Protective effects of hyperbaric oxygen and iloprost on ischemia-reperfusion injury in rabbit kidneys

Article *in* Italian Journal of Vascular and Endovascular Surgery · June 2013

CITATION
1

READS
52

9 authors, including:

Murat Aksun
Izmir Katip Celebi Universitesi
75 PUBLICATIONS **277** CITATIONS
SEE PROFILE

Sahin Bozok
Izmir Bakircay University, Faculty of Medicine
82 PUBLICATIONS **388** CITATIONS
SEE PROFILE

Berkan Ozpak
Çorlu Devlet Hastanesi
35 PUBLICATIONS **136** CITATIONS
SEE PROFILE

Tevfik Gunes
Pamukkale University
66 PUBLICATIONS **138** CITATIONS
SEE PROFILE

J VASC ENDOVASC SURG 2013;20:89-95

Protective effects of hyperbaric oxygen and iloprost on ischemia-reperfusion injury in rabbit kidneys

G. ILHAN [1], M. AKSUN [2], S. BOZOK [1], H. B. OZPAK [3], T. GUNES [3], M. E. DURAKOGLUGİL [4], H. KARA [3], A. KUPELİ [5], E. SENER [1]

Aim. **The aim of the study was to demonstrate protective effects of hyperbaric oxygen (HBO) and iloprost (IL) on renal ischemia-reperfusion (IR) injury using histopathological and biochemical methods.**

Methods. **Fourty New Zealand white male rabbits were randomly allocated into one of four study groups. HBO group (N.=10) received a single session of HBO treatment (120 min at 2.5 atm); IL group (N.=10) received an infusion of 25 ng/kg/min IL; HBO+IL group (N.=10) received both HBO and IL; control group (N.=10) received only 0.9% saline. Renal ischemia-reperfusion was established by clamping abdominal aorta for 1h. Levels of pH, PO_2, PCO_2, HCO_3, Na^+, K^+, lactate dehydrogenase, blood urea nitrogen and creatinine, plasma and renal malondialdehyde, myeloperoxidase, glutathione and superoxide dismutase were measured at onset, the end of ischemia period and the 24th hour of reperfusion. The kidneys of the sacrificed rabbits were evaluated histopathologically.**

Results. **Even though blood urea nitrogen and creatinine levels were significantly higher in control group, there were not any differences between other groups. Blood PO_2, pH, and HCO_3 concentrations were significantly elevated and malondialdehyde levels were lower in control group compared to HBO, IL and HBO+IL groups. Histopathological changes including tubular necrosis, atrophy, hydropic degeneration and regenerative atypia that reflect renal injury were also significantly higher in control group.**

Conclusion. **We suggested that both HBO and IL, either alone or in combination significantly reduced biochemical and histopathological signs of renal ischemia-reperfusion injury.**

KEY WORDS: Reperfusion injury - Hyperbaric oxygenaytion - Iloprost.

Corresponding author: G. Ilhan, MD, Department of Cardiovascular Surgery Rize University Faculty of Medicine, Rize Training and Research Hospital, Rize, Turkey. E-mail: drgknilhan@yahoo.com

[1]Department of Cardiovascular Surgery Recep Tayyip Erdogan University Faculty of Medicine Rize Training and Research Hospital, Rize, Turkey
[2]Department of Anesthesiology and Reanimation İzmir Atatürk Training and Research Hospital İzmir, Turkey
[3]Department of Cardiovascular Surgery İzmir Atatürk Training and Research Hospital İzmir, Turkey
[4]Department of Cardiology Recep Tayyip Erdogan University Faculty of Medicine Rize Training and Research Hospital, Rize, Turkey
[5]Department of Pathology, Solar Pathology Laboratory İzmir, Turkey

