Protective effects of N-acetyl-L-cysteine in endotoxemia

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Zhang, Haibo, Herbert Spapen, Duc Nam Nguyen, Malik Benlabeled, Wim A. Buurman, and Jean-Louis Vincent. Protective effects of N-acetyl-L-cysteine in endotoxemia. *Am. J. Physiol.* 266 (Heart Circ. Physiol. 35): H1746–H1754, 1994.—Because oxygen free radicals have been implicated in the endothelial cell damage and in the myocardial depression occurring during severe sepsis, we investigated whether N-acetyl-L-cysteine (NAC) could influence the oxygen extraction capabilities during an acute reduction in blood flow induced by cardiac tamponade after endotoxin challenge. Sixteen anesthetized, saline-infused, and ventilated dogs received *Escherichia coli* endotoxin (2 mg/kg) 30 min before tamponade was induced by repeated bolus injections of warm saline into the pericardial space. Thirty minutes before endotoxin administration, nine dogs received NAC (150 mg/kg, followed by a 20 mg·kg⁻¹·h⁻¹ infusion); the other seven dogs served as a control group. The NAC group maintained higher cardiac index, oxygen delivery ($\dot{V}O_2$), and left ventricular stroke work index, but lower systemic and pulmonary vascular resistance, than the control group. The oxygen uptake ($\dot{V}O_2$) levels at critical $\dot{V}O_2$ ($\dot{V}O_{2crit}$) were identical in the two groups. However, $\dot{V}O_{2crit}$ was significantly lower in the NAC than in the control group (8.1 ± 1.7 vs. 10.8 ± 1.8 ml·kg⁻¹·min⁻¹, $P < 0.01$). Critical oxygen extraction ratio and the slope of the $\dot{V}O_2$ -to- $\dot{V}O_{2crit}$ -dependent line were higher in the NAC than in the control group (72 ± 14 vs. $53 \pm 15\%$ and 0.80 vs. 0.56 , respectively; both $P < 0.05$). The peak lactate and the maximal tumor necrosis factor (TNF) levels were lower in the NAC than in the control group (5.2 ± 0.4 vs. 7.6 ± 0.4 mM, and 0.14 ± 0.03 vs. 1.21 ± 0.58 ng/ml, respectively; both $P < 0.01$). NAC significantly increased glutathione peroxidase activity. In this model of endotoxic shock, prior administration of NAC improved oxygen extraction capabilities as well as myocardial function. Enhancement of glutathione peroxidase activity and inhibition of TNF release by NAC can be implicated.

oxygen delivery; oxygen extraction; oxygen free radicals; glutathione peroxidase; superoxide dismutase; vitamin E; tumor necrosis factor; tamponade; hypoxia

IN SEPTIC CONDITIONS, tissue oxygen consumption ($\dot{V}O_2$) may become dependent on oxygen delivery ($\dot{V}O_2$), even when $\dot{V}O_2$ is preserved or above normal. The usual occurrence of lactic acidosis in these conditions suggests that the cellular oxygen requirements are not met (24, 32). An increased oxygen demand, a defective oxygen extraction capability, and a depressed myocardial contractility may contribute to the pathological $\dot{V}O_2$ - $\dot{V}O_{2crit}$ dependency in these conditions (24, 32). The pathogenesis of impaired tissue oxygen extraction capabilities in septic states could be related to various factors such as the microvascular obstruction by activated cells, the release of vasoactive and toxic substances, and the subsequent endothelial cell damage. Among other sub-

stances, oxygen free radicals can have strong damaging effects on the endothelium. They have also been implicated in the pathogenesis of the sepsis-induced myocardial depression (27).

N-acetyl-L-cysteine (NAC) can exert important antioxidant and cytoprotective effects. Its pharmacokinetics are well documented, and its toxicity is low (3, 4, 13, 23). NAC can replenish intracellular glutathione, one of the pivots of cellular defense against oxidative stress (3, 6, 19). NAC has been reported to block the acute toxicity of tumor necrosis factor (TNF) (25). According to recent studies, NAC may also stimulate endothelium-derived relaxing factor (EDRF), which exerts potent vasodilating and platelet-inhibiting effects (29).

