Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 508 (2005) 249–254

www.elsevier.com/locate/ejphar

Hyperbaric oxygen protects against lipopolysaccharide-stimulated oxidative stress and mortality in rats

Hui-Ching Lin[a,*], Fang-Jung Wan[b], Chin-Chen Wu[a], Che-Se Tung[c], Tzuei-Hsia Wu[b]

[a]*Department of Pharmacology, National Defense Medical Center, P.O. Box 90048-504, Taipei 114, Taipei, Taiwan*
[b]*Graduate Institute of Undersea Medicine, National Defense Medical Center, P.O. Box 90048-504, Taipei 114, Taipei, Taiwan*
[c]*Department of Physiology, National Defense Medical Center, P.O. Box 90048-504, Taipei 114, Taipei, Taiwan*

Received 6 September 2004; received in revised form 7 December 2004; accepted 10 December 2004
Available online 6 January 2005

Abstract

Free radicals and proinflammatory mediators have been implicated in the pathogenesis of endotoxic shock, a disease with high mortality caused by Gram-negative bacterial endotoxin. Hyperbaric oxygen is used as an adjuvant therapy for various inflammatory diseases and shows beneficial effects in lipopolysaccharide-induced shock syndrome. However, the underlying mechanisms for these effects are still to be defined. In this study, we investigated the effect of hyperbaric oxygen on inflammatory mediators, free radicals, and mortality in endotoxic rats. Wistar–Kyoto rats were injected with lipopolysaccharide (10 mg/kg) and then exposed to aminoguanidine, an inhibitor of inducible *nitric oxide (NO) synthase* (bolus injection 2 h after lipopolysaccharide), or hyperbaric oxygen (2 ATA for 60 min 1, 4, 9, and 24 h after lipopolysaccharide). Plasma tumor necrosis factor alpha (TNF-α), NO, and *superoxide anion* were detected and the vasorelaxation response and survival rate were assessed. The results demonstrated that increases in plasma TNF-α and NO, and the vasohyporeactivity induced by lipopolysaccharide treatment were significantly inhibited by hyperbaric oxygen and aminoguanidine. Mortality and vascular *superoxide anion* production of lipopolysaccharide treatment were also markedly reduced by hyperbaric oxygen treatment, but were not restored by aminoguanidine. None of the parameters was changed by hyperbaric oxygen treatment alone. Thus, repeated hyperbaric oxygen exposure significantly attenuated the inflammatory mediators, free radicals, and mortality in endotoxic rats.

Keywords: Nitric oxide; *Tumor necrosis factor alpha*; Aminoguanidine; Superoxide; Endotoxin

1. Introduction

Septic shock is a serious progressive failure of the circulation with a high mortality rate of 30–90% (Rangel-Frausto et al., 1995). Lipopolysaccharide evokes a septic shock-like state characterized by fever, hypotension, vasohyporeactivity to vasoactive agents, myocardial dysfunction, hypoglycemia, and multiple organ failure. It has become clear that lipopolysaccharide does not injure host tissues directly, but acts through a variety of inflammatory mediators, such as tumor necrosis factor alpha (TNF-α), interleukins, and nitric oxide (NO) (Chorinchath et al., 1996; Zhao et al., 1997). However, recent studies using antibodies to inflammatory mediators do not show reduced mortality in patients with septic shock (Fukumoto et al., 1996; Volman et al., 2002b). Notably, lipopolysaccharide also increases production of *reactive oxygen species* such as superoxide anions (Tsao et al., 2003; Victor and De La Fuente, 2003). Recently, pretreatment with hyperbaric oxygen has shown some beneficial effects on lipopolysaccharide-induced sepsis shock (Sunakawa and Yusa, 1997; Pedoto et al., 2003), but the underlying protective mechanism remains to be examined.