Vascular surgical procedures requiring transient clamping of abdominal aorta or peripheral arteries due to aneurysm, aortoiliac occlusive disease, thromboembolectomy, and transplantation; hypotension, hypovolemic shock, and cardiac arrest causes ischemia-reperfusion (IR) injury of the lower extremities and visceral organs. Consequently, injury of the either ischemic organ or tissue (local injury) or non-ischemic areas away from ischemic tissue (remote organ injury) may ensue. Inflammatory response characterized by increased tumor necrosis factor-α (TNF-α), and interleukin-1β levels; activation of polymorphonuclear leukocytes has the most important role in this injury.[1] Reactive oxygen species, produced initially during reperfusion, aggravate this damage.[2]

The severity of renal IR increases with longer ischemia duration. Therefore, a spectrum of clinical conditions ranging from prerenal azotemia without tissue injury to acute renal failure due to cortical necrosis is observed.[3] Despite new therapeutic options, acute renal failure is associated with high morbidity and mortality.[4]

Recently, some studies demonstrated that hyperbaric oxygen (HBO) treatment reduces the consequences of I-R injury in various tissues, including the brain, myocardium, skeletal muscle, musculocutaneous flap, small intestine, liver, and testis.[5-7] The beneficial effects of HBO in IR stem from lower endothelial adhesion molecule expression and decreased PML activation and migration. Moreover, HBO stabilizes lysosomal enzymes and promotes nitric oxide production.[6] On the other hand, iloprost (IL), a vasodilator prostaglandin, decreases oxygen consumption and production of superoxide anions, especially in IR studies.[8] IL decreases neutrophil activation and aggregation, in addition to inhibition of ROS production and release of lysosomal enzymes.[9] Natori *et al.* demonstrated that PGE1 lowered IR injury by inhibiting leukocyte adhesion, in hepatic IR setting. This action was mediated by down regulation of intercellular adhesion molecule-1 (ICAM-1).[10]

The purpose of this experimental study was to investigate and compare the effectiveness of HBO and IL treatment on renal injury induced by IR.

Materials and methods

Experimental procedure and study groups

Fourty New Zealand white male rabbits, weighing 2.5 to 3 kg, were randomly allocated into one of four study groups. HBO group (N.=10) received a single session of HBO treatment, IL group (N.=10) received an infusion of 25 ng/kg/min IL, HBO+ IL group (N.=10) received both HBO and IL; control group (N.=10) received only 0.9% saline. HBO treatment and IL infusion were administrated during 60 min. of ischemia and 60 min. of reperfusion in the treatment groups. All procedures were performed aseptically. Initial anesthesia was achieved with intramuscular ketamine (50 mg/kg) and xylazine (5 mg/kg) without endotracheal intubation followed by 25 mg/kg fractionally in order to allow the animals to have spontaneous respiration. All animals received a similar volume of maintenance fluids (0.9% sodium chloride, 20 mL/h) for the whole procedure. An arterial catheter (20-gauge) was placed in an ear artery to monitor blood pressure (Petas KMA 800, Ankara, Turkey).

Surgical procedure

Each animal received heparin (150 UI/kg) intravenously for anticoagulation. Animals were placed in the supine position. Infrarenal abdominal aorta was exposed through a midline laparotomy incision and a transperitoneal approach, with the abdominal contents reflected to the right. The aorta was exposed from the left renal artery down to the aortic bifurcation. In all animals, the aorta was cross clamped just proximal to the aortic bifurcation above to the renal artery using atraumatic arterial bulldog clamps (Vascu-statt, Scanlan International, St. Paul, MN, USA). The clamp was removed after 60 min and restoration of blood flow was verified visually.

Twenty-four hours after the initiation of renal ischemia, all animals were humanely killed by a lethal cardiac injection of pentobarbital (100 mg/kg); left nephrectomy was performed and renal biopsies were taken from the cortex of the kidneys for microscopic and biochemical evaluation. The left kidney was removed and stored at -70 °C until further testing. Three animals died during the procedure, one in the HBO group during anesthetic induction, one in the IL group due to surgical intervention. They were excluded from the study.