These effects of NAC may be protective during endotoxemia. In endotoxic animals, NAC decreased neutrophil-aggregating activity, markedly reduced pulmonary hypertension, and attenuated vascular permeability (3). In a porcine model of endotoxin-induced pulmonary edema, NAC significantly increased cardiac output and $\dot{V}O_2$, lowered pulmonary hypertension and intrapulmonary shunt, and decreased platelet aggregation (23). Although NAC has been shown to increase $\dot{V}O_2$, $\dot{V}O_{2crit}$, and oxygen extraction in patients with fulminant hepatic failure (13), its effects on oxygen extraction capabilities in septic shock have not been well defined. We hypothesized that by its antioxidant, cytoprotective, and microcirculatory effects, NAC could increase oxygen extraction capabilities after endotoxemia. The present study was therefore performed to explore the effects of NAC on hemodynamics and oxygen utilization in an endotoxic shock model in which blood flow was progressively reduced by cardiac tamponade.

MATERIALS AND METHODS

Experimental preparation. Sixteen mongrel dogs of either sex ranging in weight from 20 to 28 kg were anesthetized with pentobarbital sodium, administered as a slow intravenous bolus of 30 mg/kg, followed by a constant infusion of 4 mg·kg⁻¹·h⁻¹ with an infusion pump (Infusomat II, B. Braun, Melsungen, Germany). After endotracheal intubation, each dog was ventilated with room air (Servo ventilator 900B, Siemens-Elema, Solna, Sweden). Controlled ventilation was facilitated with pancuronium bromide, 0.15 mg/kg initially, with supplementation at 0.075 mg·kg⁻¹·h⁻¹. Respiratory rate was 12 breaths/min, and tidal volume was adapted to keep end-tidal carbon dioxide tension (47210A Capnometer, Hewlett-Packard, Waltham, MA) between 28 and 38 mmHg. The left femoral artery was catheterized for monitoring of arterial blood pressure and withdrawal of arterial blood samples. The left forepaw vein was used for intravenous administration of pentobarbital. The right forepaw vein was catheterized for intravenous fluid infusion. Through the right external jugular vein, a balloon-tipped pulmonary arterial catheter (model 93A-

131-7-F, Baxter Healthcare, Irvine, CA) was placed under guidance of pressure waves (monitor Sirecust 302A, Siemens, Erlangen, Germany). A left thoracotomy between the fourth and the fifth intercostal space was performed to introduce a 16-gauge polyethylene catheter (Intracath, Deseret Medical, Sandy, UT) with multiple side holes in the pericardial space via a 2- to 3-mm incision in the anterior pericardium. The catheter was secured with silk purse-string sutures and sealed with medical glue. The pericardial cavity was drained with replacement of 30 ml of warm sterile saline before sealing, the thoracic cavity was then carefully closed in three layers, and a chest tube (Trocath catheter A75, 28Ch-40 cm, Argyle Tullamore, Ireland) was placed through the seventh intercostal space to allow gentle evacuation of the chest. The pericardial catheter was used to inject saline into the pericardial space and to measure intrapericardial pressure.

Experimental protocol. After surgical preparation, the dog was turned to the supine position and allowed to stabilize for 30 min. The pericardial cavity was emptied using a 5-ml syringe to ensure a slightly negative intrapericardial pressure before control measurements (*baseline 1*). The animals were randomized into two groups, receiving a generous saline infusion at $20 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ either alone (control group, $n = 7$) or in combination with NAC (NAC group, $n = 9$). In the latter group, NAC (Lysomucil, Inpharlam Zambon Group, Milan, Italy) was administered separately at a loading dose of 150 mg/kg iv (100 mg/ml solution) followed by a continuous infusion of $20 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ iv (40 mg/ml) until the end of the experiment. Thirty minutes later, a second set of measurements (*baseline 2*) was obtained. Thereafter, 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 min. A third set of measurements (*baseline 3*) was performed 30 min later. Tamponade was then induced by repeated bolus injection of normal saline heated to 37°C into the pericardium. The amount of saline injected was 30 ml for the first two injections, then 20 ml for the next two injections, then 10 ml until $\dot{V}\text{O}_2$ started to fall from baseline, and finally 5 ml until mean arterial pressure fell by 80% from baseline. The experiment was then ended. After each injection, a time interval of 15 min was permitted to reach a steady state, characterized by a stable expired oxygen fraction ($\text{FO}_{2\text{exp}}$), end-tidal carbon dioxide tension, arterial pressure, and heart rate, before the next measurements were obtained. Throughout the study, the core temperature of the animal was kept constant by a heating blanket and operating lamps.