Hyperbaric oxygen therapy provides 100% inhaled oxygen at increased atmospheric pressure. It is used as an

* Corresponding author. Tel./fax: +886 2 87921405.
E-mail address: hclin@ndmctsgh.edu.tw (H.-C. Lin).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.12.021

adjuvant therapy for many disorders, including several types of inflammatory conditions and ischemic tissue injury (Tibbles and Edelsberg, 1996; Nighoghossian and Trouillas, 1997). Hyperbaric oxygen successfully protects mice with aerobic septicemia and significantly prolongs survival of the infected host through its antibacterial activity (Ross and McAllister, 1965). Luongo and coworkers have reported that hyperbaric oxygen significantly decreases symptoms, reduces TNF-α and NO levels, and improves survival of animals shocked by zymosan, a nonbacterial and non-endotoxic agent (Luongo et al., 1998). Recently, in vitro studies show that hyperbaric oxygen inhibits the endotoxin lipopolysaccharide-induced proinflammatory cytokines in monocyte-macrophages (Benson et al., 2003). Furthermore, the beneficial effect of hyperbaric oxygen is mediated by increased superoxide dismutase and glutathione peroxidase activities in experimental acute necrotizing pancreatitis (Yasar et al., 2003). In this study, we investigated the relevance of the reduction of TNF-α, NO, and superoxide anion formation to improvement of vasohyporeactivity and mortality after hyperbaric oxygen treatment in endotoxic rats.

2. Materials and methods

2.1. Materials

Eight- to ten-week-old male Wistar–Kyoto rats weighing 230–280 g were obtained from the National Animal Breeding and Research Center, Taipei, Taiwan. All reagents were obtained from Sigma Chemicals (St. Louis, MO) unless otherwise specified. The main reagents used include lipopolysaccharide from *Escherichia coli*, serotype 0127:B8, and aminoguanidine. Drugs and chemicals were dissolved in sterile normal saline for experimental use.

2.2. Hyperbaric oxygen treatment and experimental protocol

Animals were anaesthetized with pentobarbital sodium (50 mg/kg i.p.) and the carotid vein was cannulated with polyethylene tubing for taking blood samples and injecting drugs or saline.

After lipopolysaccharide treatment, animals were introduced into a cylindrical steel hyperbaric chamber with one compartment and thick glass windows to allow direct observation of animals during treatment (45 cm diameter, 75 cm long; constructed following Luongo et al., 1998). Rats were exposed to hyperbaric oxygen (100% oxygen; 2 atm absolute pressure, ATA) for a 60 min compression period 1, 4, 9, and 24 h after lipopolysaccharide treatment. To eliminate carbon dioxide accumulation, the chamber was flushed with 100% oxygen at a rate of 4 L/min every 30 min during compression. All experimental animal protocols designed by the following applicant has been evaluated by the Institutional Animal Care and Use Committee of National Defense Medical Center and permitted to perform in animal facility.

Rats were randomly divided into five groups (n=8–10 each) for treatment as follows: control (normal saline injection), LPS (lipopolysaccharide, 10 mg/kg i.v., bolus for 10–15 min, followed by normal saline), HBO (hyperbaric oxygen exposure at 2 ATA for 60 min), HBO-LPS (hyperbaric oxygen exposure after receiving lipopolysaccharide), and AG-LPS (aminoguanidine 30 mg/kg, i.v., bolus 2 h after receiving lipopolysaccharide). Blood samples were collected for evaluating plasma NO_x and TNF-α concentrations 2, 5, 9, 10, 24, and 25 h after lipopolysaccharide administration. The abbreviated group names correspond to the nomenclature used in Figs. 1–4.

2.3. Survival rate

Rats were injected with lipopolysaccharide (15 mg/kg, i.p.) and survival was monitored every 6 h for a 72 h experimental period. The treatment groups (n=20 each)

