovolemia. This initial dysfunction was rapidly corrected with large amounts of fluid. In contrast, in the treated group, both cardiac depression and hemoconcentration were not observed. This result suggests that administration of mercaptoethylguanidine prevented the systemic vascular permeability changes and the initial cardiac depression.

On the other hand, in the later phase, after 24 h, the cardiac dysfunction seen in the control group was the result of myocardial contractile dysfunction. This was shown by the relationship between pre-load and stroke work index. This study was designed to determine the concentration of $\text{NO}_2^-/\text{NO}_3^-$ (NO_x), metabolite of NO, in plasma. The difference in the level of NO_x between

the control group and the MEG group (iNOS inhibited group) should be proportional to the amount of NO associated with iNOS. The control group showed an increase in plasma iNOS–NO immediately after injury and it reached significance at 24 h after injury. This significant increase in NO_x ran in parallel with the cardiac dysfunction seen in the control group in the later period. In the iNOS inhibited group, both the increase in NO production and the cardiac depression were prohibited. These data may suggest that iNOS–NO contributes to some part of the myocardial contractile dysfunction seen in the later phase after this kind of injury.

Recent investigations have suggested that inflammatory cytokines such as TNF or IL-1 β can be cardiac depressant substances [15–17]. Other studies using isolated hearts or cultured myocytes have suggested that NO is implicated in the myocardial contractile dysfunction induced by these inflammatory cytokines [17,23,24,32]. Although our study was not designed to evaluate the changes in inflammatory cytokines, many studies have shown that these inflammatory cytokines can be produced from burned tissue or injured airway [33–36].

There is still controversy over whether decreased plasma volume is responsible for cardiac depression seen with extensive burn injury [2,4,12], since evaluating cardiac mechanical function is difficult using an in vivo model in which the cardiac function is affected by many factors [3,4,32]. However, in our study, the cardiac depression in the initial phase is most probably due to the transient decrease in CI as a result of the loss of fluid from the circulation. In the MEG group, hemoconcentration was not observed and cardiac function did not show deterioration. The mechanism of this early effect of MEG could not be defined by this study, as iNOS is not expressed before cell activation by inflammatory cytokines, and this takes several hours [21]. We speculate that the peroxynitrite that can be scavenged by MEG has some role in the occurrence of cardiac depression and vascular hyperpermeability in the early stage of burn injury. It has been demonstrated that peroxynitrite is generated from the NO synthesized by constitutive NOS after hemorrhagic shock, sepsis or ischemia-reperfusion injury [37,38].

In summary, the present study strongly suggests that NO produced from iNOS is responsible for the myocardial contractile dysfunction that may be induced by inflammatory cytokines seen with burn and smoke combination injury in the later phase. The iNOS inhibition restored this cardiac dysfunction. Additionally, mercaptoethylguanidine prevented the cardiac depression seen in the early phase after injury although the mechanism responsible for this effect is still unknown.

