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REVIEW

Role of oxidative stress in experimental sepsis and multisystem organ dysfunction

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Abstract
Massive increase in radical species can lead to oxidative stress, promoting cell injury and death. This review focuses on experimental evidence of oxidative stress in critical illnesses, sepsis and multisystem organ dysfunction. Oxidative stress could negatively affect organ injury and thus overall survival of experimental models. Based on this experimental evidence, we could improve the rationale of supplementation of antioxidants alone or in combination with standard therapies aimed to reduce oxidative stress as novel adjunct treatment in critical care.

Keywords: *Oxidative stress, critical illness, antioxidants, ROS*

Abbreviations: *ALI, Acute lung injury; ARDS, Acute respiratory distress syndrome; ATP, Adenosine triphosphate; CAT, Catalase peroxidase; GSH, Glutathione; iNOS, inducible nitric oxide synthase; IL-1*β*, Interleukin-1* β*; LPS, Lipolysaccharide; MODS, Multiple organ dysfunction syndrome; MOF, Multi organ failure; NAC,* N*-acetylcysteine; NADPH, Reduced nicotinamide-adenine dinucleotide phosphate; NF-*κ*B, Nuclear factor-* κ*B; NO, Nitric oxide; nNOS, neuronal nitric oxide synthase; RNOS, Reactive nitrogenous oxide species; ROS, Reactive oxygen species; SOD, Superoxide dismutase; TNF*α*, Tumor necrosis factor* α*; TBARS, Thiobarbituric acid reactive substances; TRAP, Total Antioxidant capacity*

Introduction

A balanced immune response from all organ systems is crucial for survival from critical illness. However, during critical illnesses, the immune response fails to protect the body. Oxidative stress defines a discrepancy in release of oxidizing chemical species and their effective removal by protective antioxidants and scavenger enzymes. Massive increase in free radicals can lead to an overwhelming inflammatory response and tissue injury. This review focuses on experimental evidence of oxidative stress in critical illnesses, characterized by tissue ischemia–reperfusion injury and by an intense systemic inflammatory response such as during sepsis and acute respiratory distress syndrome (ARDS). A better knowledge of oxygen radical-mediated mechanisms may lead to improved therapies in the treatment of critically ill patients.

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Activation of phagocytes, exceeding production of nitric oxide (NO) and reactive oxygen species (ROS), release of iron, copper ions and metalloproteins are main sources of oxidative stress during critical illnesses, such as sepsis or ARDS as well as burn trauma [1–3]. The evidence described below demonstrates the role of severe oxidative stress in critical care.

Oxidative stress in experimental models

Ischemia–reperfusion models. Tissue ischemia–reperfusion injury represents a typical form of oxidative damage. The role of ROS as mediators of the "reperfusion injury" syndrome has been shown in different organs [4–8]. ROS can induce disruption of endothelial cells with subsequent microvascular thrombosis and organ dysfunction [9].

In a rat model of hepatic ischemia/reperfusion, hepatic antioxidant glutathione (GSH) was consumed, accompanied to excessive ROS production [10]. Treatment with dimethylsulfoxide reduces hepatic GSH consumption and tissue damage. Interestingly, the protective effect of dimethylsulfoxide occurs when endogenous supply of GSH is reduced to below 30% of normal values, showing that normal liver has a functional antioxidative reserve that must be depleted before injury occurs.

Hemorrhagic shock, followed by resuscitation, represents a widespread ischemia–reperfusion insult. In a rat model of hemorrhagic shock-resuscitation, treatment with superoxide dismutase (SOD) + catalase peroxidase (CAT) did not produce any significative difference in organ blood flow and muscle depolarization during hemorrhagic shock, but significantly improved repolarization of cell membrane after resuscitation, highlighting the role of ROS in damage to excitable cell membrane [11].

Sepsis model. Sepsis, the main cause of morbidity and mortality in intensive care units in the United States [12] and in Europe [13], is a complex disease characterized by different hemodynamic and metabolic alterations, leading to multiple-organ dysfunction and death [14].

In an *in vitro* model of microvascular injury by lipopolysaccharide (LPS) (priming step) and heat shock (activation step), resembling the damage observed in multiple organ dysfunction syndrome (MODS), scavenging of the hydroxyl radical by dimethylsulfoxide, a membrane-permeable oxygen radical scavenger, and high levels of allopurinol, a xanthine oxidase inhibitor, blocked apoptosis when applied before LPS priming, suggesting a role for the hydroxyl radical as an intracellular signal in endothelial cell apoptosis [15]. Recently, an interesting study using a model of sepsis induced by cecal ligation and perforation showed that rats that went on to die of their sepsis had a significant increase of TBARS as an index of lipoperoxidation, protein carbonyls as an index of protein damage, and SOD which were early predictors of mortality. Most importantly, non-surviving rats showed a marked increase of SOD without a proportional increase of CAT. This different modulation of SOD and CAT in sepsis can lead to overproduction of hydrogen peroxide or hydroxyl radicals, increasing cell damage [16]. It is intriguing in the understanding the progression of sepsis that a sustained SOD/CAT imbalance occurs in lethal sepsis, in contrast to non lethal sepsis [17].

In a model of sepsis by cecal ligation and puncture, early inflammatory events in the lung are mediated by large quantities of nitric oxide radical produced by inducible nitric oxide synthase (iNOS) as demonstrated by decrease of arginase II, involved in generation of L-ornithine for biosynthesis of glutamate, L-proline, and polyamines, and an upregulation of iNOS [18]. Recent evidence in a similar model of sepsis confirms iNOS activity in pulmonary inflammatory cells is the major determinant of pulmonary oxidant stress [19].