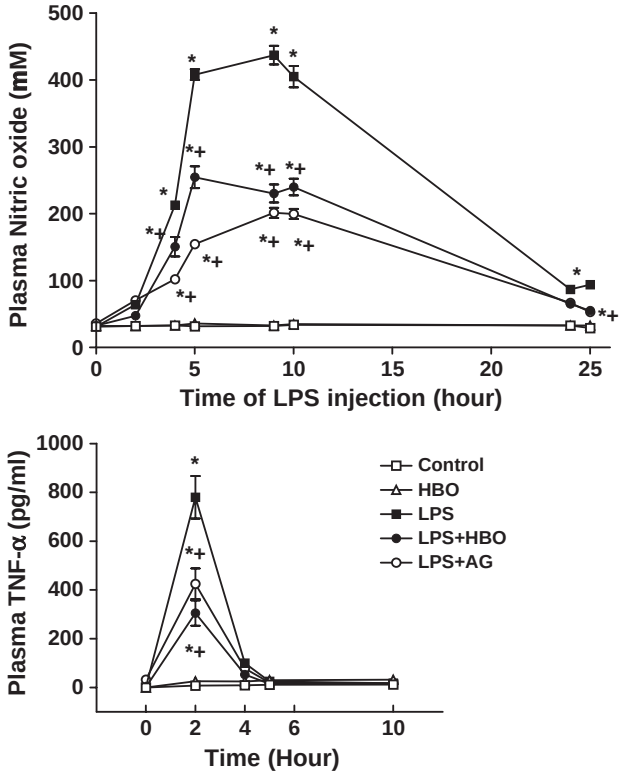

Fig. 1. Hyperbaric oxygen and aminoguanidine, an inhibitor of inducible NO synthase, reduce elevation of plasma TNF-α and NO_x produced by endotoxin. NO_x concentrations are determined by chemiluminescence analyzer and TNF-α levels are detected by enzyme-liked immunosorbent assay. Data are expressed as mean±S.E.M. (n=8 rats in each group). *P<0.05, significantly different to control; ^{+}P<0.05, significantly different to lipopolysaccharide treatment group. HBO, hyperbaric oxygen; LPS, lipopolysaccharide; AG, aminoguanidine.

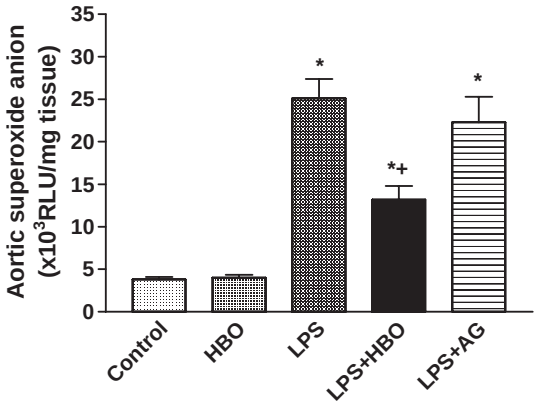

Fig. 2. Hyperbaric oxygen exposure reduces elevation of superoxide anion in aorta of rats treated with endotoxin. Aortic superoxide anions were obtained 10 h after lipopolysaccharide injection. Data from 8–10 animals are expressed as mean±S.E.M. *P<0.05, significantly different to the corresponding basal value; ^{+}P<0.05, significantly different to corresponding value in the LPS treatment group. HBO, hyperbaric oxygen; LPS, lipopolysaccharide; AG, aminoguanidine.

were LPS, HBO, HBO-LPS, and AG-LPS as described above, and the percentage of surviving rats was determined.

2.4. Measurement of plasma NO_x concentrations

Forty rats were used to determine the plasma NO_x and TNF-α concentration (n=8 each group). Plasma NO_x concentration (nitrite [NO_2^-] and nitrate [NO_3^-]), was determined with a Sievers chemiluminescence NO analyzer (280 NOA, Sievers, Boulder), as described previously (Lin et al., 1999). Briefly, plasma samples from each animal test group were obtained and deproteinized with twice the volume of ice-cold absolute ethanol. NO_x in 5 μl supernatant was reduced by VCl_3 to NO. NO was in turn reacted with O_3 to produce high-energy-state NO_2, which then emitted energy to become stable-state NO_2. The emitted energy was detected by a chemiluminescence analyzer. NO_x

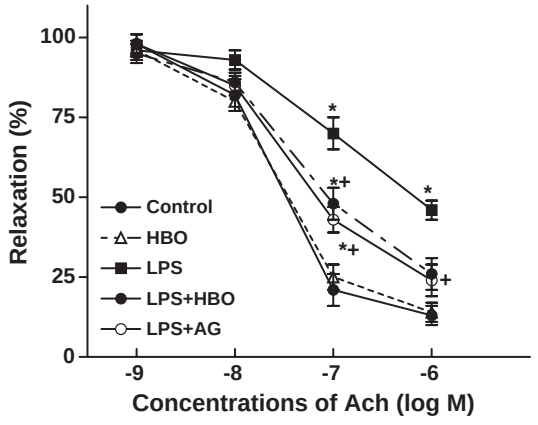

Fig. 3. Hyperbaric oxygen *partially prevents* vascular hyporeactivity induced by endotoxin. *P<0.05, significantly different to control; ^{+}P<0.05, significantly different to the lipopolysaccharide treatment group. HBO, hyperbaric oxygen; LPS, lipopolysaccharide; AG, aminoguanidine; Ach, acetylcholine.