Measurements of pressures and cardiac output. Pressures from femoral arterial, pulmonary arterial, and intrapericardial lines were monitored continuously by using pressure transducers (series 966020-01, Baxter Healthcare) with amplifiers (Hellige Servomed, Freiburg, Germany) and a pen recorder (model 2600S Gould Instruments, Cleveland, OH). All pressures were determined at end-expiration. Cardiac output was measured by the thermodilution technique (COM-2, Baxter), using three to five 5-ml bolus injections of cold 5% dextrose in ice water at end-inspiration.

Measurements of expired gases, blood gases, and hematocrit. Exhaled gases were directed through a mixing chamber for sampling of $\text{FO}_{2\text{exp}}$ (P. K. Morgan, Chatham, Kent, UK). Expired minute volume (V_{exp}) was measured with a spirometer (Haloscale Wright Respirometer, Edrington, UK) over a 2-min period. Arterial and mixed venous blood samples were simultaneously withdrawn for immediate determination of blood gases (analyzer Stat Profile 7, NOVA Biomedical, Waltham, MA). Arterial and mixed venous oxygen saturation and total hemoglobin were measured (OSM 3 Hemoximeter, calibrated for dog blood, Radiometer, Copenhagen, Denmark). Hemato-

crit was determined by capillary method (Hettich Haematokrit, Tuttlingen, Germany). VO_2 was determined from the following equation

$$\dot{V}\text{O}_2 (\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = V_{\text{exp}} (\text{ml/kg})$$

$$\times \{[(1 - \text{FO}_{2\text{exp}} - \text{FCO}_{2\text{exp}})/(1 - \text{FO}_{2\text{insp}})] \times \text{FO}_{2\text{insp}} - \text{FO}_{2\text{exp}}\}$$

where $\text{FCO}_{2\text{exp}}$ and $\text{FO}_{2\text{insp}}$ represent expired carbon dioxide fraction and inspired oxygen fraction, respectively. $\dot{V}\text{O}_2$ was derived from the product of cardiac index and arterial oxygen content. The oxygen extraction ratio (O_2ER) was calculated as the ratio of $\dot{V}\text{O}_2$ to $\dot{Q}\text{O}_2$. There was a strong correlation between the O_2ER values derived from $\dot{V}\text{O}_2/\dot{Q}\text{O}_2$ and from the ratio of arteriovenous oxygen difference to arterial oxygen content ($r = 0.94$ in the control and 0.91 in the NAC group, respectively; both $P < 0.01$).

Measurements of lactate, plasma proteins, and TNF. Blood lactate concentration was determined by a glucose-lactate analyzer (2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH). The normal value is $< 2 \text{ mM}$. Plasma proteins were determined by spectrophotometry (Lambda 15 UV/VIS spectrophotometer, Perkin-Elmer, Ueberlingen, Germany) following the Biuret method. TNF levels were measured using ligand immunoassays (W. Buurman); the limit of detection of the enzyme-linked immunosorbent assay (61E71) was 10 pg/ml .

Measurements of glutathione peroxidase activity, superoxide dismutase activity, and plasma vitamin E. Glutathione peroxidase and superoxide dismutase activities were measured spectrophotometrically from arterial blood samples (Lambda 15 UV/VIS spectrophotometer, Perkin-Elmer). Glutathione peroxidase activity of the samples was measured by recording the fall in absorbance at 340 nm of NADPH in the presence of Cumol peroxide as substrate and reduced glutathione as cofactor. Superoxide dismutase activity was measured by inhibition of the superoxide anion-mediated reduction of cytochrome *c* and was reported in units per gram of proteins. Vitamin E was measured by high-performance liquid chromatography with α -tocopherol acetate as internal standard (Jones Chromatography, Hengoed, Wales, UK).

Statistics. A dual-line regression method was used to determine the critical $\dot{V}\text{O}_2$ ($\dot{V}\text{O}_{2\text{crit}}$) from a plot of $\dot{V}\text{O}_2$ vs. $\dot{Q}\text{O}_2$ in each individual animal. For each plot, linear regression by best fit was used to calculate straight lines for the supply dependency and independency (24). The point of intersection of their regression lines defined the $\dot{V}\text{O}_{2\text{crit}}$ and the corresponding critical $\dot{V}\text{O}_2$. The critical O_2ER ($\text{O}_{2\text{ERcrit}}$) was derived by $\dot{V}\text{O}_{2\text{crit}}/\dot{Q}\text{O}_{2\text{crit}}$. An analysis of variance followed by Dunnett's test was used for statistical analysis. The slopes of $\dot{V}\text{O}_2/\dot{Q}\text{O}_2$ were tested by covariance analysis. A P value < 0.05 was considered statistically significant. All values are expressed as means $\pm$ SD unless otherwise indicated.