NO has been implicated in the pathogenesis of cardiovascular alterations in septic shock [20–22]. Large amounts of NO are produced as consequence of the increase in iNOS activity in response to bacterial endotoxin or inflammatory cytokines; the enhanced formation of NO contributes to vascular collapse and myocardial dysfunction, mediating the depressant effects of proinflammatory cytokines, namely tumor necrosis factor α (TNFα) and interleukin-1 β (IL-1β), in septic shock [23–26]. Endotoxin administration produced less hypotension in iNOS deficient mice [27], and in mice treated with a selective pharmacological inhibitor of iNOS [28].

The increased production of NO, induced by neuronal nitric oxide synthase isoform (nNOS), mediated the deficit in arteriolar conducted vasoconstriction sepsis related [29].

NO reduces myocardial contractility by reducing calcium affinity of contractile apparatus and may cause direct myocyte damage by peroxynitrite production [30]. Proposed alternative mechanisms by which NO can impair the cardiovascular system during septic shock include inactivation of alpha-adrenoreceptor by peroxynitrite [31] and inhibition of vasopressin release [32]. It has been suggested that NO could have beneficial effects in sepsis related to counteraction of the released vasoconstrictors substances, to inhibition of leukocyte rolling and adhesion and to inhibition of nuclear factor-κB (NF-κB) [20] (Figure 1).

An excess of NO synthesized by iNOS may play an important role in septic diaphragmatic failure [33–35]; increased levels of NO, by production of peroxynitrite, harm diaphragmatic mitochondrial function, which can contribute to the impairment of muscle contractility [36].

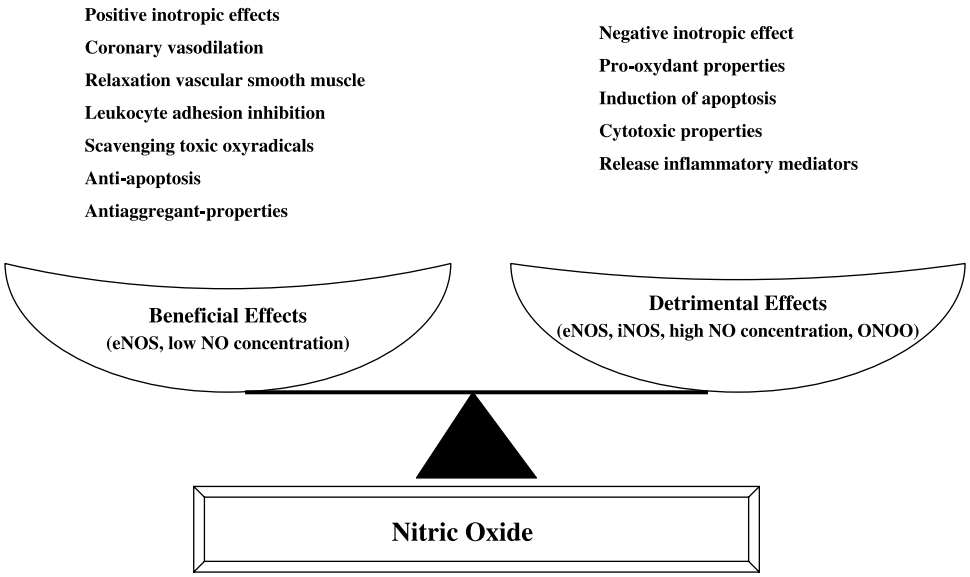

Figure 1. Different effects of Nitric oxide. Endothelial nitric oxide synthase (eNOS) produces physiologically small amounts of NO, which produce beneficial effects. Inducible isoform (iNOS), activated in response to proinflammatory signals, is mainly responsible for larger and more persistent production of NO and, consequently, for its detrimental effects. A constantly upregulated eNOS activity could also lead to large NO concentrations which may contribute to deleterious effects.

The overproduction of NO has been shown to contribute to increased gut epithelial permeability in different models of inflammation [37]; moreover, iNOS might be involved in intestinal ischemia–reperfusion-induced loss of gut barrier function, playing an important role in the development of systemic inflammation and distant organ failure [38].

Endotoxic shock by LPS induced severe oxidative injury reflected by increased plasma levels of lipoperoxides malondialdehyde and 4-hydroxynonenal, reduced plasma total antioxidant capacity and high concentration of nitrites/nitrates. Interestingly, depletion of the liver glutathione yielded higher levels of lipoperoxides and lower plasma antioxidant capacity, confirming the important role of glutathione as antioxidant, but, unexpectedly, blunted the increase in iNOS and plasma nitrites/nitrates, probably because of iNOS dependence of reduced thiols. These results highlight the complex interaction exisisting among oxidants, antioxidant systems and nitric oxide [39].

ALI/ARDS models. ARDS is an inflammatory disease initiated by a wide variety of systemic and/or pulmonary insults, that leads to disruption of the alveolar-capillary unit and to a breakdown in the barrier and gas exchange functions of the lung [40].