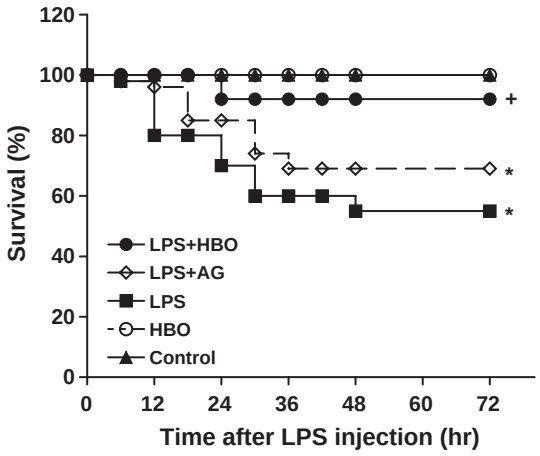

Fig. 4. Effects of hyperbaric oxygen on survival rate in rats treated with lipopolysaccharide. Depicted are changes in survival of rats injected intraperitoneally with lipopolysaccharide (15 mg/kg, n=20), lipopolysaccharide plus aminoguanidine (30 mg/kg 2 h after lipopolysaccharide injection, n=20), lipopolysaccharide plus hyperbaric oxygen (2 ATA, n=20), hyperbaric oxygen alone (n=20), or control (n=20) during the 72 h experimental period. Data are expressed as percentage of control (mean±S.E.M.) at each time point. *P<0.05, significantly different to control; ^{+}P<0.05, significantly different to lipopolysaccharide treatment group. HBO, hyperbaric oxygen; LPS, lipopolysaccharide; AG, aminoguanidine.

calibration was achieved by comparison with a standard solution of sodium nitrite.

2.5. TNF-α enzyme-linked immunosorbent assay

Plasma TNF-α levels were measured by enzyme-linked immunosorbent assay (ELISA) (Cytoscreen Immunoassay Kit, BioSource International Inc, Camarillo, CA) according to the manufacturer's instructions. Briefly, recombinant TNF-α standards from 0 to 1000 pg/ml were used. Samples and standards were pipetted into 96-well microtiter plates coated with a TNF-α-specific antibody. Samples were incubated with a secondary biotinylated antibody for 1.5 h at room temperature and then streptavidin-peroxidase was added. Absorbance of the color reaction product was measured spectrophotometrically at 450 nm.

2.6. Measurement of superoxide anion

In this experiment, forty-two rats were used (n=8–10 each group). Ten hours after lipopolysaccharide (10 mg/kg, i.v.) injection, the rat aortic rings of 4–6 mm thickness were cut and mounted under isometric conditions in modified Krebs–HEPES solution (composition in mM: NaCl, 118; HEPES, 20; KCl, 4.7; $MgSO_4$ anhydrous, 1; $CaCl_2 \cdot 2H_2O$, 1.8; glucose, 5.5; $NaHCO_3$, 25; KH_2PO_4, 1.03; pH 7.4), gassed (with 5% CO_2 and 95% O_2) then warmed to 37 °C. Stabilization was allowed for 1 h during which the solution was changed every 15 min. The generation of vascular oxidant stress was primarily in arterial vascular smooth muscle, where *superoxide anion* generation was confirmed by chemiluminescence (Microplate Luminometer LB96V,

Berthold, Germany). Briefly, stabilized vascular rings were placed in a 96-well microtiter plate containing 200 μl modified Krebs–HEPES, and 50 μl lucigenin (1.25 mM) was injected and allowed to react with released *superoxide anion* for 15 min. The amount of *superoxide anion* produced was calculated in reactive luminescence units (RLU) per milligram dry vascular ring weight using the following formula: ($RLU_{Vascular\ ring} - RLU_{background}$)/dry vascular ring weight=*superoxide anion* concentration (RLU/mg).

