Eur J Appl Physiol (2001) 85: 96–103
DOI 10.1007/s004210100391

ORIGINAL ARTICLE

Zhong-jin Yang · Gerardo Bosco · Anthony Montante
Xiao-lan Ou · Enrico M. Camporesi

Hyperbaric O_2 reduces intestinal ischemia-reperfusion-induced TNF-α production and lung neutrophil sequestration

Accepted: 8 January 2001 / Published online: 21 March 2001

Abstract Treatment with hyperbaric O_2 (HBO) ameliorates ischemia-reperfusion (I/R) injury. Since tumor necrosis factor-α (TNF-α) plays an important role in I/R injury, we hypothesized that the effect of HBO in I/R injury may be due to its ability to inhibit TNF-α production. In this study, one group of rats received HBO during 60 min of ischemia (HBO group, $n=9$), while control rats endured the same procedure but did not receive HBO (non-HBO, $n=9$). A group of sham-operated control rats (SHAM, $n=6$) underwent laparotomy without occlusion of the artery and HBO treatment. Intestinal I/R led to an increase in serum TNF-α concentration to [mean (SEM)] 165 (32) pg/ml ($P<0.01$ vs SHAM rats). HBO attenuated this increase [34 (9) pg/ml; $P<0.05$ vs non-HBO group]. Intestinal I/R also resulted in a marked increase in lung myeloperoxidase content [0.62 (0.04) U/g vs 0.17 (0.02) U/g of SHAM rats, $P<0.01$]. HBO suppressed this increase [0.40 (0.04) U/g, $P<0.05$ vs non-HBO rats]. HBO ameliorated the injury to the intestine and lung. The number of neutrophils sequestered in the lung was reduced in HBO rats compared to non-HBO rats [6.4 (0.9) neutrophils/per oil field and 10.9 (2) neutrophils/per oil field, respectively; $P<0.05$]. These findings demonstrate that HBO inhibits TNF-α production during intestinal I/R, and this reduced TNF-α production may be attributed to the beneficial effects of HBO.

Keywords Hyperbaric O_2 · Ischemia-reperfusion · TNF-α · Lung injury · Neutrophils

Z. Yang (✉) · A. Montante · E.M. Camporesi
Cardiopulmonary Research Laboratory,
Department of Anesthesiology,
New York Upstate Medical Center,
State University of New York, Syracuse, NY 13210, USA
E-mail: yangz@upstate.edu
Tel.: +1-315-4649932
Fax: +1-315-4649930

G. Bosco
Department of Biomedical Sciences,
Human Physiology Laboratory, School of Medicine,
University of Chieti, Italy

X. Ou
Department of Pathology, University Hospital,
Upstate Medical University, State University of New York,
Syracuse, NY 13210, USA

Introduction

Hyperbaric O_2 (HBO) is a therapeutic modality that has beneficial effects on selected clinical disorders, such as decompression sickness, carbon monoxide poisoning, smoke inhalation, gas gangrene, and refractory osteomyelitis (Hampson 1999). HBO enhances restoration of the circulation and function of injured limbs (Marucha et al. 1991), it improves healing of skin grafts and flaps (Zamboni et al. 1993a), promotes functional recovery of injured peripheral nerves (Zao 1991) and limbs (Bouachour et al. 1996), and ameliorates lymphadenopathy, hypergammaglobulinemia, butterfly rash and protenuria of patients with systemic lupus erythematosus (Inamoto et al. 1991). In animal studies, it has been shown that HBO reduces post-ischemic edema (Nylander et al. 1985), stimulates aerobic metabolism to preserve energy-rich compounds (Nylander et al. 1987; Yamada et al. 1994), reduces venular neutrophil adherence and inhibits progressive adjacent arteriolar vasoconstriction of ischemic muscle (Zamboni et al. 1993b), accelerates the recovery of crush injured peripheral nerves (Bradshaw et al. 1996), rescues fibers from ischemic degeneration (Kihara et al. 1995), and salvages testes from torsion (Kolski et al. 1998). HBO reduces lung neutrophil sequestration in rats with intestinal ischemia-reperfusion injury (Tjarnstrom et al. 1999), and suppress inflammatory processes (Inamoto et al. 1991).

Reperfusion of an ischemic tissue or organ, such as in acute pancreatitis (Guice et al. 1989), sepsis (Brigham et al. 1979) and intestinal ischemia-reperfusion injury (Caty et al. 1989), may cause acute lung injury. Intestinal ischemia-reperfusion injury is accompanied by a high complication rate, which is characterized by adult

respiratory distress syndrome, hypotension and renal failure (Wilson et al. 1987). The fact that restoration of blood flow can save the intestine, but results in multi-organ dysfunction and death (Klass 1953) has led to extensive clinical and experimental studies. The involvement of neutrophil in intestinal ischemia-reperfusion-induced lung injury is important (Caty et al. 1989; Schmeling et al. 1989; Tjarnstrom et al. 1999). Neutrophils are activated during intestinal ischemia, and their adhesiveness and rigidity increase. During reperfusion, activated neutrophils are trapped within the lung and adhere to endothelial cells, generating oxidants and proteases (Turnage et al. 1994). Many mediators induce neutrophil activation, including tumor necrosis factor-α (TNF-α). The involvement of TNF-α in ischemia-reperfusion injury is evidenced by the facts that (1) the production of TNF-α increases in intestinal ischemia-reperfusion injury (Caty et al. 1989; Sorkine et al. 1995), and (2) the blockade of TNF-α activity reduces intestinal ischemia-reperfusion-induced lung injury (Sorkine et al. 1995). Strategies for minimizing ischemic damage, neutrophil activation, sequestration, and inflammation process have been sought as treatments to prevent injuries after post-ischemic reperfusion and host-defense responses (Nighoghossian and Trouillas 1997).