ROS and reactive nitrogen oxide species (RNOS) play an important role in the pathogenesis of ARDS [41,42]. Neutrophils and alveolar macrophage are considered the most important source of ROS in acute lung injury (ALI). In addition, many structural cells such as epithelial cells, endothelial cells and interstitial cells can contribute to ROS production [43]. Reduced nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase complex, xanthine oxidase and iNOS are the main pathways of oxidants production. Evidence of oxidative stress has been shown in different models of ALI/ARDS [44–46] and during mechanical ventilation with elevated tidal volume[47,48]. ROS and RNOS increase epithelial and endothelial permeability, impair ion transport and adenosine triphosphate (ATP) synthesis in epithelial cells, and reduce the synthesis of surfactant, so contributing to the most important pathologic findings of ARDS [40]. Endotoxin-induced lung injury is reduced by NADPH oxidase and iNOS inhibition [49,50] as well as in iNOS gene deficient mice [51]; moreover, iNOS knockout mice show a decreased inflammatory response characterized by reduced neutrophil accumulation and cytokine expression [52]. Interestingly, the increased production of ROS induced by cyclic mechanical stretch may participate to development of ventilatory induced lung injury [53].

Antioxidant and scavenger therapy

Restoring endogenous antioxidants (i.e. antioxidants or scavenger SOD or SOD mimetics) or supplementing exogenous agents with antioxidant properties (i.e. *N*-acetylcysteine) or administering drugs that reduce oxidant production (i.e. allopurinol, a xanthine inhibitor, or desferoxamine, a iron scavenger) represent different modalities of antioxidant therapy [54–57].

Antioxidant therapy in preclinical models

Several therapeutic approaches have been evaluated in different animal models in order to reduce oxidative damage (Table I).

Superoxide dismutase. In a sepsis model, administration of scavenger SOD has improved survival in rats when administered 24 h before induction of sepsis, whereas it did not show any beneficial effect when administered after induction of sepsis [58]. Recombinant human SOD improved both endotoxin and tumor necrosis factor-induced ALI in sheep [59,60]. Pre- or post-treatment with EUK-8, a synthetic SOD with both SOD-mimetic and catalase mimetic properties, reduced lung injury in endotoxemic models [61,62]. SOD mimetics have been proposed as novel therapeutic agents, showing beneficial effects in ischemia–reperfusion injury, septic shock and zymosan-induced shock [63,64]. In a LPS induced lung injury model, administration of catalase prevented the severity of oxidative stress and the development of lung injury [65].

Tempol. Tempol, a membrane-permeable radical scavenger able to interfere with many radicals (superoxide anions, hydroxyl radical, and peroxynitrate), attenuated the degree of multiorgan failure (MOF) induced by zymosan [66]; there is increasing evidence that tempol may be useful in the therapy of ischemia–reperfusion injury, shock and sepsis [67–70].

N-acetylcysteine. *N*-acetylcysteine (NAC) has been widely used as an antioxidant in experimental models. NAC has direct and indirect (by conversion, *in vivo*, to L-cysteine, which repletes intracellular glutathione stores) antioxidant properties. In endotoxic shock, pre-treatment with NAC decreased NF-κB activation [71], attenuated TNF-α production, reduced blood lactate levels and increased survival [72]. In a model of fluid resuscitated endotoxic shock, administration of NAC before administration of the endotoxin improved oxygen extraction with an enhanced regional blood flow in mesenteric, renal and femoral vasculatures [73]. However, when NAC infusion was started after 12 h of endotoxin administration, there was no improvement in local and regional hemodynamics, metabolism, or oxygen exchange despite the increased glutathione concentration [74]. The lack of efficacy of delayed NAC administration highlights the importance of early antioxidant supplementation before that the hemodynamic and metabolic effects induced by sepsis are fully established [56].

In different models of inflammation, NAC has reduced lung [75–78] and intestinal damage [79,80]. Intratracheal administration of NAC by liposomal encapsulation prolonged the protective effect in a model of lung injury [81]. In models of septic shock co-administration of NAC with α-tocopherol [82] suppressed NF-κB, co-administration with vitamin E and β-carotene [83] reduced lipid peroxidation and with desferoxamine [84] reduced TBARS production, improved the balance between catalase and SOD activities, limiting neutrophil infiltration and mithocondrial dysfunction. In a model of ALI induced by intratracheal LPS, the co-administration of NAC with deferoxamine reduces TBARS production, mithocondrial superoxide production, blunting the inflammatory response [85]. NAC may prevent the septic phrenic nerve dysfunction [86,87].

Vitamin E. Vitamin E, the most important antioxidant against lipid peroxidation, has been shown to reduce oxidative stress in sepsis models [88]. Administration of liposomal α-tocopherol has reduced lipid peroxidation in a rat model of hypoxia-induced lung injury [89] and decreased the number of neutrophils in the airways, preventing lung injury in a mice model of ALI (ALI) induced by aerosolized LPS [90]. α-tocopherol showed protective effect, reducing lipid peroxidation, in models of mild and severe brain injury [91].

iNOS inhibition. Selective inhibition of iNOS has been shown to be protective, probably by reducing peroxynitrite generation, in hemorrhagic and endotoxic shock [92–94]. Interestingly, the beneficial effects of ascorbate on the impaired arteriolar vasoconstriction in sepsis may be related to iNOS inhibition [95]. However, a clinical trial of a non-selective inhibitor of NO in patients with septic shock resulted in an increase in mortality in the treated group [96]. This implies a dose-dependent effect of NO in sepsis. Thus, the role of iNOS inhibition in sepsis is still controversial [97].

Conclusions

There is increasing evidence that oxidative stress play an important role in the beginning and establishment of the inflammatory diseases, representing a common pathway for life-threatening critical illnesses such as septic shock, ARDS.

Supplementation with antioxidants seems to be the logical answer to reduced levels of antioxidants but the benefit of this therapy may depend on many variables including class of drugs, dose and timing of administration, differences in patient population and size of the samples.