2.7. Vascular reactivity studies

On completion of the in vivo experiments, the rat thoracic aorta was excised and placed in cold Krebs–HEPES solution (4 °C). Fat and connective tissues were removed and the aorta was cut into rings 3–4 mm thickness. The rings were suspended in oxygenated (95% O_2+5% CO_2) Krebs–HEPES solution and connected to FT03 force transducers and a Grass Model 7 oscillographic recorder (Grass Instrument, Quincy, MA) by microfilament stainless steel hooks and silk ligatures. The rings were equilibrated for 1 h under a 2.0 g resting tension and then precontracted with 100 nM norepinephrine. The concentration-dependent vasorelaxation was then evaluated with acetylcholine (1 nM to 1 μM). Total forty rats were used in this experiment (n=8 each group).

2.8. Statistical analysis

Data were analyzed with a statistical software program (Prism, version 3) and results expressed as means±S.E.M. Statistical analysis was performed by a two-way analysis of variance (ANOVA) followed by the Bonferroni test as post hoc test. Superoxide products were analyzed by one-way ANOVA followed by the Bonferroni test. Survival data were calculated for each time period and compared by a Chi-square test. Statistical significance was accepted at $P<0.05$.

3. Results

3.1. Effect of hyperbaric oxygen and aminoguanidine treatment on TNF-α and NO_x release

Plasma TNF-α and NO_x concentrations for the five rat groups are shown in Fig. 1. Significant increases in plasma TNF-α (from 0.8±1.3 to 780±87 pg/ml; $P<0.05$) at 2 h and NO_x (from 31.2±1.3 to 435±16 μM; $P<0.05$) at 9 h were noted in lipopolysaccharide-treated rats compared with the control group. In lipopolysaccharide-treated rats, administration of aminoguanidine significantly decreased plasma TNF-α production by 54% ($P<0.05$) and NO_x by 46% ($P<0.05$), and exposure to hyperbaric oxygen significantly inhibited the increase in plasma TNF-α by 39% ($P<0.05$) and NO_x by 54% ($P<0.05$). Baseline values for TNF-α and NO_x of control rats were not significantly different from those of rats exposed to hyperbaric oxygen alone.

3.2. Effect of hyperbaric oxygen and aminoguanidine on superoxide anion production

In lipopolysaccharide-treated rats, hyperbaric oxygen significantly inhibited vascular *superoxide anion* production by 52.5% (from 25.1±2.3 to 13.2±1.6×10^3 RLU/mg, $P<0.05$; Fig. 2), although this was still higher than production in control rats ($P<0.05$). Hyperbaric oxygen treatment alone did not produce any statistical modification of basal *superoxide anion* concentration; values were 3.8±1×10^3 RLU/mg in control rats and 4.0±1.8×10^3 RLU/mg in hyperbaric oxygen-exposed rats. Aminoguanidine had no effect on vascular *superoxide anion* production either in lipopolysaccharide-treated rats (22.3±3×10^3 RLU/mg, $P>0.05$; Fig. 2) or control rats (data not shown).

3.3. Effect of hyperbaric oxygen and aminoguanidine treatment on vasorelaxation

Concentration–response curves for relaxation to acetylcholine (1 nM to 1 μM) on norepinephrine (100 nM)-contracted aortic rings in control, lipopolysaccharide-, hyperbaric oxygen plus lipopolysaccharide-, and aminoguanidine plus lipopolysaccharide-treated rats are shown in Fig. 3. Hyperbaric oxygen significantly inhibited the hypovasoreactivity of relaxation to acetylcholine in lipopolysaccharide-treated rats ($P<0.05$), but had no effect on control rats. The phenomenon resembled that found in the aminoguanidine plus lipopolysaccharide group ($P<0.05$).

3.4. Survival rate analysis

Administration of lipopolysaccharide (15 mg/kg) to rats resulted in a 55% survival rate over 72 h (11/20 animals). In contrast, hyperbaric oxygen treatment of lipopolysaccharide-treated rats yielded a survival rate of 92% ($P<0.05$ when compared with lipopolysaccharide treatment alone) and had no effect on control rats (Fig. 4). Treatment of lipopolysaccharide-treated rats with aminoguanidine did not significantly affect the survival rate compared with lipopolysaccharide treatment alone.