HBO has a beneficial effect on ischemia-reperfusion injury, and a suppressive effect on immune function. Based on this, we hypothesized that the suppressing effect of HBO on TNF-α production contributes to a reduced ischemia-reperfusion-induced acute lung injury. This experiment was designed to investigate the impact of HBO on serum TNF-α production and lung neutrophil sequestration during intestinal ischemia-reperfusion in the rat.

Methods

Animal model

Pathogen-free male Sprague-Dawley rats weighing between 280 and 300 g (Taconic Farm, Germantown, N.Y., USA) were used. All rats were kept in wire mesh cages for 1 week to acclimate them to the study environmental conditions: 12 h dark and 12 h light cycle (light between 6 a.m. and 6 p.m.); room temperature 26 ± 1°C; 45% relative humidity. Rat chow (Diet no. 5008, Ralston Purina, St. Louis, Mo., USA) and tap water were provided ad libitum. The proposed study was approved by the State University of New York, Upstate Medical University, Syracuse Committee for the Humane Use of Animals, and was in accordance with the guidelines established by the National Institutes of Health. All surgical procedures were performed with the rats under general anesthesia, which was induced by intramuscular injection of a mixture of ketamine and xylazine (150:30 mg/ml) at a dose of 0.6 ml/kg.

Surgical procedures

A midline laparotomy was performed. The superior mesenteric artery was identified and occluded with a Schwarz-microvascular clip. The ischemia status was confirmed by disappearance of the distal pulse. The laparotomy incision was closed with 3-O silk running suture, and the skin was closed with surgical clips. The animals from one group of rats were placed into an HBO chamber to receive 60 min of treatment with HBO during the intestinal ischemia period (HBO group, $n = 9$). The rats were then removed from the HBO chamber and the laparotomy incision was reopened and the clip was removed. Reperfusion was confirmed by the return of a pulse to the mesenteric vascular arcade. The incision was again closed and the rat was subjected to reperfusion for 30 min. Control rats (non-HBO group, $n = 9$) underwent an identical procedure, except that they were not treated with HBO. Sham-operated rats (SHAM group, $n = 6$) underwent the laparotomy procedure without the occlusion of the superior mesenteric artery and HBO treatment.

HBO treatment

HBO was accomplished by placing the rats individually into a cylindrical pressure chamber (Sechrist Model 1300B, Sechrist Industries, Anaheim, Calif., USA). Each treatment consisted of 100% of oxygen for 60 min during the ischemia period at 2.8 atm pressure (where 1 atm = 101.3 kN/m^2).

TNF-α assay

A blood sample was obtained from the right ventricle and allowed to clot for 2 h at room temperature, then centrifuged at 2,000 *g* for 20 min. The serum samples were stored at –20°C until assay. TNF-α assays were performed according to the method described by Pizarro et al. (1993) using the quantitative sandwich enzyme immunoassay technique (Quantikine M, R&D Systems, Minneapolis, Minn., USA). Briefly, the serum sample was diluted at 1:2 with Calibrator Diluent RD5-17 and added to a 96-well enzyme-linked immunosorbent assay plate coated with a monoclonal antibody specific for rat TNF-α. The plate was washed and incubated sequentially with an enzyme-linked polyclonal antibody specific for rat TNF-α. The enzyme reaction yielded a blue product that turned yellow when Stop Solution was added. The result was measured within 30 min using a microplate spectrophotometer set to 450 nm (SpectraMax 250, Molecular Devices, Sunnyvale, Calif., USA). The minimum detectable dose of rat TNF-α was 12.5 pg/ml.

Myeloperoxidase assay

Myeloperoxidase (MPO), an enzyme found primarily within neutrophils, is a sensitive marker for quantifying neutrophil content in tissue. Assaying MPO in the lung is a quantitative method of detecting neutrophil sequestration.

After the blood sample was withdrawn, the left lung was resected, the surface washed free of blood, blotted dry and immediately frozen in liquid nitrogen and stored at –20°C until assay. The thawed sample was weighed and then homogenized in 1 g tissue/5 ml of 50 mM KH_2PO_4 and 5 mM ethylenediaminetetraacetic acid (pH 7.4) using the Virtis model 45 homogenizer. The homogenate was centrifuged at 10,000 *g* at 4°C for 20 min, the supernatant decanted, and the pellet resuspended in 3 ml of 50 mM KH_2PO_4 with 0.5% hexadecyltriethyl-ammonium bromide at pH 6.0. The resulting suspension was frozen at –70°C overnight. The pellet was then rehomogenized and sonicated (Fischer Model 550 Sonic Dismembrator, Fischer Scientific, Pittsburgh, Pa., USA) for 5 min and centrifuged at 10,000 *g* for 20 min. The final supernatant was collected for MPO assay.

The assay mixture contained 0.2 ml of 0.4 M KH_2PO_4, 0.1 ml of 16 mM 3,3,5,5, tetramethylbenzidine in *N*,*N*,dimethylformamide, and 0.5 ml of 150 mM KH_2PO_4 in 154 mM saline. Three minutes after the addition of 0.1 ml of 3 mM H_2O_2, the reaction was stopped with 4 ml of 0.2 mM sodium acetate at pH 3.0. Absorbance was measured spectrophotometrically at 655 nm (Spectronic Genesys 5, Spectronic Instruments, Rochester, N.Y., USA) and compared with a standard curve generated from human MPO

(Sigma) to determine the units of MPO per gram of tissue (U/g, wet weight).