HBO treatment procedure

Animals received HBO therapy in an animal monoplace chamber during 60 minutes of ischemia and 60 minutes of reperfusion period at a pressure 2.5 atm. Before pressurization, 100% medical oxygen was flushed through the chamber for 10 min to displace ambient air. The oxygen pressure was then increased slowly and reached 2.5 atm in 5 min. The chamber was ventilated during HBO therapy to avoid carbon dioxide (CO_2) accumulation. The concentration of CO_2 was not allowed to rise above 0.1%. An environmental control system maintained inner temperature and relative humidity at 25±1 °C and 50±20%, respectively. After 120 min at 2.5 atm, the chamber was decompressed to normal atmospheric pressure in 5 min.

TABLE I.—*Histopathological scoring.*

	0	1	2	3
Tubular necrosis	Absent	Focal	Multifocal	Diffuse
Tubular atrophy	Absent	<25%	25-50%	>50%
Regenerative atypia	Absent	Focal	Multifocal	Diffuse
Hydropic degeneration/vacuolization	Absent	Focal	Multifocal	Diffuse
Interstitial fibrosis	Absent	Focal	Multifocal	Diffuse
Brush border disappearance	Absent	Focal	Multifocal	Diffuse

Serum and tissue biochemical evaluation

Blood oxygen-binding properties take part in the antioxidant system by the establishment of oxygen transport conditions and pO_2 values. Blood pH, PO_2 (mmHg), PCO_2 (mmHg) and HCO_3 (mmol/L) values were determined at the end of the reperfusion period. Blood pH, pO_2, pCO_2 and HCO_3, values were determined using a blood-gas analyzer (Ciba-Corning Blood Gas Analyzer Model 860, Ciba Corning Diagnostics Corp., Irvine, CA, USA). Venous blood samples were processed in an automatic analyzer (Hitachi 717; Boehringer Mannheim, Indianapolis, IN, USA) to measure blood urea nitrogen (BUN) and creatinine levels. BUN and creatinine levels were measured at onset, the end of ischemia and the 24th hour of reperfusion to investigate renal functions.

Malondialdehyde (MDA), an indirect parameter of oxidative stress, and antioxidant peptide glutathione (GSH) levels were measured in the kidney tissue samples. Tissue levels of MDA and GSH were measured as described previously.[11] The activity of superoxide dismutase, an enzyme that inactivates superoxide (O_2^-), was determined in order to understand level of oxidative stress, via methods of Spitz, and Woolliams.[12, 13] Myeloperoxidase (MPO) activity, a sensitive marker of PML infiltration in tissues was calculated by hydrogen peroxide dependent oxidation of tetrametylbenzidine, a reaction catalyzed by myeloperoxidase.[14]

Histopathological evaluation

Portions of the fixed renal biopsy materials were examined microscopically (Nikon model Eclipse E600W). The specimens were fixed in 10% formalin. Paraffin blocks were cut at 5 μm and stained with hematoxylin eosin. Areas corresponding to the kidney specimens were graded for the degree of renal damage based on each of the following parameters: tubular necrosis and atrophy, regenerative atypia, hydropic degeneration, interstitial fibrosis, loss of supranuclear cytoplasms and brush border disappearance. Renal injury was scored semiquantatively according to these characteristics as; grade 0 as normal, grade 1 as mild (focal), grade 2 as moderate (multifocal) and grade 3 as severe (diffuse) pathologic changes (Table I) by the same pathologist, who was blinded to the study.[15] Neutrophil infiltration was determined by counting the number of neutrophils in 10 high-power fields (X 400) for each kidney slide.[16]

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) software (version 10.0 for Windows). All differences associated with a chance probability of 0.05 or less were considered statistically significant. All data are presented as mean ± SD. Statistical analysis was performed using analysis of variance (ANOVA) test. One-way ANOVA test was followed by *post-hoc* Dunnett's test.

Results

Biochemical findings and renal functions

There were not any statistically significant differences between the groups according to the physiological parameters in the preischemic and postischemic periods. Table II presents mean values and standard deviations of pH, pO2, pCO2, HCO3, BUN and creatinine levels. Blood pO2 concentration was significantly elevated in both HBO and HBO+ IL groups. There were not any significant differences in other parameters between HBO, IL and HBO+ IL groups.