An intense study of oxygen radical-mediated mechanisms may lead to improve current therapies in critical care medicine.

Table I. Antioxidants in preclinical models.

Authors	Model	Antioxidants	Main results
Warner [58]	Sepsis (CLP)	SOD	Pre-treatment improved survival; no survival improvement when SOD administered after sepsis induction
Amari [59]	TNF-induced lung injury	Rh-SOD	Attenuated pulmonary hypertension; reduced thromoboxane A2/prostacycline metabolites
Koyama [60]	Endotoxin-induced lung injury	rh-SOD	Attenuated lung injury
Gonzalez [62]	LPS induced ARDS	EUK-8 (synthetic superoxide dismutase)	Attenuated lung injury
Cuzzocrea [63]	Zymosan-induced shock	M40401 (selective superoxide dimutase mimetic)	Attenuated organ failure and systemic inflammation
Milligan [65]	Endotoxin-induced lung injury	Catalase	Attenuated lung injury
Cuzzocrea [66]	Zymosa-induced MOF	Tempol	Attenuated degree of MOF
Zacharowski [68]	Endotoxin-induced MODS	Tempol	Attenuated renal/hepatocellular dysfunction
Matejovic [69]	Bacteremia (continuous infusion of pseudomonas aeruginosa)	Tempol	Partially attenuation of endothelial/coagulation and oxidative stress by tempol post-treatment
Liaw [70]	Sepsis (CLP)	Tempol	Attenuated organ dysfunction and reduced mortality
Blackwell [71]	Endotoxin-induced lung injury	NAC	Decreased lung injury and NF-κB activation
Zhang [72]	*Escherichia coli* endotoxin	NAC	Attenuated TNF-α production, reduced lactate levels, increased survival
Zhang [73]	*Escherichia coli* endotoxin	NAC	Improved O_2 extraction, enhanced regional blood flow
Vassilev [74]	Endotoxic shock	NAC (postendotoxemia)	No hemodynamics improvement
Cuzzocrea [75]	Carrageenan-induced lung injury	NAC	Reduced oxidant lung injury
Bernard [76]	Endotoxin-induced ARDS	NAC	Attenuated lung injury
Davreux [77]	Endotoxin-induced acute lung injury	NAC	Attenuated lung injury
Ozdulger [78]	Sepsis (CLP)	NAC	Reduced lung apoptosis
Cuzzocrea [79]	Zymosan-induced MOF	NAC	Reduced oxidative stress and organ injury
Cuzzocrea [80]	Ischemia–reperfusion injury	NAC	Reduced inflammatory response
Fan [81]	Ischemia–reperfusion injury	Liposomal NAC	Reduced lung injury
Fox [82]	LPS-cell activation	NAC + alpha-tocopherol	Suppressed kupffer cell activation
Kheir-eldin [83]	Endotoxin brain injury	NAC + vitamin E + betacarotene	Reduced brain oxidative stress
Ritter [84]	Sepsis (CLP)	NAC + desferoxamine	Reduced oxidative stress, improved survival
Ritter [85]	LPS-induced lung injury	NAC + desferoxamine	Attenuated lung oxidative damage
Atis [87]	Sepsis (CLP)	NAC	Attenuated phrenic nerve dysfunction
Minko [89]	Hypoxic lung injury	Liposomal alpha-tocopherol	Reduced lipid peroxidation
Rocksen [90]	Inhaled endotoxin-induced lung injury	Alpha-tocopherol	Reduced neutrophils migration and airway inflammation
Inci [91]	Brain injury	Alpha-tocopherol	Reduced brain lipid peoxidation
Arkovitz [92]	Endotoxin-induced pulmonary transvascular	Selective iNOS inhibitors	Prevented increase of pulmonary index flux
Saetre [93]	Group A streptococcal sepsis	Aminoethyl–isothiourea (nitric oxide synthase inhibitor and radical scavenger)	Prolonged survival, counteracted hemodynamic deterioration
Szabo [94]	Hemorrhagic shock	Mercaptoethylguanidine (iNOS inhibitor)	Improved hemodynamics, improved survival, reduced intestinal lipid peroxidation
Wu [95]	Sepsis (CLP)	Ascorbate	Reduced nitric oxide production, increased survival

ARDS, acute respiratory distress syndrome; CLP, cecal ligation and puncture; iNOS, inducible nitric oxide synthase; LPS, lipolysaccharide; MODS, multiple organ dysfunction syndrome; MOF, multi organ failure; NAC, *N*-acetylcysteine; NF-κB, nuclear factor-κB; rh-SOD, recombinant human superoxide dismutase; SOD, superoxide dismutase; TNF, tumor necrosis factor.