Morphological analysis

Intestinal injury

A specimen of the small intestine was taken at an equidistant point along its length. The specimens were immediately fixed in 10% formaldehyde-saline solution. The fixed tissue was embedded in paraffin, sectioned, and stained with hematoxylin and eosin by a pathologist (X.O.). The injury was evaluated according to a mucosal injury score, as defined by Chiu et al. (1970), in a blind fashion by a pathologist. The mucosal damage was evaluated as:

1. Grade 0: normal mucosal villi.
2. Grade 1: development of a subepithelial space, usually at the tip of the villus, with capillary congestion.
3. Grade 2: extension of the subepithelial space with moderate lifting of the epithelial layer.
4. Grade 3: massive epithelial lifting down the sides of villi.
5. Grade 4: denuded villi with lamina propria and dilated capillaries exposed. Increased cellularity of the lamina propria.
6. Grade 5: digestion and disintegration of the lamina propria, hemorrhage, and ulceration.

For easy comparison, damage deserving of a grade greater than or including 4 was defined as severe damage.

Lung neutrophil sequestration

The right lung was removed and immersion-fixed in formalin. Fixed specimens were paraffin-embedded, sectioned and stained with hematoxylin-eosin for light microscopic analysis. All microscope sections were read in a blind fashion by a pathologist. Lung neutrophil sequestration was quantitated by counting alveolar septal wall neutrophils. Microscope fields containing other structures such as airways and larger vessels were excluded. Neutrophil sequestration is expressed as the mean number of neutrophils per eight oil fields (OF; magnification ×100).

Statistical analysis

The results are presented as the mean (SEM). Statistical analysis was performed using analysis of variance for repeated measures and Bonferoni test for post-hoc paired comparisons. The level of statistical significance was set at $P < 0.05$. Morphological analysis between HBO and non-HBO rats was performed with a paired Student's *t*-test.

Results

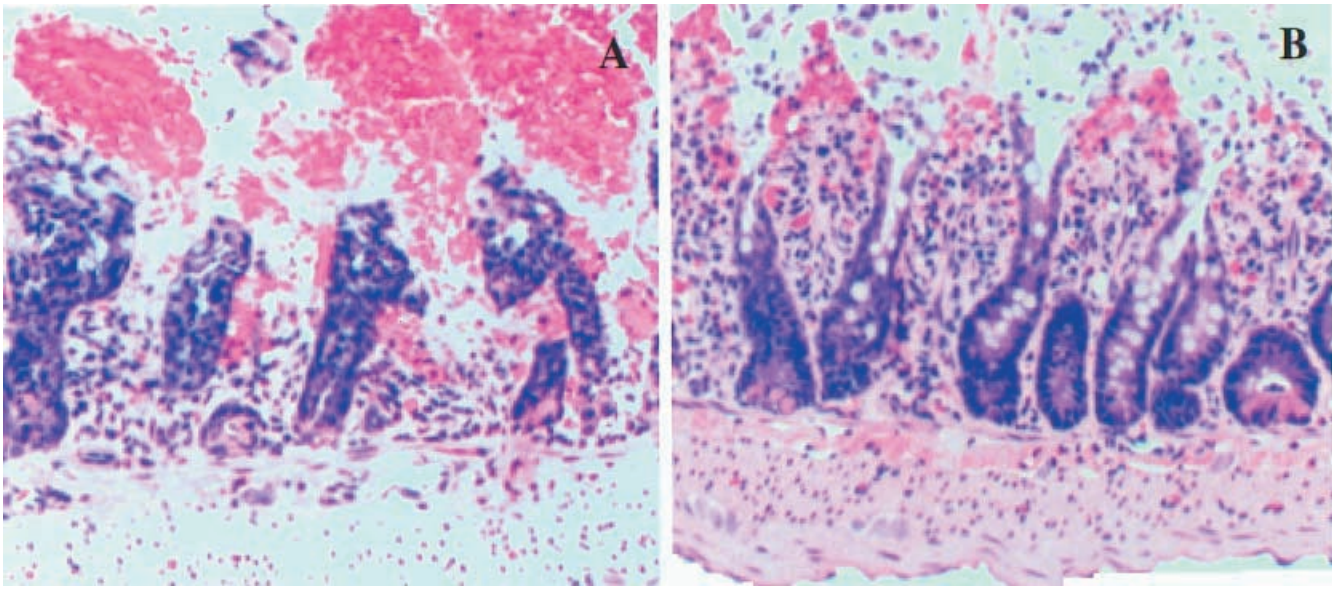
Effect of HBO on intestinal ischemia-reperfusion injury

A 60-min period of intestinal ischemia followed by 30 min reperfusion resulted in a severe damage rate of 84 (7)%, and treatment with HBO reduced this rate to 60 (11)% (Fig. 1).

Effect of HBO on lung neutrophil sequestration during intestinal ischemia-reperfusion

A 60-min period of intestinal ischemia and 30 min reperfusion resulted in a marked increase in lung MPO content to 0.62 (0.04) U/g, compared to 0.17 (0.02) U/g in the SHAM group ($P < 0.01$, Fig. 2). During the period of ischemia, HBO attenuated the increase in lung MPO content [0.40 (0.04) U/g compared to the non-HBO group]. Neutrophil sequestration in the lung following intestinal ischemia-reperfusion is evidenced by morphological examination (Fig. 3). The number of neutrophils in HBO rats was lower than in non-HBO rats [5.1 (0.2) neutrophils/OF and 8.7 (1.6) neutrophils/OF, respectively].