Malondialdehyde (MDA) levels of the medicated

TABLE II.—*Blood pH, PO_2, PCO_2, HCO3 serum BUN and creatinine levels determined at the end of the reperfusion period.*

Groups	pH	pO_2 (mmHg)	pCO_2 (mmHg)	HCO_3 (mmol/L)	BUN (mg/dL)	Creatinine (mg/dL)
HBO group	7.21±0.3	186.8±7.02**	43.4±2.6	18.4±5.3	57.2±4.3	1.43±0.4
IL group	7.23±0.50	86.7±6.35	40.7±3.3	17.4±5.2	48.6±65.1	1.2±0.3
HBO + IL group	7.24±0.2	165.7±7.06**	41.3±2.1	19.7±2.8	50.1±8.9	1.35±0.4
Control group	7.1±0.10*	54.10±6.5*	49.8±5.3+	9.5±5.6*	136.1±14.7+	4.8±1.7+

*= Significantly lower than the treatment groups by ANOVA test (p=0.006); **= Significantly higher than the control, sham and IL groups by ANOVA test (P=0.001); += Significantly higher than the treatment groups by ANOVA test (P=0.002). BUN=blood urea nitrogen; HBO=hyperbaric oxygen; IL=iloprost.

TABLE III.—*Tissue MDA, GSH; SOD and MPO levels.*

Groups	MDA (renal) (nmol/g)	GSH (μmol/mg)	SOD (U/mg protein)	MPO (ΔA/dk/g)
Group HBO	58±8.2	2.2±0.2	5.5± 1.2	1.7±0.5
Group IL	33±6	2.4±0.3	4.2±0.9	1.5±0.3
Group HBO+IL	38±7.3	2.9±0.6	3.9±0.8	1.6±0.5
Group Control	102.3±12.6*#	1.2±0.1*#	7.8±2.2*#	5.3±1.3*#

*= P<0.05 *vs.* Sham group; #= P<0.05 *vs.* and treatment groups by ANOVA test (P<0.05). MDA=malondialdehyde; GSH= glutathione; SOD=superoxidedismutase; MPO=myeloperoxidase; HBO=hyperbaric oxygen; IL=iloprost.

TABLE IV.—*Histopathological score of the groups and neutrophil count in kidneys after 24 h data are expressed as mean ± SEM.*

Groups	Grade 0	Grade 1	Grade 2	Grade3	Mean±SD	Neutrophil infiltration
Group HBO	3	4	3		1.00±0.38	8.2±1.3
Group IL	3	6	1		0.85±0.42	7.3±1.1
Group HBO+IL	4	4	2		0.87±0.40	7.1±0.9
Group Control		2	5	3	2.12±0.88*#	18.7±3.4*#

*= Significantly higher than the Sham group by ANOVA test (P=0.001); #= Significantly higher than the treatment groups by ANOVA test (P=0.01). HBO=hyperbaric oxygen; IL=iloprost.

groups were significantly lower than control group (HBO *vs.* IL, P=0.130; HBO *vs.* Control, P=0.01; IL *vs.* Control, P=0.005; HBO+IL *vs.* Control, P=0.001). Although statistically not significant, tissue MDA concentration was higher in HBO group. Interestingly, we observed a trend in tissue MDA concentrations, that were lower in HBO+ IL group compared to HBO (HBO+IL *vs.* HBO, P=0.085).

SOD and MPO levels were higher in control group compared to other groups (P=0.005). Even though SOD and MPO concentrations were mildly elevated in HBO, IL and HBO +IL groups, this did not reach statistical significance ($p > 0.05$). Even though GSH levels were elevated in HBO, IL and HBO+IL rabbits compared to controls, this result was not statistically significant (P=0.15) (Table III). This data supported that the IR model was well formed in the animals.