Table 1
Summary of data

Variable/group	Time (h) after injury						
	Baseline	3	6	12	24	36	48
<i>pH</i>							
Control	7.49 ± 0.01	7.51 ± 0.03	7.50 ± 0.01	7.51 ± 0.02	7.52 ± 0.03	7.45 ± 0.03	7.47 ± 0.04
MEG	7.49 ± 0.01	7.56 ± 0.02	7.56 ± 0.01	7.51 ± 0.02	7.50 ± 0.02	7.46 ± 0.01	7.44 ± 0.02
<i>MAP (mmHg)</i>							
Control	99 ± 3	106 ± 4	106 ± 4	108 ± 5	104 ± 3	96 ± 4	99 ± 3
MEG	101 ± 5	114 ± 5	114 ± 5	110 ± 5	110 ± 7	100 ± 7	98 ± 6
<i>PAP (mmHg)</i>							
Control	21 ± 2	27 ± 2†	29 ± 2†	32 ± 2†	33 ± 2†	32 ± 2†	31 ± 2†
MEG	20 ± 1	22 ± 2†	28 ± 2†	31 ± 1†	30 ± 1†	31 ± 1†	31 ± 2†
<i>CVP (mmHg)</i>							
Control	5.8 ± 1.3	6.7 ± 1.1†	8.0 ± 1.2†	9.3 ± 1.7†	12.0 ± 1.5†	12.0 ± 2.8†	11.8 ± 2.6†
MEG	3.8 ± 0.4†	4.8 ± 0.7†	7.8 ± 1.1†	8.7 ± 0.9†	9.3 ± 0.8†	8.2 ± 0.6†	8.2 ± 0.7†
<i>LAP (mmHg)</i>							
Control	7.2 ± 0.7	8.8 ± 1.0	10.6 ± 1.3	11.8 ± 2.8†	16.6 ± 3.7†	14.2 ± 1.5†	11.8 ± 2.6†
MEG	6.0 ± 0.4	6.8 ± 0.8	8.6 ± 0.8	10.0 ± 1.3†	10.6 ± 0.7†	8.8 ± 1.0†	8.6 ± 0.5†
<i>CI (l min⁻¹ m⁻²)</i>							
Control	6.1 ± 0.5	5.2 ± 0.5†	4.8 ± 0.3	5.0 ± 0.4	4.6 ± 0.7†	5.6 ± 0.8	5.8 ± 0.2
MEG	5.6 ± 0.2	5.9 ± 0.4*	5.7 ± 0.3*	6.3 ± 0.3*	6.4 ± 0.4*	7.1 ± 0.3	7.5 ± 0.5†
<i>SVI (ml beat⁻¹ m⁻²)</i>							
Control	63 ± 4	33 ± 4†	44 ± 5†	42 ± 5†	33 ± 4†	42 ± 6†	43 ± 2†
MEG	56 ± 2	49 ± 3*	48 ± 4*	54 ± 3*	51 ± 4*	58 ± 3*	58 ± 6*
<i>SVRI (dyn s cm⁻⁵ m⁻²)</i>							
Control	1212 ± 128	1970 ± 358†	1381 ± 151	1539 ± 162	1337 ± 136	1201 ± 127	1141 ± 130
MEG	1412 ± 108	1772 ± 254†	1492 ± 82	1311 ± 130	1306 ± 147	978 ± 107†	974 ± 116†

All animals received a 40% third-degree burn and insufflated with smoke. The MEG group was treated with MEG and the control group was with 0.9% NaCl. †: Significant difference from baseline value, $P < 0.05$. *: Significant difference from the control group, $P < 0.05$. MAP, mean arterial pressure; PAP, pulmonary arterial pressure; CVP, central venous pressure; LAP, left atrial pressure; CI, cardiac index; SVI, stroke volume index; SVRI, systemic vascular resistance index.