Fig. 1A, B Morphologic examination shows the protective effect of hyperbaric O_2 (*HBO*) on the small intestine. **A** Severe hemorrhage of the villi of the small intestine villi in rats that were not treated with HBO (*non-HBO*). **B** Microvilli in the villi of the small intestine of HBO rats, exhibiting a much lesser degree of hemorrhage than those in **B**



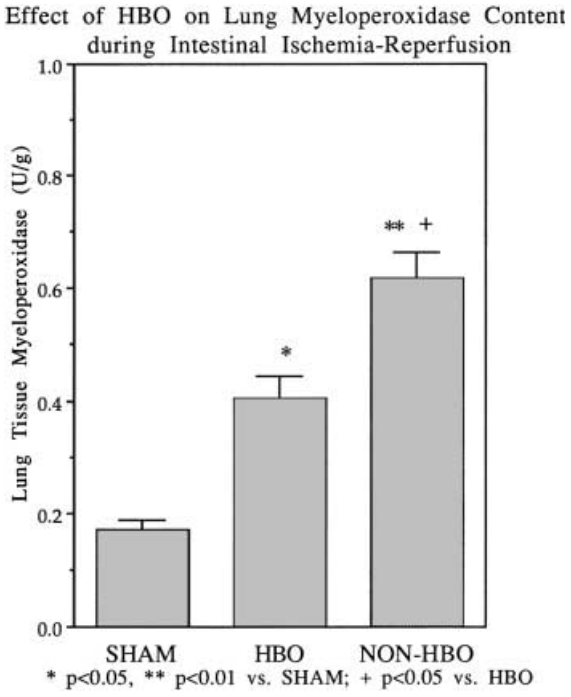


Fig. 2 Lung tissue myeloperoxidase content expressed as U/g wet tissue in rats subjected to intestinal ischemia-reperfusion with (HBO, $n=9$) or without HBO treatment (non-HBO, $n=9$). Control rats underwent sham operation without HBO treatment (*SHAM*, $n=6$). Lung tissue myeloperoxidase content increased significantly in HBO and non-HBO rats compared with SHAM rats, being [mean (SEM)] 0.40 (0.04) U/g ($P<0.05$), 0.619 (0.044) U/g ($P<0.01$) and 0.17 (0.02) U/g, respectively. HBO treatment significantly alleviated the increase in lung myeloperoxidase content ($P<0.05$)

Effect of HBO on serum TNF-α concentration during intestinal ischemia reperfusion

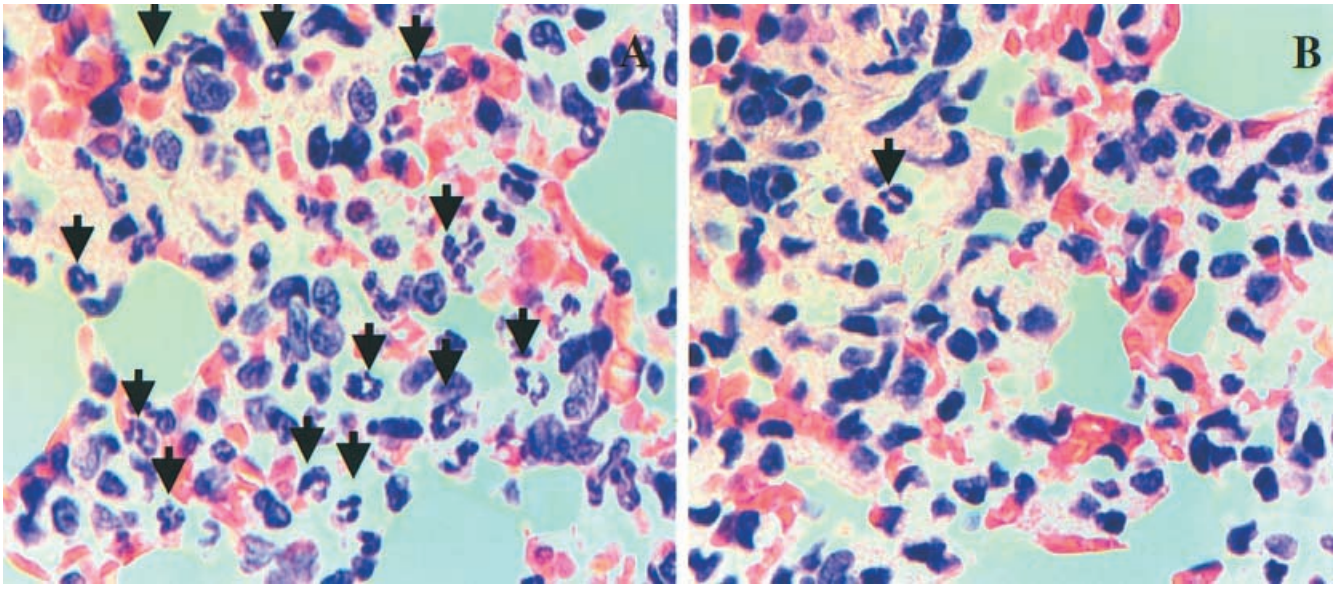
Serum TNF-α concentration was undetectable in all but one of the sham-operated rats (Fig. 4). Intestinal ischemia and subsequent reperfusion resulted in an increase in serum TNF-α concentration [to 165 (32) pg/ml] compared to the SHAM group. During the period of ischemia, HBO attenuated the increase in serum TNF-α [to 34 (9) pg/ml] compared to non-HBO rats.

Discussion

The main findings of this study are that: (1) 60 min of intestinal ischemia and 30 min reperfusion resulted in intestine and lung injury, (2) the serum TNF-α concentration was elevated in intestinal ischemia-reperfusion injury, (3) HBO attenuated TNF-α production, and (4) HBO alleviated ischemia-perfusion-induced intestine and lung injury. These data demonstrate a connection between HBO treatment and alleviated intestine injury, decreased TNF-α production, and reduced lung injury.