4. Discussion

Previous studies have shown that hyperbaric oxygen has beneficial effects on experimental acute necrotizing pancreatitis and zymosan-induced shock models (Luongo et al., 1998; Pedoto et al., 2003; Yasar et al., 2003). Our study further demonstrated that TNF-α and NO_x release, vascular superoxide anion production, hypovasoreactivity and survival rate in lipopolysaccharide-treated rats were improved by serial exposure to hyperbaric oxygen (2 ATA). The

effects of aminoguanidine, an inhibitor of inducible NOS, resembled some effects of hyperbaric oxygen, but failed to show a beneficial effect on limiting lipopolysaccharide-induced *superoxide anion* release and mortality. Consistent with this observation, Volman et al. have found that there is no alleviation of zymosan-induced multiple organ dysfunction with aminoguanidine treatment (Volman et al., 2002a). In fact, aminoguanidine and other nonselective inhibitors exacerbate lipopolysaccharide-induced liver damage and show no effect on mortality (Farghali et al., 2003).

In this study, we found that hyperbaric oxygen reduced overproduction of vascular *superoxide anion* by lipopolysaccharide, but had no effect on control animals. *Reactive oxygen species* are scavenged by glutathione peroxidase, superoxide dismutase and catalase. In support of our data, Yasar et al. (2003) have demonstrated the beneficial effect of hyperbaric oxygen on oxidative stress by increasing the levels of glutathione peroxidase and Cu/Zn-superoxide dismutase activity in experimental acute necrotizing pancreatitis. Others have also shown that hyperbaric oxygen treatment increases Mn-superoxide dismutase activity (Gregorevic et al., 2001) and attenuates glutathione depletion in postischemic muscle (Haapaniemi et al., 1995). In addition, hyperbaric oxygen exposure of lymphocytes increases cellular ferritin levels, suggesting that hyperbaric oxygen may also prevent generation of hydroxyl radicals through the Fenton reaction pathway (Rothfuss and Speit, 2002).

Our data contrast to those from studies demonstrating that repeated exposure to hyperbaric oxygen leads to significant accumulation of plasmatic *reactive oxygen species* and decreases erythrocyte superoxide dismutase and catalase activity (Benedetti et al., 2004). The reason for this discrepancy is not fully understood. Formation of ROS has been proposed as one mechanism by which hyperbaric oxygen produces its toxicity (Jerrett et al., 1973); indeed, cytotoxicity can be produced when a single hyperbaric oxygen exposure beyond 3 h or higher than 3 ATA is applied (Juttner et al., 2003; Benson et al., 2003). Nevertheless, human studies provide evidence that the toxic effects are not elicited by single or repetitive hyperbaric oxygen exposure of 2.5 ATA and 90 min compression (Juttner et al., 2003). Our data showed that hyperbaric oxygen at 2 ATA with 60 min compression did not induce significant production of superoxide and inflammatory mediators in nonstimulated control animals. In contrast, the data showed that hyperbaric oxygen treatment attenuated the superoxide generation by vascular ring in our experimental sepsis model.

Investigators have found that hyperbaric oxygen suppresses the lipopolysaccharide-, lipid A-, and PHA-induced interleukin-1β and TNF-α production (Benson et al., 2003). It is suggested that mortality in the zymosan-induced shock model could be due to secretion of endogenous TNF-α. In contrast to this point of view, others suggest that *reactive oxygen species* are the determining mediator of the animal's mortality. Thus, several studies provide evidence that levels of TNF-α are not significantly different between lethal and nonlethal shock animals. Nevertheless, *reactive oxygen species* production, and superoxide in particular, is more closely related to mortality in lipopolysaccharide-treated animals (Victor et al., 1999; Victor and De la Fuente, 2000, 2003). Moreover, TNF-α pretreatment did not increase mortality induced by lipopolysaccharide (Murphey and Traber, 2000). Recent studies further demonstrated that propofol, an antioxidant and anti-inflammatory agent, promotes survival of animals with endotoxic shock by decreasing superoxide anion production, and not through its effects on TNF-α and NO (Taniguchi et al., 2000, 2002; Tsao et al., 2003). Our current data show that, although aminoguanidine reduced plasma levels of TNF-α and NO levels, it could not *prevent superoxide anion* production and mortality, thus implicating *superoxide anion* in the mortality of lipopolysaccharide-shocked animals.