Histopathological findings

Histological evidence of reperfusion injury was the presence of tubular necrosis and atrophy, regenerative atypia, hydropic degeneration, interstitial fibrosis, loss of supranuclear cytoplasms and brush border disappearance. Kidney samples from control group rabbits revealed severe damage, including tubular cell swelling, interstitial edema, tubular dilatation, necrosis of the epithelium, and hyaline casts. Mean histopathological scores of HBO, IL and HBO+IL groups were significantly lower than control group (HBO *vs.* Control, P=0.005; IL *vs.* Control, P=0.001; HBO+IL *vs.* Control, P=0.002). There was no significant difference between HBO and IL groups (P=0.331) (Table IV). Neutrophil count was lower in HBO, IL, and HBO +IL group in comparison to control group (P<0.05) (Table IV).

Discussion

Varying levels of non-immunologic tissue damage occur after diminished or disrupted renal blood flow, and reperfusion.[3] Severe reduction in renal blood flow, depletion of high-energy phosphate products, and subsequent impairment in cell membrane functions result in cellular injury. The severity of this damage differs with respect to ischemia duration and abundance of collateral circulation. Paradoxically, restoration of normal blood flow causes more harm, called as reperfusion injury.[3] The reperfusion of endothelial cells results in production of reactive oxygen species, which in turn leads to secretion of a systemic inflammatory cytokines such as interleukin-1, interleukin-8, platelet-activating factor and TNF-α. This IR process causes leukocyte dependent micro-vascular dysfunction, leukocyte plugging, impaired endothelium-dependent vasodilation, enhanced capillary fluid filtration, and plasma protein extravasations all of which affect distal organs.[17] Evidently, an increase in vascular permeability leads to reduction of renal cortical blood flow.[18]

Both HBO and IL has shown protective effects on renal IR injury in separate studies.[8, 16] To date, there is not any information regarding the comparative and additive influence of HBO and IL on renal IR.

HBO treatment involves inspiration of 100% oxygen intermittently for 60-90 min at a pressure higher than normal atmospheric pressure. Exposure to HBO raises dissolved oxygen concentration in arterial blood. The associated increase in oxygen tension enhances diffusion of oxygen into poorly perfused tissues . Oxygen pressure may raise to 10 to 15 times above its normal level when the patient breathes 100% oxygen at 2.8 atm. HBO therapy is often used to treat diseases related to hypoxia, including acute carbon monoxide poisoning, decompression sickness, air embolism, soft tissue infections and recalcitrant wounds.

In the present study, both HBO and IL therapies were administered at the beginning of ischemia and continued during reperfusion period. Compared to control group, tissue MDA, an indicator of IR injury and lipid peroxidation, and MPO, a sign of PML infiltration, and renal histopathologic damage and neutrophil infiltration was lower in either IL, HBO, and HBO +IL groups. Studies in animal models showed that HBO enhances fibroblast replication and collagen formation in scar tissue, improves neovascularization of ischemic tissue and decreases edema.[19] HBO also lower leukocyte adherence to venular endothelium in IR of skeletal muscle, suggesting a reduction in inflammation of the postischemic tissue.[5] The mechanisms behind this effect are not clarified, but the limited amount of available experimental data indicates that HBO, directly or indirectly, interacts with mediators that control the local inflammatory response. Since HBO has been shown to exert anti-inflammatory effects in experimental models of skeletal muscle ischemia, it may also have anti-inflammatory effects in other types of inflammation as well, although the most probable explanation for the reduction in the local inflammation in ischemia is a reduction in local ischemic tissue damage. In an intestinal IR injury model, the rats underwent 90 min. of reperfusion after a 2 hour-ischemia period. HBO was administered during the reperfusion period and a significant reduction in lung neutrophil sequestration was observed.[6] The beneficial effect of HBO on lung neutrophil sequestration could be achieved when administered during either the period of ischemia or the reperfusion. HBO treatment after reperfusion had none of these effects. Rossman *et al.* reported that perfusing the lumen of the intestine with oxygenated perfluorocarbon during ischemia reduced mucosal and associated lung injury.[20] Ueno *et al.* showed that early post-hepatectomy HBO treatment decreased neutrophil activation and improved outcome.[21] Convincingly, HBO should be administered as early as possible during IR in order to obtain a favorable effect.