The duration of ischemia in the present study was determined according to Schmeling et al. (1989), whereby it can be predicted that once ischemia persists beyond 60 min, intestinal injury and acute lung injury will occur. The length of the reperfusion period was chosen based on observations that the serum TNF-α concentration reach their highest level during the first 30 min of reperfusion after either 60 min (Sorkine et al. 1995) or 120 min of intestinal ischemia (Caty et al. 1989). Our data are in agreement with these reports. The occurrence of maximal TNF-α elevation after a short period of reperfusion is probably due to: (1) the time needed for activated inflammatory cells to produce and

Fig. 3A, B Morphological examination revealed the protective effect of HBO on neutrophil infiltration of the alveolar septa of the lung. **A** Small-intestinal ischemia-reperfusion resulted in a marked increase in neutrophil infiltration in the alveolar septa of the lung. **B** HBO significantly reduced the number of neutrophils present in the alveolar septa. The *dark arrow* indicates the infiltrated neutrophils



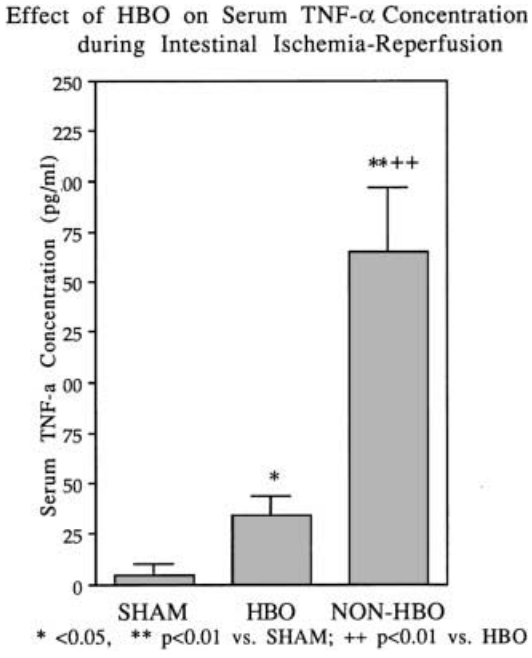


Fig. 4 Serum tumor necrosis factor-α (*TNF-α*) concentration expressed as pg/ml in rats subjected to intestinal ischemia-reperfusion with (HBO, $n=9$) or without HBO treatment (non-HBO, $n=9$). Sham control rats (SHAM, $n=6$) underwent sham operation without HBO treatment. Serum TNF-α concentration was almost undetectable in SHAM rats. Serum TNF-α concentration was significantly increased in HBO and non-HBO rats compared with SHAM rats, being 34 (9.4) pg/ml ($P<0.05$), 165 (32) pg/ml ($P<0.01$) and 5 (5) pg/ml, respectively. HBO treatment significantly reduced the increase in serum TNF-α concentration ($P<0.01$)

release TNF-α (Remick et al. 1989), (2) augmentation of the release of TNF-α oxygen free radicals that are introduced by the reperfusion, (3) increased TNF-α production from activated neutrophils and other inflammatory cells sequestered in the liver and the lung (Simpson et al. 1993), and (4) a simple "washout" from the damaged intestine during the initiation of reperfusion. Clearly, the damaged intestine is most responsible for the induction of TNF-α. HBO decreases TNF production during massive hemorrhage, and this is thought to be attributable to improved oxygenation (Yamashita and Yamashita 2000). In our study, HBO attenuated local ischemic damage, thus contributing to a reduction in TNF-α production.

Ischemia-reperfusion-induced acute lung injury is characterized by basement membrane disruption, interstitial edema and neutrophil sequestration. In our study, 60 min of ischemia followed by 30 min of reperfusion led to neutrophil sequestration of the lung, suggesting an early stage of lung injury. Lung injury occurs primarily during the reperfusion period (Schmeling et al. 1989), suggesting that during that time, activated mediators that target the pulmonary vascular bed are released into the circulation. Numerous mediators are thought to be involved, including neutrophils (Simpson et al. 1993; Vedder et al. 1989). In the present study, increased lung MPO content corresponded with lung neutrophil infiltration, supporting the involvement of neutrophils in mediating intestinal ischemia-reperfusion-induced lung injury. The activated neutrophils sequestrate in the lung and may mediate lung injury by (1) plugging capillaries, (2) interacting with endothelial cells, (3) generating oxygen-derived free radicals, (4) releasing proteases, and (5) producing leukotriene and cytokines (Schmeling et al. 1989; Sorkine et al. 1995). The reduction or prevention of neutrophil activation and sequestration would reduce the incidence of ischemia-reperfusion-induced lung injury. This hypothesis has been supported by studies in which it has been shown that the depletion of neutrophils ameliorates ischemia-reperfusion-induce lung injury (Klausner et al. 1988). The beneficial effects of HBO may be attributable to its ability to reduce neutrophil activation and sequestration in ischemia-reperfusion injury. HBO may achieve its beneficial effects by (1) preserving high-energy compounds (Yamada et al. 1994), (2) limiting hypoxic-ischemic-induced lipid peroxidation (Thom 1990), (3) reducing leukocyte adherence (Haapaniemi et al. 1991), (4) decreasing sequestration in reperfused tissue (Zamboni et al. 1996), and (5) modifying the inflammatory process (Slotman 1998). The effect of HBO on ischemia-reperfusion-induced lung injury has been examined. In one animal study (Nahum et al. 1991), HBO was observed to reduce pedicle arterial neutrophil concentration in the blood flow to the gracillis muscle flap, but HBO did not change lung neutrophil sequestration. As pointed out by Tjarnstrom et al. (1999), the ischemic tissue volume in this model was small and, consequently, the degree of lung neutrophil sequestration was minor. In an intestinal ischemia-reperfusion model, the rats underwent 90 min of reperfusion after 2 h of ischemia. HBO was administered during the reperfusion period. A significant reduction in lung neutrophil sequestration was observed (Tjarnstrom et al. 1999). The recruitment of lung neutrophils started during the period of ischemia and continued during reperfusion for a prolonged period of time (Chiu et al. 1970; Kihara et al. 1995). Intestinal ischemia-reperfusion induces a systemic neutrophil activation (Nahum et al. 1991; Windsor et al. 1993). The beneficial effect of HBO on lung neutrophil sequestration may be achieved when it is administered during either the period of ischemia or the reperfusion. HBO may be able to inhibit the activation of neutrophils and to dislodge the sequestrated neutrophils (reduce adhesion). This is supported by recent studies demonstrating that HBO reduces neutrophil activation and deregulates intercellular adhesion molecule-1 (Buras et al. 2000; Cuzzocrea et al. 2000). Other mediators, for example reduced TNF-α may also be responsible.