RESULTS

The results are shown in Tables 1 and 2 and in Figures 1-8.

Effects of NAC alone. NAC administration was followed by no significant change in any monitored parameters, except for a reduction in hematocrit from 42.9 ± 4.8 to $40.7 \pm 4.2\%$ ($P < 0.05$), which was not observed in the control group (from 43.3 ± 7.9 to $42.6 \pm 7.1\%$, $P = \text{NS}$).

Effects of NAC after endotoxin. In the control group, endotoxin administration was followed by a fall in mean arterial pressure from 104 ± 15 to $79 \pm 24 \text{ mmHg}$ ($P <$

Table 1. Selected hemodynamic parameters

	n	Baseline 1	Baseline 2	Baseline 3	IPP, mmHg						
					0	2	4	6	8	10	12
Heart rate, beats/min											
Control	7	165 ± 16	161 ± 15	166 ± 39	166 ± 28	165 ± 36	190 ± 42	194 ± 39	150 ± 72	167 ± 79	141 ± 34
NAC	9	176 ± 18	167 ± 15	185 ± 20	188 ± 42	184 ± 17	192 ± 19	186 ± 23	163 ± 70	175 ± 29	140 ± 44
Stroke volume, ml											
Control	7	27.2 ± 12.0	27.2 ± 7.6	25.9 ± 15.9	24.0 ± 11.3	15.8 ± 9.4	10.0 ± 7.4	7.6 ± 5.8	5.3 ± 5.1	2.0 ± 1.0	1.3 ± 0.9
NAC	9	26.0 ± 6.8	28.7 ± 6.6	26.0 ± 7.2	27.2 ± 7.6	21.5 ± 6.5*	16.2 ± 6.7*	12.4 ± 5.2*	10.1 ± 6.6*	5.5 ± 4.4	3.3 ± 1.3
LVSWI, g·m·kg ⁻¹											
Control	7	1.51 ± 0.75	1.50 ± 0.56	1.18 ± 0.90	1.10 ± 0.71	0.66 ± 0.47	0.43 ± 0.32	0.27 ± 0.25	0.22 ± 0.10	0.05 ± 0.06	0.003 ± 0.002*
NAC	9	1.51 ± 0.45	1.65 ± 0.42	1.41 ± 0.50*	1.40 ± 0.50*	1.00 ± 0.40*	0.70 ± 0.31*	0.48 ± 0.30	0.42 ± 0.37	0.25 ± 0.16*	0.04 ± 0.03*
RVSWI, g·m·kg ⁻¹											
Control	7	0.14 ± 0.08	0.16 ± 0.07	0.15 ± 0.10	0.13 ± 0.08	0.09 ± 0.05	0.05 ± 0.03	0.02 ± 0.02	0.02 ± 0.01	0.002 ± 0.003	0.0002 ± 0.0001
NAC	9	0.13 ± 0.06	0.17 ± 0.09	0.15 ± 0.08	0.14 ± 0.07	0.10 ± 0.04	0.07 ± 0.02	0.04 ± 0.03	0.02 ± 0.02	0.003 ± 0.003	0.003 ± 0.003*
PAOP, mmHg											
Control	7	2.9 ± 0.6	3.1 ± 0.9	3.0 ± 0.9	3.0 ± 1.0	5.0 ± 1.0	6.2 ± 1.1	8.6 ± 0.5	10.2 ± 0.4	11.8 ± 0.5	13.0 ± 1.1
NAC	9	2.9 ± 0.7	3.7 ± 1.2	3.1 ± 1.0	3.4 ± 1.0	5.3 ± 1.0	6.5 ± 1.2	8.9 ± 1.6	10.9 ± 0.6	12.3 ± 0.5	14.0 ± 1.4
RAP, mmHg											
Control	7	2.3 ± 0.2	2.4 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	4.0 ± 1.0	6.2 ± 0.4	8.0 ± 1.0	10.2 ± 0.4	12.0 ± 0.8	13.0 ± 0.8
NAC	9	2.4 ± 0.3	2.5 ± 0.3	2.4 ± 0.2	2.7 ± 0.4	4.8 ± 0.8	6.4 ± 0.7	8.9 ± 0.6	11.0 ± 0.5	12.8 ± 0.5	13.5 ± 0.7

Values are means ± SD; n, no. of dogs. IPP, intrapericardial pressure; LVSWI, left ventricular stroke work index; RVSWI, right ventricular stroke work index; PAOP, pulmonary artery occlusion pressure; RAP, right atrial pressure. **P* < 0.05 vs. control.