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References

[1] Gutteridge JMC, Mitchell J. Redox imbalance in the critically ill. Br Med Bull 1999;55:49–75.
[2] Crimi E, Sica V, Williams-Ignarro S, Zhang H, Slutsky AS, Ignarro LJ, Napoli C. The role of oxidative stress in adult critical care. Free Radic Biol Med 2006;40:398–406.
[3] Horton JW. Free radicals and lipid peroxidation mediated injury in burn trauma: The role of antioxidant therapy. Toxicology 2003;189:75–88.
[4] Adkison D, Hollwarth ME, Benoit JN, Parks DA, McCord JM, Granger DN. Role of free radicals in ischemia–reperfusion injury to the liver. Acta Physiol Scand 1986;548:101–107.
[5] Hinder RA, Stein HJ. Oxygen derived free radicals. Editorial Arch Surg 1991;126:104–105.
[6] McCord JM. Oxygen derived free radicals in postischemic tissue injury. N Engl J Med 1985;312:159–163.
[7] Stein HJ, Esplugues J, Whittle BJ, Bauerfeind P, Hinder RA, Blum AL. Direct cytotoxic effect of oxygen radicals on the gastrica mucosa. Surgery 1989;106:318.
[8] Idris AH, Roberts 2nd, LJ, Caruso L, Showstark M, Layon AJ, Becker LB, Vanden Hoek T, Gabrielli A. Oxidant injury occurs rapidly after cardiac arrest, cardiopulmonary resuscitation, and reperfusion. Crit Care Med 2005;33:2043–2048.
[9] Schiller HJ, Reilly PM, Bulkley GB. Antioxidant therapy. Crit Care Med 1993;21:S92–S102.
[10] Stein HJ, Oosthuizen MM, Hinder RA, Lamprechts H. Oxygen free radicals and glutathione in hepatic ischemia/reperfusion injury. J Surg Res 1991;50:398–402.
[11] Yokota J, Chiao JJC, Shires GT. Oxygen free radicals affect cardiac and skeletal cell membrane potential during hemorrhagic shock in rats. Am J Physiol 1992;262:H84–H90.
[12] Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001;29:1303–1310.
[13] Salvo I, de Cian W, Musicco M, Langer M, Piadena R, Wolfler A, Montani C, Magni E. The Italian SEPSIS study: Preliminary results on the incidence and evolution of SIRS, sepsis, severe sepsis and septic shock. Intensive Care Med 1995;21:S244–S249.
[14] Awad SS. State-of-the-art therapy for severe sepsis and multisystem organ dysfunction. Am J Surg 2003;186:23S–30S.
[15] Abello PA, Fidler SA, Bulkley GB, Buchman TG. Antioxidants modulate induction of programmed endothelial cell death (apoptosis) by endotoxin. Arch Surg 1994;129:134–140.
[16] Ritter C, Andrades M, Frota Jr., ML, Bonatto F, Pinho RA, Polydoro M, Klamt F, Pinheiro CT, Menna-Barreto SS, Moreira JC, Dal-Pizzol F. Oxidative parameters and mortality in sepsis induced by cecal ligation and perforation. Intensive Care Med 2003;29:1782–1789.
[17] Andrades M, Ritter C, Moreira JC, Dal-Pizzol F. Oxidative parameters differences during non-lethal and lethal sepsis development. J Surg Res 2005;125:68–72.
[18] Carraway MS, Piantadosi CA, Jenkinson CP, Huang YC. Differential expression of arginase and iNOS in the lung in sepsis. Exp Lung Res 1998;24:253–268.
[19] Razavi HM, Wang L, Weicker S, Quinlan GJ, Mumby S, McCormack DG, Mehta S. Pulmonary oxidant stress in murine sepsis is due to inflammatory cell nitric oxide. Crit Care Med 2005;33:1333–1339.
[20] Massion PB, Moniotte S, Balligand JL. Nitric oxide: Does it play a role in the heart of the critically ill? Curr Opin Crit Care 2001;7:323–336.
[21] Murray PT, Wylam ME, Umans JG. Nitric oxide and septic vascular dysfunction. Anesth Analg 2000;90:89–101.
[22] Vincent JL, Zhang H, Szabo C, Preiser JC. Effects of nitric oxide in septic shock. Am J Respir Crit Care Med 2000;161:1781–1785.
[23] Hollenberg SM, Easington CR, Osman J, Broussard M, Parrillo JE. Effects of nitric oxide synthase inhibition on microvascular reactivity in septic mice. Shock 1999;12:262–267.
[24] Kinugawa K, Takahashi T, Kohmoto O, Yao A, Aoyagi T, Momomura S, Hirata Y, Serizawa T. Nitric oxide-mediated effects of interleuking-6 on [Ca^{2+}] and cell contraction in cultured chick ventricular myocytes. Circ Res 1994;75:285–295.
[25] Kumar A, Brar R, Wang P, Dee L, Skorupa G, Khadour F, Schulz R, Parrillo JE. Role of nitric oxide and cGMP in human septic serum-induced depression of cardiac myocyte contractility. Am J Physiol 1999;276:R265–R276.
[26] Stein B, Frank P, Schmitz W, Scholz H, Thoenes M. Endotoxin and cytokines induce direct cardiodepressive effects in mammalian cardiomyocytes via induction of nitric oxide synthase. J Mol Cell Cardiol 1996;28:1631–1639.
[27] Ullrich R, Scherrer-Crosbie M, Bloch KD, Ichinose F, Nakajima H, Picard MH, Zapol WM, Quezado ZM. Congenital deficiency of nitric oxide synthase 2 protects against endotoxin-induced myocardial dysfunction in mice. Circulation 2000;102:1440–1446.
[28] Strunk V, Hahnenkamp K, Schneuing M, Fischer LG, Rich GF. Selective iNOS inhibition prevents hypothension in septic rats while preserving endothelium-dependent vasodilation. Anesth Analg 2001;92:681–682.
[29] McKinnon RL, Lidington D, Bolon M, Ouellette Y, Kidder GM, Tyml K. Reduced arteriolar conducted vasoconstriction in septic mouse cremaster muscle is mediated by nNOS-derived NO. Cardiovasc Res 2006;69:236–244.
[30] Szabo C, Cuzzocrea S, Zingarelli B, O'Connor M, Salzman AL. Endothelial dysfunction in a rat model of endotoxic shock: Importance of the activation of the poly (ADP-ribose) synthetase by peroxynitrite. J Clin Invest 1997;100:723–735.
[31] Takakura K, Taniguchi T, Muramatsu I, Takeuchi K, Fukuda S. Modification of alpha1-adrenoceptors by peroxynitrite as a possible mechanism of systemic hypotension in sepsis. Crit Care Med 2002;30:894–899.
[32] Giusti-Paiva A, De Castro M, Antunes-Rodrigues J, Carnio EC. Inducible nitric oxide synthase pathway in the central nervous system and vasopressin release during experimental septic shock. Crit Care Med 2002;30:1306–1310.
[33] Boczkowski J, Lanone S, Ungureanu-Longrois D, Danialou G, Fournier T, Aubier M. Induction of diaphragmatic nitric oxide synthase after endotoxin administration in rats. J Clin Invest 1996;98:1550–1559.
[34] el-Dwairi Q, Comtois A, Guo Y, Hussain SN. Endotoxin-induced skeletal muscle contractile dysfunction: contribution of nitric oxide synthases. Am J Physiol 1998;247:C770–C779.
[35] Gath I, Closs EI, Godtel-Armbrust U, Schmitt S, Nakane M, Wessler I, Forstermann U. NO Inducible synthase II and neuronal NO synthase I are constitutively expressed in different structures of guinea pig skeletal muscle: Implications for contractile function. FASEB J 1996;10:1614–1620.
[36] Boczkowski J, Lisdero CL, Lanone S, Samb A, Carreras MC, Boveris A, Aubier M, Poderoso JJ. Endogenous peroxynitrite mediates mitochondrial dysfunction in rat diaphragm during endotoxemia. FASEB J 1999;13:1637–1646.
[37] Chavez AM, Menconi MJ, Hodin RA, Fink MP. Cytokine-induced intestinal epithelial hyperpermeability: Role of nitric oxide. Crit Care Med 1999;27:2246–2251.