In summary, we have shown that repeated exposure to hyperbaric oxygen is beneficial in rats challenged with endotoxin. Although several protective phenomena have been observed, our study provides novel evidence that hyperbaric oxygen decreases superoxide anion production and reduces mortality in a lipopolysaccharide-induced shock model. Although the role of superoxide production in mortality induced by endotoxic shock warrants further investigation, hyperbaric oxygen exposure appears to offer an alternative tool in the treatment of septic shock.

Acknowledgments

This work was supported by NSC 91-2314-B-016-036 Grants from National Science Council and C.Y. Foundation for Advancement of Education, Sciences and Medicine, Taiwan.

References

Benedetti, S., Lamorgese, A., Piersantelli, M., Pagliarani, S., Benvenuti, F., Canestrari, F., 2004. Oxidative stress and antioxidant status in patients undergoing prolonged exposure to hyperbaric oxygen. Clin. Biochem. 37, 312–317.

Benson, R.M., Minter, L.M., Osborne, B.A., Granowitz, E.V., 2003. Hyperbaric oxygen inhibits stimulus-induced proinflammatory cytokine synthesis by human blood-derived monocyte-macrophages. Clin. Exp. Immunol. 134, 57–62.

Chorinchath, B.B., Kong, L.Y., Mao, L., McCallum, R.E., 1996. Age-associated differences in TNF-alpha and nitric oxide production in endotoxic mice. J. Immunol. 156, 1525–1530.

Farghali, H., Canova, N., Kucera, T., Martinek, J., Masek, K., 2003. Nitric oxide synthase inhibitors modulate lipopolysaccharide-induced hepatocyte injury: dissociation between in vivo and in vitro effects. Intern. Immunopharmacol. 3, 1627–1638.

Fukumoto, T., Matsukawa, A., Ohkawara, S., Takagi, K., Yoshinaga, M., 1996. Administration of neutralizing antibody against rabbit IL-1

receptor antagonist exacerbates lipopolysaccharide-induced arthritis in rabbits. Inflamm. Res. 45, 479–485.

Gregorevic, P., Lynch, G.S., Williams, D.A., 2001. Hyperbaric oxygen modulates antioxidant enzyme activity in rat skeletal muscles. Eur. J. Appl. Physiol. 86, 24–27.

Haapaniemi, T., Sirsjo, A., Nylander, G., Larsson, J., 1995. Hyperbaric oxygen treatment attenuates glutathione depletion and improves metabolic restitution in postischemic skeletal muscle. Free Radic. Res. 23, 91–101.

Jerrett, S.A., Jefferson, D., Mengel, C.E., 1973. Seizures, H_2O_2 formation and lipid peroxides in brain during exposure to oxygen under high pressure. Aerosp. Med. 44, 40–44.

Juttner, B., Scheinichen, D., Bartsch, S., Heine, J., Ruschulte, H., Elsner, H.A., Franko, W., Jaeger, K., 2003. Lack of toxic side effects in neutrophils following hyperbaric oxygen. Undersea Hyperb. Med. 30, 305–311.

Lin, H.C., Wan, F.J., Kang, B.H., Wu, C.C., Tseng, C.J., 1999. Systemic administration of lipopolysaccharide induces release of nitric oxide and glutamate and c-fos expression in the nucleus tractus solitarii of rats. Hypertension 33, 1218–1224.

Luongo, C., Imperatore, F., Cuzzocrea, S., Filippelli, A., Scafuro, M.A., Mangoni, G., Portolano, F., Rossi, F., 1998. Effects of hyperbaric oxygen exposure on a zymosan-induced shock model. Crit. Care Med. 26, 1972–1976.

Murphey, E.D., Traber, D.L., 2000. Pretreatment with tumor necrosis factor-alpha attenuates arterial hypotension and mortality induced by endotoxin in pigs. Crit. Care Med. 28, 2015–2021.