Virtually all intestinal injury is sustained during periods of ischemia; the incidence of reperfusion injury appears to be almost negligible (Wilson et al. 1987). However, damage to the distant organs, such as the lung, is progressive. Therefore, the attenuation of either localized ischemic tissue injury or distant-organ injury

may reduce ischemia-reperfusion injury. HBO ameliorates local ischemic tissue injury and suppress the inflammatory process. Yamada et al. (1994) found that treatment with HBO during intestinal ischemia resulted in an improvement in the survival rate and energy charge of the intestine before reperfusion and intestinal mucosal injury, and longitudinal muscle degeneration was ameliorated. Treatment with HBO after reperfusion had none of these effects. Rossman et al. (1997) reported that perfusing the lumen of the intestine with oxygenated perfluorocarbon during a period of ischemia reduced mucosal injury and associated lung injury. Ueno et al. (1999) showed that early post-hepatectomy HBO treatment decreased neutrophil activation and improved the outcome. Convincingly, HBO should be administered as early as possible during ischemia-reperfusion in order to obtain a favorable effect.

TNF-α is considered to be a primary mediator involved in moderating the cascade of the inflammatory processes that lead to ischemia-reperfusion lung injury (Caty et al. 1989; Khimenko et al. 1998; Welbourn et al. 1991). This is evidenced by the facts that:

1. Intravenous injection of low doses of TNF-α causes a lung injury that mimics that seen in ischemia-reperfusion-induced lung injury (Okusawa et al. 1988).
2. Anti-TNF-α antibody has a protective effect against ischemia-reperfusion-induced lung injury (Colleti et al. 1990).
3. Concentrations of TNF-α are increased in ischemia-reperfusion injury.
4. TNF-α activates neutrophils and endothelial cells resulting in a rapid up-regulation of the adhesion receptors, which enhances the adhesion of neutrophils and endothelial cells (Bevilacqua et al. 1987; Strieter et al. 1987).
5. TNF-α enhances the macrophage respiratory burst, which may also lead to oxidative tissue injury (Mayer et al. 1993; Phillips et al. 1990).
6. TNF-α influences the production of other inflammatory cytokines by neutrophils (Marucha et al. 1991).
7. TNF-α promotes the interaction between sequestered neutrophils and the vascular endothelium, resulting in an increase in neutrophil-dependent vascular permeability (Gibbs et al. 1990).
8. TNF-α may directly injure the endothelial cells.

In our study, elevated serum TNF-α may be responsible for ischemia-reperfusion-induced lung injury.

Macrophages, mast cells, epithelial cells and neutrophils are capable of producing significant quantities of TNF-α (Xing et al. 1993). The likely source of TNF-α in intestinal ischemia-reperfusion is from activated macrophages and neutrophils leaking into the systemic circulation from the ischemic intestine, and from nearly all organs containing mononuclear cells, including circulating blood monocytes (Beutler et al. 1985; Dubravec et al. 1990). Since the liver and the lungs have a large resident population of macrophages, and are the first organs in the path of recirculation for the translocated endotoxin, they may be another major source of TNF-α. One of the early studies showed that patients with rheumatoid arthritis who were treated with HBO achieved a remarkable recovery, suggesting that HBO has an immunosuppressive effect (Kamada 1985). This is supported by animal studies in which it was shown that HBO inhibits interleukin (IL)-1 production (Inamoto et al. 1991; Saito et al. 1991). One recent study has shown that HBO attenuates the production of TNF-α, IL-6, and their respective hepatic mRNAs after massive hemorrhage (Yamashita and Yamashita 2000). Our data provide further evidence that HBO inhibits TNF-α production in intestinal ischemia-reperfusion injury.

In conclusion, intestinal ischemia-reperfusion results in damage to the intestine and acute lung injury. The production of TNF-α is enhanced during intestinal ischemia-reperfusion. A connection exists between enhanced TNF-α production and lung neutrophil sequestration. During ischemia, HBO attenuates TNF-α production and ameliorates intestinal and acute lung injury. These data suggest that the immunosuppressive effect of HBO is at least partially attributed to its beneficial effect in intestinal ischemia-reperfusion injury.

Acknowledgements The authors are grateful for the technical support provided by the staff in the laboratories of Dr. M.M. Meguid, Dr. G. Threatte, and Dr. S. Badaway.