[38] Suzuki Y, Deitch EA, Mishima S, Lu Q, Xu D. Inducible nitric oxide synthase gene knockout mice have increased resistance to gut injury and bacterial translocation after an intestinal ischemia–reperfusion injury. Crit Care Med 2000;28: 3692–3696.

[39] Carbonell LF, Nadal JA, Llanos MC, Hernandez I, Nava E, Diaz J. Depletion of liver glutathione potentiates the oxidative stress and decreases nitric oxide synthesis in a rat endotoxin shock model. Crit Care Med 2000;28:2002–2006.

[40] Ware LB, Matthay MA. The acute respiratory distress syndrome. N Engl J Med 2000;342:1334–1349.

[41] Chow CW, Herrera Abreu MT, Suzuki T, Downey GP. Oxidative stress and acute lung injury. Am J Respir Cell Mol Biol. 2003;29:427–431.

[42] Fink MP. Role of reactive oxygen and nitrogen species in acute respiratory distress syndrome. Curr Opin Crit Care 2002;8:6–11.

[43] Sanders KA, Huecksteadt T, Xu P, Sturrock AB, Hoidal JR. Regulation of oxidant production in acute lung injury. Chest 1999;116:56S–61S.

[44] Hammerschmidt S, Buchler N, Wahn H. Tissue lipid peroxidation and reduced glutathione depletion in hypochlorite-induced lung injury. Chest 2002;121:573–581.

[45] Matsuo N. The role of intrapulmonary nitric oxide generation in the development of adult respiratory distress syndrome. Surg Today 1999;29:1068–1074.

[46] Yang C, Moriuchi H, Takase J, Ishitsuka Y, Irikura M, Irie T. Oxidative stress in early stage of acute lung injury induced with oleic acid in guinea pigs. Biol Pharm Bull 2003;26:424–428.

[47] Hammerschmidt S, Sandvoss T, Gessner C, Schauer J, Wirtz H. High in comparison with low tidal volume ventilation aggravates oxidative stress-induced lung injury. Biochim Biophys Acta 2003;1637:75–82.

[48] Hammerschmidt S, Schiller J, Kuhn H, Meybaum M, Gessner C, Sandvoss T, Arnold K, Wirtz H. Influence of tidal volume on pulmonary NO release, tissue lipid peroxidation and surfactant phospholipids. Biochim Biophys Acta 2003;1639:17–26.

[49] Mikawa K, Nishina K, Tamada M, Takao Y, Maekawa N, Obara H. Aminoguanidine attenuates endotoxin-induced acute lung injury in rabbits. Crit Care Med 1998;26:905–911.

[50] Wang W, Suzuki Y, Tanigaki T, Rank DR, Raffin TA. Effect of the NADPH oxidase inhibitor apocynin on septic lung injury in guinea pigs. Am J Respir Crit Care Med 1994;150:1449–1452.

[51] Kristof AS, Goldberg P, Laubach V, Hussain SN. Role of inducible nitric oxide synthase in endotoxin-induced acute lung injury. Am J Respir Crit Care Med 1998;158:1883–1889.

[52] Shanley TP, Zhao B, Macariola DR, Denenberg A, Salzman AL, Ward PA. Role of nitric oxide in acute lung inflammation: Lessons learned from the inducible nitric oxide synthase knockout mouse. Crit Care Med 2002;30:1960–1968.

[53] Chapman KE, Sinclair SE, Zhuang D, Hassid A, Desai LP, Waters CM. Cyclic mechanical strain increases reactive oxygen species production in pulmonary epithelial cells. Am J Physiol Lung Cell Mol Physiol 2005;289:L834–L841.

[54] Bulger EM, Maier RV. Antioxidant in critical illness. Arch Surg 2001;136:1201–1207.