0.01) and a slight decrease in cardiac index and left ventricular stroke work index (NS). Systemic vascular resistance decreased from $2,059 \pm 185$ to $1,666 \pm 206$ dyn·s·cm⁻⁵ (*P* < 0.05), but pulmonary vascular resistance increased from 181 ± 22 to 299 ± 46 dyn·s·cm⁻⁵ (*P* < 0.05). $\dot{V}O_2$ decreased slightly, but $\dot{V}O_2$ was preserved by an increase in O_2ER .

In the NAC group, the fall in arterial pressure was significantly less severe than in the control group (from 110 ± 22 to 93 ± 21 mmHg, *P* < 0.01). There was no decrease in cardiac index or left ventricular stroke work index. Systemic vascular resistance decreased from $2,014 \pm 193$ to $1,549 \pm 151$ dyn·s·cm⁻⁵ (*P* < 0.05). The increase in pulmonary vascular resistance was totally prevented. $\dot{V}O_2$ remained stable, but $\dot{V}O_2$ and O_2ER increased.

In the control, like in the NAC group, endotoxin challenge was followed by significant increases in hematocrit and blood lactate levels. TNF increased from 0.21 ± 0.13 to 0.79 ± 0.22 ng/ml (*P* < 0.01) in the control group, but from 0.10 ± 0.02 to only 0.14 ± 0.03 ng/ml (*P* < 0.05) in the NAC group.

Effects of NAC during tamponade. In the control group, the stepwise increase in intrapericardial pressure

resulted in progressive reductions in arterial pressure, cardiac index, stroke volume, and left ventricular stroke work index. Systemic and pulmonary vascular resistances initially remained relatively stable but increased later during the experiment.

In the NAC group, mean arterial pressure remained at similar levels, but cardiac index, stroke volume, and left ventricular stroke work index were maintained at higher levels than in the control group. The late increase in systemic and pulmonary vascular resistance was largely attenuated. For all data, the pressure-flow relationships indicated that, for any pressure in the systemic or the pulmonary circulation, the cardiac index was higher in the NAC than in the control dogs.

In the control group, despite a progressive reduction in $\dot{V}O_2$, $\dot{V}O_2$ was initially maintained by an increase in O_2ER . $\dot{V}O_2$ declined dramatically at higher intrapericardial pressure levels when O_2ER appeared to reach a plateau. The $\dot{V}O_{2crit}$ was found at 10.8 ± 1.8 ml·kg⁻¹·min⁻¹.

In the NAC group, $\dot{V}O_2$ remained higher, and $\dot{V}O_2$ was longer maintained. When intrapericardial pressure reached 11 mmHg, $\dot{V}O_2$ had fallen by only 55% from baseline in the treated animals but by 90% in the control group (*P* < 0.01). $\dot{V}O_{2crit}$ was found at 8.1 ± 1.7 ml·kg⁻¹·min⁻¹ (*P* < 0.01 vs. control). O_2ER increased later than in the control group and did not appear to reach a plateau. O_2ER_{crit} was significantly higher in the NAC than in the control group (72 ± 14 vs. $53 \pm 15\%$, *P* < 0.05). The slope of $\dot{V}O_2$ - $\dot{V}O_2$ dependency was also steeper in the NAC than in the control group (0.80 ± 0.22 vs. 0.56 ± 0.20 , *P* < 0.05). The maximal O_2ER was 83% in the NAC vs. 70% in the control group (*P* < 0.05).