References

Beutler BA, Milsark IW, Cerami S (1985) Cachectin/tumor necrosis factor: production, distribution and metabolic fate in vivo. J Immunol 135:3972–3977

Bevilacqua MJ, Prober J, Mendrick D, Cotran R, and Gimbrone M (1987) Identification of an inducible endothelial-leukocyte adhesion molecule. Proc Natl Acad Sci USA 84:9238–9242

Bouachour G, Cronier P, Gouello JP, Toulemonde JL, Talha A, Alquier P (1996) Hyperbaric oxygen therapy in the management of crush injuries: a randomized double-blind placebo-controlled clinical trial. J Trauma-Injury Infect Crit Care 41:333–339

Bradshaw PO, Nelson AG, Fanton JW, Yates T, Kagan-Hallet KS (1996) Effect of hyperbaric oxygenation on peripheral nerve regeneration in adult male rabbits. Undersea Hyperbaric Med 23:107–113

Brigham KL, Bowers R, Haynes J (1979) Increased sheep lung vascular permeability caused by *Escherichia coli* endotoxin. Circ Res 45:292–297

Buras JA, Stahl GL, Svoboda KKH, Reenstra WR (2000) Hyperbaric oxygen downregulates ICAM-1 expression induced by hypoxia and hypoglycemia: the role of NOS. Am J Physiol 278:C292–C302

Caty MG, Guice KS, Oldham KT, Remick DG, Kunkel SL (1989) Evidence for tumor necrosis factor-induced pulmonary microvascular injury after intestinal ischemia-reperfusion injury. Ann Surg 212:694–700

Chiu CJ, McArdle AH, Brown R, Scott HJ, Gurd FN (1970) Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. Arch Surg 101:478–483

Colleti LM, Remick DG, Burtch GD, Kunkel SL, Strieter RM, Cambell DA Jr (1990) Role of tumor necrosis factor-a in the pathophysiologic alterations after hepatic ischemia/reperfusion injury in the rat. J Clin Invest 85:1936–1943

Cuzzocrea S, Imperatore F, Costantino G, Luongo C, Mazzon E, Scafuro M, Mangoni G, Caputi AP, Rossi F, Filippelli A (2000) Role of hyperbaric oxygen exposure in reduction of lipid peroxidation and in multiple organ failure induced by zymosan administration in the rat. Shock 13:197–203

Dubravec DB, Spriggs DR, Mannick JA, Rodrick ML (1990) Circulating human peripheral blood granulocytes synthesize and secrete tumor necrosis factor alpha. Proc Natl Acad Sci U S A 87:6758–6761

Gibbs LS, Lai L, Malik AB (1990) Tumor necrosis factor enhances the neutrophil-dependent increase in endothelial permeability. J Cell Physiol 145:496–500

Guice KS, Oldham KT, Caty MG, Johnson KJ, Ward PA (1989) Neutrophil-dependent, oxygen-radical mediated lung injury associated with acute pancreatitis. Ann Surg 210:740–707

Haapaniemi T, Nylander G, Sirsjo A, Larsson J (1991) Hyperbaric oxygen reduces ischemia-induced skeletal muscle injury. Plast Reconstr Surg 97:602–607

Hampson NB (ed) (1999) Hyperbaric oxygen therapy: 1999 committee report. Undersea and Hyperbaric Medical Society, Kensington, Md

Inamoto Y, Okuno F, Saito K, Tanaka Y, Watanabe K, Morimoto I, Yamashita U, Eto S (1991) Effect of hyperbaric oxygenation on macrophage function in mice. Biochem Biophys Res Commun 179:886–891

Kamada T (1985) Superoxide dismutase and hyperbaric oxygen therapy of the patient with rheumatoid arthritis. J Jpn Orthop Assoc 59:17–26

Khimenko PL, Bagby GJ, Fuseler J, Taylor AE (1998) Tumor necrosis factor-a in ischaemia and reperfusion injury in rat lungs. J Appl Physiol 85:2005–2011

Kihara M, McManis PG, Schmelzer JD, Kihara Y, Low PA (1995) Experimental ischemic neuropathy: salvage with hyperbaric oxygenation. Ann Neurol 37:89–94

Klass AA (1953) Acute mesenteric arterial occlusion: restoration of blood flow by embolectomy. J Int Coll Surg 20:687–694

Klausner JM, Paterson IS, Kobzik L, Shepro D, Hechtman HB (1988) Lower torso ischaemia induced lung injury is leukocyte-dependent. Ann Surg 208:761–767

Kolski JM, Mazolewski PJ, Stephenson LL, Texter J, Grigoriev VE, Zamboni WA (1998) Effect of hyperbaric oxygen therapy on testicular ischemia-reperfusion injury. J Urol 160:601–604

Marucha PT, Zeff RA, Kreutzer DL (1991) Cytokine-induced IL-1b gene expression in the human polymorphonuclear leukocyte: transcriptional and post-transcriptional regulation by tumor necrosis factor and IL-1. J Immunol 147:2603–2608

Mayer AM, Pittner RA, Lipscomb GE, Spitzer JA (1993) Effect of in vivo TNF administration on superoxide production and PKC activity of rat alveolar macrophages. Am J Physiol 264:L43–L52

Nahum A, Chamberlin W, Sznajder JI (1991) Differential activation of mixed venous and arterial neutrophils in patients with sepsis syndrome and acute lung injury. Am Rev Respir Dis 143:1083–1087

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

Nylander G, Lewis D, Nordstrom H, Larsson J (1985) Reduction of postischemic edema with hyperbaric oxygen. Plast Reconstr Surg 76:596–603

Nylander G, Nordstrom H, Lewis D, Larsson J (1987) Metabolic effects of hyperbaric oxygen in postischemic muscle. Plast Reconstr Surg 79:91–97