References

- [1] Reynolds EM, Ryan DP, Sheridan RL, Doody DP. Left ventricular failure complicating severe pediatric burn injuries. *J Pediatr Surg* 1995;30:264–9.
- [2] Elgjo GI, Mathew BP, Poli de Figueriedo LF, Schenarts PJ, Dubick MA, Kramer GC. Resuscitation with hypertonic saline dextran improves cardiac function in vivo and ex vivo after burn injury in sheep. *Shock* 1998;9:375–83.
- [3] Adams HR, Baxter CR, Izenberg SD. Decreased contractility and compliance of the left ventricle as complications of thermal trauma. *Am Heart J* 1984;108:1477–87.
- [4] Wolfe RR, Miller HI. Cardiovascular and metabolic responses during burn shock in the guinea pig. *Am J Physiol* 1976;231:892–7.
- [5] Sugi K, Theissen JL, Traber LD, Herndon DN, Traber DL. Impact of carbon monoxide on cardiopulmonary dysfunction after smoke inhalation injury. *Circ Res* 1990;66:69–75.
- [6] Horton JW, Kaufman TM, White DJ, Mahony L. Cardiac contractile and calcium transport function after burn injury in adult and aged guinea pigs. *J Surg Res* 1993;55:87–96.
- [7] Hilton JG, Marullo DS. Effects of thermal trauma on cardiac force of contraction. *Burns Incl Therm Inj* 1986;12:167–71.
- [8] Bowen BD, Bert JL, Gu X, Lund T, Reed RK. Microvascular exchange during burn injury: III. Implications of the model. *Circ Shock* 1989;28:221–33.
- [9] Lund T, Bert JL, Onarheim H, Bowen BD, Reed RK. Microvascular exchange during burn injury. I: a review. *Circ Shock* 1989;28:179–97.
- [10] Lund T, Wiig H, Reed RK. Acute postburn edema: role of strongly negative interstitial fluid pressure. *Am J Physiol* 1988;255:H1069–74.
- [11] Herndon DN, Hilton JG, Traber DL, Barrow RE. Burn shock and its resuscitation. *Prog Clin Biol Res* 1987;236:539–57.
- [12] Shoemaker WC, Vladeck BC, Bassin R, Printen K, Brown RS, Amato JJ, Reinhard JM, Kark AE. Burn pathophysiology in man. I. Sequential hemodynamic alterations. *J Surg Res* 1973;14:64–73.
- [13] Bevilacqua MP, Pober JS, Wheeler ME, Cotran RS, Gimbrone MA Jr. Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. *J Clin Invest* 1985;76:2003–11.
- [14] Baxter CR, Cook WA, Shires GT. Serum myocardial depressant factor of burn shock. *Surg Forum* 1966;17:1–2.
- [15] Giroir BP, Horton JW, White DJ, McIntyre KL, Lin CQ. Inhibition of tumor necrosis factor prevents myocardial dysfunction during burn shock. *Am J Physiol* 1994;267:H118–24.
- [16] Cain BS, Meldrum DR, Dinarello CA, Meng X, Joo KS, Harken AH. Tumor necrosis factor-alpha and interleukin-1beta synergistically depress human myocardial function. *Crit Care Med* 1999;27:1309–18.