In the control group, hematocrit remained stable, and lactate progressively increased. In the NAC group, hematocrit initially tended to be lower and significantly

Table 2. Values at critical $\dot{V}O_2$ and slopes of $\dot{V}O_2$ - $\dot{V}O_2$ relationship

	$\dot{V}O_{2crit}$, ml·kg ⁻¹ ·min ⁻¹	$\dot{V}O_2$, ml·kg ⁻¹ ·min ⁻¹	O_2ER , %	$\dot{V}O_2$ - $\dot{V}O_2$ Dependency Slope
Control	10.8 ± 1.8	6.0 ± 1.3	52.9 ± 15.0	0.56 ± 0.20
NAC	8.1 ± 1.7	5.8 ± 0.5	72.4 ± 13.9	0.80 ± 0.22
<i>P</i> value	0.008	0.555	0.038	0.038

Values are means ± SD. $\dot{V}O_2$, oxygen delivery; $\dot{V}O_2$, oxygen uptake; $\dot{V}O_{2crit}$, critical $\dot{V}O_2$; O_2ER , oxygen extraction ratio; NAC, N-acetyl-L-cysteine.

Fig. 1. Relationships among mean arterial pressure (MAP), mean pulmonary arterial pressure (MPAP), and cardiac index (CI) and intrapericardial pressure (IPP) in control (circles) and *N*-acetyl-L-cysteine (NAC)-treated (triangles) groups. B1-B3, baselines 1-3. Data are means $\pm$ SE. *Statistical significance ($P < 0.05$) between NAC-treated and control groups.

decreased toward the end of the study. The increase in blood lactate was less significant in the NAC group than in the control group, especially toward the end of the experiment. The eventual lactate level was 5.2 ± 0.4 mM in the NAC but 7.6 ± 0.4 mM in the control group ($P < 0.01$). The TNF levels remained significantly lower in the NAC than in the control group after endotoxin challenge ($P < 0.01$).

After endotoxin, the glutathione peroxidase activity decreased in the control group but was well preserved in the NAC group ($P < 0.01$). The superoxide dismutase activity tended to be higher in the NAC group, but the differences failed to reach statistical significance. Vita-

min E concentration decreased similarly in the two groups.

DISCUSSION

A main finding of our study is that the alterations in oxygen extraction capabilities induced by endotoxin were markedly attenuated by prior administration of NAC. Normal anesthetized dogs generally extract between 60 and 75% of the delivered oxygen before $\dot{V}O_2$ becomes supply dependent at a $\dot{D}O_{2crit}$ of 7-9 ml·kg⁻¹·min⁻¹ (24, 32). In septic states, this supply-dependency phenomenon can occur at a higher $\dot{D}O_2$ value (24, 32). In a recent study using the present tamponade model (32), we observed that $\dot{D}O_{2crit}$ was 9 ml·kg⁻¹·min⁻¹ in control animals but 12 ml·kg⁻¹·min⁻¹ in endotoxic animals; O_2ER_{crit} was 60% in control but 47% in endotoxic animals. In the present study, endotoxic animals had a $\dot{D}O_{2crit}$ of 10.8 ml·kg⁻¹·min⁻¹ and an O_2ER_{crit} of 53%. NAC reduced $\dot{D}O_{2crit}$ from 10.8 to 8.1 ml·kg⁻¹·min⁻¹ and increased O_2ER_{crit} from 53 to 72%. The slope of the dependent portion of the $\dot{V}O_2$ - $\dot{D}O_2$ relationship was also greater in the NAC-treated dogs than in the other animals. Of note is the fact that we maintained a sufficient blood volume by a generous saline infusion throughout these endotoxic experiments (32). Indeed, differences in fluid status between animals have been shown to influence markedly the extraction capabilities in endotoxic shock (7).

In the 30 min preceding the endotoxin challenge, NAC had minimal hemodynamic effects because it has a

Fig. 2. Relationship between systemic (SVR) and pulmonary (PVR) vascular resistance and IPP in the 2 groups. See Fig. 1 legend for details.

Fig. 3. Pressure-flow relationship for systemic and pulmonary vasculature in the 2 groups of animals. MAP-RAP, mean arterial pressure-right atrial pressure; MPAP-PAOP, mean pulmonary arterial pressure-pulmonary artery occlusion pressure.

relatively slow onset of action (4). Nevertheless, a slight decrease in hematocrit and a slight increase in cardiac index were probably related to some vasodilating effect of NAC. After endotoxin administration and during tamponade, NAC better maintained cardiac index and $\dot{V}O_2$ by altering both the peripheral vasculature and the myocardial function.