Okusawa S, Gelfand J, Ikejima T, Connolly R, Dinarello C (1988) Interleukin 1 induces a shock-like state in rabbits. Synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. J Clin Invest 81:1162–1172

Phillips WA, Croatto M, Hamilton JA (1990) Priming the macrophage respiratory burst with IL-4: enhancement with TNF-α but inhibition by IFN-y. Immunology 70:498–503

Pizarro TT, Malinowska K, Kovacf EJ, Clancy L, Robinson JA, Piccinini LA (1993) Induction of TNF and TNF/β (lymphotoxin) gene expression in rat cardiac transplants during allograft rejection. Transplantation 56:399–404

Remick DG, Strieter RM, Lynch JP, Nguyen D, Eskandari M, Kunkel SL (1989) In vivo dynamics of murine tumor necrosis factor-alpha gene expression. Kinetics of dexamethasone-induced suppression. Lab Invest 60:766–771

Rossman JE, Caty MG, Zheng S, Karamanoukian HL, Thusu K, Azizkhan RG, Dandona P (1997) Mucosal protection from intestinal ischemia-reperfusion reduces oxidant injury to the lung. J Surg Res 73:41–46

Saito K, Tanaka Y, Ota T, Eto S, Yamashita U (1991) Suppressive effect of hyperbaric oxygenation on immune responses of normal and autoimmune mice. Clin Exp Immunol 86:322–327

Schmeling DJ, Caty MG, Oldham KT, Guice KS, Hinshaw DB (1989) Evidence for neutrophil-related acute lung injury after intestinal ischemia-reperfusion. Surgery 106:195–202

Simpson R, Alon R, Kobzik L, Valeri R, Shepro D, Hechtman HB (1993) Neutrophil- and non-neutrophil-mediated injury in intestinal ischemia-reperfusion. Ann Surg 218:444–454

Slotman GJ (1998) Hyperbaric oxygen in systemic inflammation. HBO is not just a movie channel anymore. Crit Care Med 26:1932–1933

Sorkine P, Setton A, Miller A, Rudick V, Marmor S, Klausner JM, Goldman G (1995) Soluble tumor necrosis factor receptors reduce bowel ischemia-induced lung permeability and neutrophil sequestration. Crit Care Med 23:1377–1381

Strieter RM, Kunkel SL, Showell HJ, Remick G, Phan SH, Ward PA, Marks RM (1989) Endothelial cell expression of a neutrophil chemotactic factor by TNF-alpha, LPS, and IL-1-beta. Science 243:1467–1469

Thom SR (1990) Antagonism of carbon monoxide-mediated brain lipid peroxidation by hyperbaric oxygen. Toxicol Appl Pharmacol 105:340–344

Tjarnstrom J, Wikstrom T, Bagge U, Risberg B, Braide M (1999) Effects of hyperbaric oxygen treatment on neutrophil activation and pulmonary sequestration in intestinal. Eur Surg Res 31:147–154

Turnage RH, Guice KS, Oldham KT (1994) Pulmonary microvascular injury following intestinal reperfusion. New Horiz 2:463–475

Ueno S, Tanabe G, Kihara K, Aoki D, Arikawa K, Dogomori H, Aikou T (1999) Early post-operative hyperbaric oxygen therapy modifies neutrophil activation. Hepatogastroenterology 46:1798–1799

Vedder NB, Fouty BW, Winn RE, Harlan JM, Rice CL (1989) Role of neutrophils in generalized reperfusion injury associated with resuscitation from shock. Surgery 106:509–516

Welbourn R, Goldman G, O'Riordain M, Lindsay TF, Paterson IS, Kobzik L, Valeri CR, Shepro D, Hechtman HB (1991) Role for tumor necrosis factor as a mediator of lung injury following lower torso ischemia. J Appl Physiol 70:2645–2649

Wilson C, Gupta R, Gilmour DG, Imrie CW (1987) Acute superior mesenteric ischemia. Br J Surg 74:279–281

Windsor ACJ, Mullen PG, Fowler AA, Sugerman GJ (1993) Role of the neutrophil in adult respiratory distress syndrome. Br J Surg 80:10–17

Xing Z, Kirpalani H, Torry D, Jordana M, Gauldie J (1993) Polymorphonuclear leukocytes as a significant source of tumor necrosis factor-a in endotoxin-challenged lung tissue. Am J Pathol 143:1009–1115

Yamada T, Taguchi T, Hirata Y, Toyohara T, Hirose R, Suita S, Yagi H (1994) The effect of hyperbaric oxygenation on ischemia-reperfusion injury of the small intestine. Transplant Proc 26:1506–1507

Yamashita M, Yamashita M (2000) Hyperbaric oxygen treatment attenuates cytokine induction after massive hemorrhage. Am J Physiol 278:E811–E816

Zamboni WA, Roth AC, Russell RC, Smoot EC (1993a) The effect of hyperbaric oxygen on reperfusion of ischemic axial skin flaps: a laser Doppler analysis. Am Plast Surg 28:339–341

Zamboni WA, Roth AC, Russell RC, Graham B, Suchy H, Kucan JO (1993b) Morphologic analysis of the microcirculation during reperfusion of ischemic skeletal muscle and the effect of hyperbaric oxygen. Plast Reconstr Surg 91:1110–1123

Zamboni WA, Wong HP, Stephenson LL (1996) Effect of hyperbaric oxygen on neutrophil concentration and pulmonary sequestration in reperfusion injury. Arch Surg 131:756–760

Zao DW (1991) Therapeutic effect of hyperbaric oxygen on recovery of surgically repaired peripheral nerve injury. Chung-Hua Wai Ko Tsa Chih 29:118–120