- [17] Quezado ZM, Karzai W, Danner RL, Freeman BD, Yan L, Banks SM, Cobb JP, Cunnion RE, Quezado MJS, Natanson C. Effects of L-NMMA and fluid loading on TNF-induced cardiovascular dysfunction in dogs. *Am J Respir Crit Care Med* 1998;157:1397–405.
- [18] Rees DD, Palmer RM, Moncada S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci USA* 1989;86:3375–8.
- [19] Moncada S, Higgs A. The L-arginine–nitric oxide pathway. *N Engl J Med* 1993;329:2002–12.
- [20] Minc-Golomb D, Tsarfaty I, Schwartz JP. Expression of inducible nitric oxide synthase by neurones following exposure to endotoxin and cytokine. *Br J Pharmacol* 1994;112:720–2.
- [21] Szabo C, Salzman AL, Ischiropoulos H. Endotoxin triggers the expression of an inducible isoform of nitric oxide synthase and the formation of peroxynitrite in the rat aorta in vivo. *FEBS Lett* 1995;363:235–8.
- [22] Szabo C. Alterations in nitric oxide production in various forms of circulatory shock. *New Horizons* 1995;3:2–32.
- [23] Kumar A, Brar R, Wang P, Dee L, Skorupa G, Khadour F, Parrillo JE. Role of nitric oxide and cGMP in human septic serum-induced depression of cardiac myocyte contractility. *Am J Physiol-Regul Integrat Comp Physiol* 1999;45:R265–76.
- [24] Kwon S, George SC. Synergistic cytokine-induced nitric oxide production in human alveolar epithelial cells. *Nitric Oxide* 1999;3(4):348–57.
- [25] Werdan K, Muller-Werdan U, Reithmann C, Boekstegers P, Fuchs R, Kainz I, Stadler J. Nitric oxide dependent and independent effects of tumor necrosis factor- on cardiomyocyte beating activity and signal transduction pathways. In: Schlag G, Redl H, editors. *Shock, sepsis, and organ failure — nitric oxide*. Berlin: Springer, 1995:286.
- [26] Joe EK, Schussheim AE, Longrois D, Maki T, Kelly RA, Balligand JL. Regulation of cardiac myocyte contractile function by inducible nitric oxide synthase (iNOS): mechanisms of contractile depression by nitric oxide. *J Mol Cell Cardiol* 1998;30:303–15.
- [27] Szabo C, Ferrer-Sueta G, Zingarelli B, Southan GJ, Salzman AL, Radi R. Mercaptoethylguanidine and guanidine inhibitors of nitric-oxide synthase react with peroxynitrite and protect against peroxynitrite-induced oxidative damage. *J Biol Chem* 1997;272:9030–6.
- [28] Southan GJ, Zingarelli B, O'Connor M, Salzman AL, Szabo C. Spontaneous rearrangement of aminoalkylisothioureas into mercaptoalkylguanidines, a novel class of nitric oxide synthase inhibitors with selectivity towards the inducible isoform. *Br J Pharmacol* 1996;117:619–32.
- [29] Szabo A, Hake P, Salzman AL, Szabo C. Beneficial effects of mercaptoethylguanidine, an inhibitor of the inducible isoform of nitric oxide synthase and a scavenger of peroxynitrite, in a porcine model of delayed hemorrhagic shock. *Crit Care Med* 1999;27(7):1343–50.
- [30] Sakurai H, Schmalstieg F, Traber L, Hawkins H, Traber D. Role of L-selectin in physiological manifestations after burn and smoke inhalation injury in sheep. *J Appl Physiol* 1999;86:1151–9.
- [31] Sakurai H, Traber LD, Traber DL. Altered systemic organ blood flow after combined injury with burn and smoke inhalation. *Shock* 1998;9:369–74.
- [32] Panas D, Khadour FH, Szabo C, Schulz R. Proinflammatory cytokines depress cardiac efficiency by a nitric oxide-dependent mechanism. *Am J Physiol* 1998;275:H1016–23.
- [33] Drost AC, Burlison DG, Cioffi WG, Jordan BS, Mason AD Jr., Pruitt BA Jr. Plasma cytokines following thermal injury and their relationship with patient mortality, burn size, and time postburn. *J Trauma* 1993;35:335–9.
- [34] Yamada Y, Endo S, Inada K. Plasma cytokine levels in patients with severe burn injury — with reference to the relationship between infection and prognosis. *Burns* 1996;22:587–93.
- [35] Mester M, Carter EA, Tompkins RG, Gelfand JA, Dinarello CA, Burke JFC. Thermal injury induces very early production of interleukin-1 alpha in the rat by mechanisms other than endotoxemia. *Surgery* 1994;115:588–96.
- [36] Endo S, Inada K, Yamada Y, Kasai T, Takakuwa T, Nakae H, Kamei Y, Suzuki T, Taniguchi S, Yoshida M. Plasma levels of interleukin-1 receptor antagonist (IL-1ra) and severity of illness in patients with burns. *J Med* 1996;27:57–71.
- [37] Szabo C, Salzman AL, Ischiropoulos H. Peroxynitrite-mediated oxidation of dihydrorhodamine 123 occurs in early stages of endotoxic and hemorrhagic shock and ischemia-reperfusion injury. *FEBS Lett* 1995;372:229–32.
- [38] Yasmin W, Strynadka KD, Schulz R. Generation of peroxynitrite contributes to ischemia-reperfusion injury in isolated rat hearts. *Cardiovasc Res* 1997;33:422–32.