The NAC-treated dogs maintained a lower systemic vascular resistance than the control animals. In addition, the typical increase in pulmonary vascular resistance observed after endotoxin challenge in animals was blunted in these animals. Because pulmonary artery pressures were somewhat lower and cardiac index was significantly higher in the NAC-treated than in the control animals, we are confident that NAC decreased the pulmonary vascular tone. In addition, the multi-point plots of pressure-flow relationship for the systemic and the pulmonary vasculature showed that NAC-treated animals had lower intravascular pressure at any blood flow. These observations are in keeping with previous experimental and clinical studies reporting that NAC induces a significant systemic and pulmonary

vasodilation (3, 13, 23). The vasodilating effects of NAC are due to both a direct relaxing action on vascular smooth muscle and an enhanced extracellular NAC-nitrosothiol formation with subsequent indirect stimulation of guanylate cyclase and enhanced guanosine 3',5'-cyclic monophosphate production (14, 30). The pulmonary vascular effects of NAC in endotoxic animals have also been related to an inhibition of the rise in thromboxane B_2 (3). The lower hematocrit level in the treated animals was also consistent with a vasodilating effect of NAC, resulting in a hemodiluting effect. Although a lower systemic vasomotor response to a reduction in blood flow may have detrimental effects, this was not the case in the present study, probably because the

Fig. 4. Relationships among oxygen delivery ($\dot{V}O_2$), oxygen uptake ($\dot{V}O_2$), and oxygen extraction ratio (O_2ER) and IPP in the 2 groups. See Fig. 1 legend for details.

Fig. 5. Individual values for $\dot{V}O_2$ and $\dot{D}O_2$ in the 2 groups of animals.

mean arterial pressure followed a similar course in the two groups.

Indeed, a major observation was a higher cardiac index and a higher stroke volume in the NAC-treated than in the control group. On this model in which cardiac index was reduced by an obstruction to venous return, right atrial pressure, like intrapericardial pressure, was elevated to identical levels in the two groups of animals so that differences in preload or in ventricular compliance could hardly account for the difference in stroke volume. A lower ventricular afterload may contribute to the higher stroke volume in the NAC-treated dogs

Fig. 6. Relationships among hematocrit, blood lactate, and IPP in the 2 groups. See Fig. 1 legend for details.

Fig. 7. Time course of plasma tumor necrosis factor (TNF) levels in the 2 groups. NAC completely attenuated release of TNF after endotoxemia. See Fig. 1 legend for details.

but cannot be entirely responsible, since arterial pressure was identical in the two groups. Thus differences in myocardial contractility must be incriminated, as evidenced by the greater left ventricular stroke work index in the NAC group than in the control group. In animal models of myocardial ischemia, NAC has been reported to improve markedly myocardial contractility (8, 9) not only by increasing coronary blood flow but also presumably by increasing the cellular thiol pool of the myocardium (22). As a consequence, the calcium gradient from sarcoplasmic reticulum to cytosol may be increased (8). Furthermore, the release of oxygen free radicals has been implicated in the myocardial depression associated with sepsis (27) so that the oxygen free radical scavenging effects of NAC may inhibit the endotoxin-induced myocardial depression.

Before intrapericardial pressure reached critical levels around 8 mmHg, NAC-treated dogs had higher $\dot{V}O_2$ than the control dogs. We attribute this greater $\dot{V}O_2$ to the higher blood flow in these animals, whose left ventricular stroke work index nearly doubled. A direct stimulating effect of NAC on cellular metabolism is unlikely and would be expected to be associated with a higher O_2ER . It was only when intrapericardial pressure reached high levels that $\dot{V}O_2$ was maintained by a higher oxygen extraction in the NAC group. In other words, the NAC-treated animals kept their oxygen extraction reserve by a greater oxygen supply to the tissues and used this reserve when tissue blood flow became critically reduced.

NAC may exert these protective effects after endotoxemia by several intertwined mechanisms. An important one is the scavenging effect of NAC on oxygen free radicals, since glutathione is one of the main intracellular defense mechanisms against oxidative stress, and cellular glutathione levels have been shown to be reduced during severe sepsis (18). NAC is a low-molecular-weight precursor to glutathione, which can cross the cell membrane and thereby replenish intracellular glutathione stores (3, 12, 19). We did not measure intracellular glutathione levels, but we determined the plasma activity of glutathione peroxidase, the important enzyme reducing hydrogen peroxide (H_2O_2) as well as organic hydroperoxides, through the oxidation of reduced gluta-

Fig. 8. Changes in glutathione peroxidase activity, superoxide dismutase (SOD) activity, and vitamin E in the 2 groups. NAC significantly enhanced glutathione peroxidase activity after endotoxemia. See Fig. 1 legend for details.

thione (20). The blood activity of this enzyme is considered to reflect the tissue antioxidant activity (20). As the glutathione peroxidase activity is tightly coupled to the cellular concentrations of glutathione, its higher activity reflected the replenishment of intracellular glutathione levels under NAC administration (2, 12). We thought that the antioxidant efficacy of NAC in endotoxic shock might be indirectly monitored through its sparing effects on plasma vitamin E, another important oxygen free radical scavenger, but this was not observed. A possible explanation is that vitamin E predominantly scavenges lipid peroxy radicals (21), whereas NAC primarily reacts with hydroxyl radical and hydrogen peroxide (2). Although NAC may increase superoxide

dismutase activity, it had no such effect in the present study.