[55] Furhman MP. Antioxidant supplementation in critical illness: What do we know? Nutrition 2000;16:470–471.

[56] Rotstein OD. Antioxidant therapy in critical illness. Crit Care Med 2004;32:610–611.

[57] Oldham KM, Bowen EP. Oxidative stress in critical care: Is antioxidant supplementation beneficial? J Am Diet Assoc 1998;98:1001–1008.

[58] Warner BW, Hasselgren PO, James JH, Bialkowska H, Rigel DF, Ogle C, Fischer JE. Superoxide dismutase in rats with sepsis. Effect on survival rate and amino acid transport. Arch Surg 1987;122:1142–1146.

[59] Amari T, Kubo K, Kobayashi T, Sekiguchi M. Effects of recombinant human superoxide dismutase on tumor necrosis factor-induced lung injury in awake sheep. J Appl Physio 1993;74:2641–2648.

[60] Koyama S, Kobayashi T, Kubo K, Sekiguchi M, Ueda G. Recombinant-human superoxide dismutase attenuates endotoxin-induced lung injury in awake sheep. Am Rev Respir Dis 1992;145:1404–1409.

[61] Gonzalez PK, Zhuang J, Doctrow SR, Malfroy B, Benson PF, Menconi MJ, Fink MP. Delayed treatment with EUK-8, a novel synthetic superoxide dismutase (SOD) and catalase (CAT) mimetic, ameliorates acute lung injury in endotoxemic pigs. Surg Forum 1995;46:72–73.

[62] Gonzalez PK, Zhuang J, Doctrow SR, Malfroy B, Benson PF, Menconi MJ, Fink MP. EUK-8, a synthetic superoxide dismutase and catalase mimetic, ameliorates acute lung injury in endotoxemic swine. J Pharmacol Exp Ther 1995; 275:798–806.

[63] Cuzzocrea S, Mazzon E, Di Paola R, Genovese T, Serraino I, Dugo L, Cuzzocrea E, Fulia F, Caputi AP, Salvemini D. Protective effects of M40401, a selective superoxide dismutase mimetic, on zymosan-induced nonseptic shock. Crit Care Med 2004;32:157–167.

[64] Salvemini D, Cuzzocrea S. Therapeutic potential of superoxide dismutase mimetics as therapeutic agents in critical care medicine. Crit Care Med 2003;31:S29–S38.

[65] Milligan SA, Hoeffel JM, Goldstein IM, Flick MR. Effect of catalase on endotoxin-induced acute lung injury in unanesthetized sheep. Am Rev Respir Dis 1988;137:420–428.

[66] Cuzzocrea S, McDonald MC, Mazzon E, Filipe HM, Centorrino T, Lepore V, Terranova ML, Ciccolo A, Caputi AP, Thiemermann C. Beneficial effects of tempol, a membrane-permeable radical scavenger, on the multiple organ failure induced by zymosan in the rat. Crit Care Med 2001;29:102–111.

[67] Thiemermann C. Membrane-permeable radical scavengers (tempol) for shock, ischemia–reperfusion injury, and inflammation. Crit Care Med 2003;31:S76–S84.

[68] Zacharowski K, Olbrich A, Cuzzocrea S, Foster SJ, Thiemermann C. Membrane-permeable radical scavenger, tempol, reduces multiple organ injury in a rodent model of gram-positive shock. Crit Care Med 2000;28:1953–1961.

[69] Matejovic M, Krouzecky A, Martinkova V, Rokyta Jr, R, Radej J, Kralova H, Treska V, Radermacher P, Novak I. Effects of tempol, a free radical scavenger, on long-term hyperdynamic porcine bacteremia. Crit Care Med 2005;33:1057–1063.

[70] Liaw WJ, Chen TH, Lai ZZ, Chen SJ, Chen A, Tzao C, Wu JY, Wu CC. Effects of a membrane-permeable radical scavenger, tempol, on intraperitoneal sepsis-induced organ injury in rats. Shock 2005;23:88–96.

[71] Blackwell TS, Blackwell TR, Holden EP, Christman BW, Christman JW. *In vivo* antioxidant treatment suppresses nuclear factor-kappa B activation and neutrophilic lung inflammation. J Immunol 1996;157:1630–1637.

[72] Zhang H, Spapen H, Nguyen DN, Benlabed M, Buurman WA, Vincent JL. Protective effects of *N*-acetyl-L-cysteine in endotoxemia. Am J Physiol 1994;266:H1746–H1754.

[73] Zhang H, Spapen H, Nguyen DN, Rogiers P, Bakker J, Vincent JL. Effects of *N*-acetyl-L-cysteine on regional blood flow during endotoxic shock. Eur Surg Res 1995;27: 292–300.

[74] Vassilev D, Hauser B, Bracht H, Ivanyi Z, Schoaff M, Asfar P, Vogt J, Wachter U, Schelzig H, Georgieff M, Bruckner UB, Radermacher P, Froba G. Systemic, pulmonary, and hepatosplanchnic effects of *N*-acetylcysteine during long-term porcine endotoxemia. Crit Care Med 2004;32:525–532.

[75] Cuzzocrea S, Mazzon E, Dugo L, Serraino I, Ciccolo A, Centorrino T, De Sarro A, Caputi AP. Protective effects of *n*-acetylcysteine on lung injury and red blood cell modification induced by carrageenan in the rat. FASEB J 2001;15: 1187–1200.