Nighoghossian, N., Trouillas, P., 1997. Hyperbaric oxygen in the treatment of acute ischemic stroke: an unsettled issue. J. Neurol. Sci. 150, 27–31.

Pedoto, A., Nandi, J., Yang, Z.J., Wang, J., Bosco, G., Oler, A., Hakim, T.S., Camporesi, E.M., 2003. Beneficial effect of hyperbaric oxygen pretreatment on lipopolysaccharide-induced shock in rats. Clin. Exp. Pharmacol. Physiol. 30, 482–488.

Rangel-Frausto, M.S., Pittet, D., Costigan, M., Hwang, T., Davis, C.S., Wenzel, R.P., 1995. The natural history of the systemic inflammatory response syndrome (SIRS). A prospective study. JAMA 273, 117–123.

Ross, R.M., McAllister, T.A., 1965. Protective action of hyperbaric oxygen in mice with pneumococcal septicaemia. Lancet 1, 579–581.

Rothfuss, A., Speit, G., 2002. Overexpression of heme oxygenase-1 (HO-1) in V79 cells results in increased resistance to hyperbaric oxygen (HBO)-induced DNA damage. Environ. Mol. Mutagen. 40, 258–265.

Sunakawa, M., Yusa, T., 1997. The effect of hyperbaric oxygenation on nitrite and nitrate production in rats treated with endotoxin. J. Anesth. 46, 1447–1453.

Taniguchi, T., Yamamoto, K., Ohmoto, N., Ohta, K., Kobayashi, T., 2000. Effects of propofol on hemodynamic and inflammatory responses to endotoxemia in rats. Crit. Care Med. 28, 1101–1106.

Taniguchi, T., Kanakura, H., Yamamoto, K., 2002. Effects of posttreatment with propofol on mortality and cytokine responses to endotoxin-induced shock in rats. Crit. Care Med. 30, 904–907.

Tibbles, P.M., Edelsberg, J.S., 1996. Hyperbaric-oxygen therapy. N. Engl. J. Med. 334, 1642–1648.

Tsao, C.M., Ho, S.T., Chen, A., Wang, J.J., Tsai, S.K., Wu, C.C., 2003. Propofol ameliorates liver dysfunction and inhibits aortic superoxide level in conscious rats with endotoxic shock. Eur. J. Pharmacol. 477, 183–193.

Victor, V.M., De la Fuente, M., 2000. Comparative study of peritoneal macrophage functions in mice receiving lethal and non-lethal doses of LPS. J. Endotoxin Res. 6, 235–241.

Victor, V.M., De La Fuente, M., 2003. Changes in the superoxide production and other macrophage functions could be related to the mortality of mice with endotoxin-induced oxidative stress. Physiol. Res. 52, 101–110.

Victor, V.M., Guayerbas, N., Garrote, D., Del Rio, M., De la Fuente, M., 1999. Modulation of murine macrophage function by *N*-acetylcysteine in a model of endotoxic shock. BioFactors 10, 347–357.

Volman, T.J., Goris, R.J., Van Der Jagt, M., Van De Loo, F.A., Hendriks, T., 2002a. Organ damage in zymosan-induced multiple organ dysfunction syndrome in mice is not mediated by inducible nitric oxide synthase. Crit. Care Med. 30, 1553–1559.

Volman, T.J., Hendriks, T., Verhofstad, A.A., Kullberg, B.J., Goris, R.J., 2002b. Improved survival of TNF-deficient mice during the zymosan-induced multiple organ dysfunction syndrome. Shock 17, 468–472.

Yasar, M., Yildiz, S., Mas, R., Dundar, K., Yildirim, A., Korkmaz, A., Akay, C., Kaymakcioglu, N., Ozisik, T., Sen, D., 2003. The effect of hyperbaric oxygen treatment on oxidative stress in experimental acute necrotizing pancreatitis. Physiol. Res. 52, 111–116.

Zhao, K., Kirman, I., Tschepen, I., Schwab, R., Weksler, M.E., 1997. Peritoneal lavage reduces lipopolysaccharide-induced elevation of serum TNF-alpha and IL-6 mortality in mice. Inflammation 21, 379–390.