NAC can also exert important anti-inflammatory effects on neutrophils and monocytes (19). In our study, this decreased inflammatory response was reflected by a complete inhibition of TNF release by NAC. In a mouse endotoxic model, Peristeris et al. (25) recently demonstrated that pretreatment with NAC significantly inhibited TNF production both in the serum and in the spleen. This attenuating effect of NAC on the TNF production is most likely due to a decreased release of oxygen free radicals. Spin trap techniques can decrease TNF levels and protect mice from endotoxic shock (26). Like glutathione, NAC has no direct effect on TNF production in murine peritoneal cells, but glutathione depletion potentiates TNF production (6, 15). As a consequence, NAC may break a vicious circle in that TNF can increase the production of oxygen free radicals, which in turn may activate inflammatory cells and induce further release of TNF (16).

The increased oxygen extraction in the NAC-treated dogs could also be related to the associated vasodilating effects that may reduce the distance for oxygen diffusion from the capillary to the cells. We did not evaluate the effects of NAC on the distribution of blood flow, which may also have improved with this agent. These effects of NAC are at least in part related to the action of EDRF, an endogenous vasodilator that relaxes vascular smooth muscle and inhibits the aggregation and adhesion of platelets (29). NAC can contribute to EDRF enhancement by several mechanisms. Sulfhydryl compounds, including NAC, promote the generation of nitrites and nitric oxide (NO) in the vascular smooth muscle (5). In this regard, thiol specificity may be important. Sulfhydryl compounds can also react with NO to form S-nitrosothiols, and both compounds stimulate guanylate cyclase to increase intracellular guanosine 3',5'-cyclic monophosphate levels (29). The production of nitroso-compounds may also occur outside the cell when exogenous thiol is provided (1). The attenuation of TNF and oxygen free radical production by NAC may potentiate the EDRF action as TNF and oxygen free radicals either prevent the synthesis or destroy the biological activity of EDRF (10, 11). A recent study by Sunman et al. (30) on isolated rat and human resistance arteries demonstrated that the removal of the vascular endothelium did not alter the vasodilating response to NAC, suggesting a direct endothelium-independent, relaxing action of NAC on vascular smooth muscle. It is intriguing to observe that NAC may inhibit the NO production by blocking the TNF release and at the same time promote an EDRF activity. Because inhibition of NO has not always been found beneficial in endotoxic shock (31), TNF blockade, while maintaining some EDRF activity, may be a rational therapeutic approach provided by NAC.

Some clinical studies have examined the effects of NAC in patients with sepsis and adult respiratory distress syndrome (ARDS). In a preliminary trial in 30 patients with sepsis-induced ARDS, Bernard (2) reported that intravenous administration of NAC markedly increased plasma cysteine as well as plasma and red

cell glutathione levels, which were initially decreased in these patients. They found some beneficial effects of NAC on chest radiograph edema scores, pulmonary vascular resistance, static compliance, $\dot{V}O_2$, and $\dot{V}O_{2E}$. In 66 ARDS patients, Jepsen et al. (17) demonstrated that NAC acts as an anticoagulant with probably beneficial effects on pulmonary microcirculation. In 61 patients with severe respiratory failure, Schaller et al. (28) recently showed that NAC improved blood oxygenation and decreased mortality rate from 35 to 22%. In 20 patients with fulminant hepatic failure, Harrison et al. (13) reported that NAC at an initial dose of 150 mg/kg followed by 50 mg/kg over 4 h enhanced $\dot{V}O_2$ by 46% through increasing both $\dot{V}O_2$ and $\dot{V}O_{2E}$. These clinical results, although still preliminary, are consistent with the present observations. As our study only investigated the effects of pretreatment with NAC, an important question remains whether NAC could exert these protective effects once the septic response has already developed.

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