[76] Bernard GR, Lucht WD, Niedermeyer ME, Snapper JR, Ogletree ML, Brigham KL. Effect of *N*-acetylcysteine on the pulmonary response to endotoxin in the awake sheep and upon in vitro granulocyte function. J Clin Invest 1984;73: 1772–1784.

[77] Davreux CJ, Soric I, Nathens AB, Watson RW, McGilvray ID, Suntres ZE, Shek PN, Rotstein OD. *N*-acetyl cysteine attenuates acute lung injury in the rat. Shock 1997; 8:432–438.

[78] Ozdulger A, Cinel I, Koksel O, Cinel L, Avlan D, Unlu A, Okcu H, Dikmengil M, Oral U. The protective effect of *N*-acetylcysteine on apoptotic lung injury in cecal ligation and puncture-induced sepsis model. Shock 2003;19:366–372.

[79] Cuzzocrea S, Costantino G, Mazzon E, Caputi AP. Protective effect of *N*-acetylcysteine on multiple organ failure induced by zymosan in the rat. Crit Care Med 1999;27:1524–1532.

[80] Cuzzocrea S, Mazzon E, Costantino G, Serraino I, De Sarro A, Caputi AP. Effects of *n*-acetylcysteine in a rat model of ischemia and reperfusion injury. Cardiovasc Res 2000;47:537–548.

[81] Fan J, Shek PN, Suntres ZE, Li YH, Oreopoulos GD, Rotstein OD. Liposomal antioxidants provide prolonged protection against acute respiratory distress syndrome. Surgery 2000; 128:332–338.

[82] Fox ES, Brower JS, Bellezzo JM, Leingang KA. *N*-acetylcysteine and alpha-tocopherol reverse the inflammatory response in activated rat Kupffer cells. J Immunol 1997;158: 5418–5423.

[83] Kheir-Eldin AA, Motawi TK, Gad MZ, Abd-ElGawad HM. Protective effect of vitamin E, beta-carotene and *N*-acetylcysteine from the brain oxidative stress induced in rats by lipopolysaccharide. Int J Biochem Cell Biol 2001;33: 475–482.

[84] Ritter C, Andrades ME, Reinke A, Menna-Barreto S, Moreira JC, Dal-Pizzol F. Treatment with *N*-acetylcysteine plus deferoxamine protects rats against oxidative stress and improves survival in sepsis. Crit Care Med 2004;32:342–349.

[85] Ritter C, da Cunha AA, Echer IC, Andrades M, Reinke A, Lucchiari N, Rocha J, Streck EL, Menna-Barreto S, Moreira JC, Dal-Pizzol F. Effects of *N*-acetylcysteine plus deferoxamine in lipopolysaccharide-induced acute lung injury in the rat. Crit Care Med 2006;34:471–477.

[86] Nayci A, Atis S, Comelekoglu U, Ozge A, Ogenler O, Coskun B, Zorludemir S. Sepsis induces early phrenic nerve neuropathy in rats. Eur Respir J 2005;26:686–692.

[87] Atis S, Nayci A, Ozge A, Comelekoglu U, Gunes S, Bagdatoglu O. N-acetylcysteine protects the rats against phrenic nerve dysfunction in sepsis. Shock 2006;25:30–35.

[88] Goode HF, Webster NR. Free radicals and antioxidants in sepsis. Crit Care Med 1993;21:1770–1776.

[89] Minko T, Stefanov A, Pozharov V. Selected contribution: Lung hypoxia: Antioxidant and antiapoptotic effects of liposomal alpha-tocopherol. J Appl Physiol 2002;93:1550–1560.

[90] Rocksen D, Ekstrand-Hammarstrom B, Johansson L, Bucht A. Vitamin E reduces transendothelial migration of neutrophils and prevents lung injury in endotoxin-induced airway inflammation. Am J Respir Cell Mol Biol 2003;28:199–207.

[91] Inci S, Ozcan OE, Kilinc K. Time-level relationship for lipid peroxidation and the protective effect of alpha-tocopherol in experimental mild and severe brain injury. Neurosurgery 1998;43:330–335.

[92] Arkovitz MS, Wispe JR, Garcia VF, Szabo C. Selective inhibition of the inducible isoform of nitric oxide synthase prevents pulmonary transvascular flux during acute endotoxemia. J Pediatr Surg 1996;31:1009–1015.

[93] Saetre T, Hoiby EA, Aspelin T, Lermark G, Egeland T, Lyberg T. Aminoethyl-isothiourea, a nitric oxide synthase inhibitor and oxygen radical scavenger, improves survival and counteracts hemodynamic deterioration in a porcine model of streptococcal shock. Crit Care Med 2000;28:2697–2706.

[94] 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;27:1343–1350.

[95] Wu F, Wilson JX, Tyml K. Ascorbate protects against impaired arteriolar constriction in sepsis by inhibiting inducible nitric oxide synthase expression. Free Radic Biol Med 2004;37: 1282–1289.

[96] Lopez A, Lorente JA, Steingrub J, Bakker J, McLuckie A, Willatts S, Brockway M, Anzueto A, Holzapfel L, Breen D, Silverman MS, Takala J, Donaldson J, Arneson C, Grove G, Grossman S, Grover R. Multiple-center, randomized, placebo-controlled, double-blind study of the nitric oxide synthase inhibitor 546C88: Effect on survival in patients with septic shock. Crit Care Med 2004;32:21–30.

[97] Hauser B, Bracht H, Matejovic M, Radermacher P, Venkatesh B. Nitric oxide synthase inhibition in sepsis? Lessons learned from large-animal studies. Anesth Analg 2005;101:488–498.