In the present study, we started directly at the definitive level in order to obtain the maximal physiologic effect before aortic occlusion. We did not perform a separate dose-response effect experiment. IL infusion was administrated during 60 minutes of ischemia and 60 minutes of reperfusion period in the treatment groups. IL, a stable prostacyclin analog, acts as a membrane stabilizator and inhibits neutrophil functions which are potential mediators of IR injury. IL also decreases white blood cell aggregation and adhesion to vascular endothelium, superoxide radical production from stimulated canine and human neutrophils, and free radical formation in myocardium subject to IR injury. IL infusion demonstrated protective effects during the clamping period in previous studies.[22] We chose the dosage of IL as used in a previous study by Katircioglu *et al.*[22] In that study, the administration was started at a lower rate before the aortic occlusion, and increased to the definitive dose of 25 ng/kg per minute.

There have been concerns regarding the use of

HBO for IR, based on the hypothesis that providing extra oxygen would increase free radical production and tissue damage. Pablos *et al.* reported increased lipid peroxidation products including MDA; higher total and oxidized glutathione (GSH) levels, and decreased glutathione reductase (GR) and glutathione peroxidase (GPx) activity in liver and brain tissue of rats under 100% oxygen at 4 atm pressure.[23] On the contrary, Boadi *et al.* demonstrated higher GSH, GR, GPx and superoxide dismutase (SOD) concentrations that increased even more after adding antioxidants to medium (vitamin E, riboflavin, selenium) in brain, lung, liver, brain and blood samples of rats undergoing HBO at 4.5 atm.[24, 25] Additionally, Puglia and coworkers notified that HBO higher than 2.8 atm may cause central nervous system toxicity which could occur swiftly in the event of SOD inhibition.[26] Moreover, even at normal pressure, 100% O_2 has been shown to induce SOD activity in lungs. On the other hand, HBO has been shown to decrease lipid peroxidation in a number of IR studies. Chen *et al.* reported that HBO pretreatment decreased lipid peroxidation in hepatic IR.[7] Similarly, Gurer and coworkers presented data indicating decreased production of free oxygen radicals, and MDA, among animals pretreated with HBO.[27] The protective effect of HBO treatment was proposed to be due to suppression of specific enzymes that catalyze lipid peroxidation and through oxygen-mediated termination reactions.

Even though Jamieson *et al.* showed that HBO increases reactive oxygen species, particularly superoxide and hydrogen peroxide, they could not demonstrate a significant increase in MDA concentrations with higher pressures in rat models.[28, 29] MDA, SOD, and MPA concentrations were significantly lower in treatment groups in comparison to controls. Another interesting point was that these markers of oxidative stress were lower in HBO+IL group compared to HBO only group, although difference was not statistically significant. We think that this issue is due to the fact that IL prevented oxidative stress and lipid peroxidation.

Conclusions

In conclusion, combined HBO and IL treatment may prevent renal IR injury both biochemically and histapathologically by protecting against reactive oxygen species, inhibiting neutrophil activation and sequestration.

References

1. Yassin MM, Harkin DW, Barros D'Sa AA, Halliday MI, Rowlands BJ. Lower limb ischemia-reperfusion injury triggers a systemic inflammatory response and multiple organ dysfunction. World J Surg 2002;26:115-21.
2. Nelson K, Herndon B, Reisz G. Pulmonary effects of ischemic limb reperfusion: evidence for a role for oxygenderived radicals. Crit Care Med 1991;19:360-3.
3. Woolfson RG, Millar CG, Neild GH. Ischaemia and reperfusion injury in the kidney: current status and future direction. Nephrol Dial Transplant 1994;9:1529-31.
4. Edelstein CL, Ling H, Schrier R. The nature of renal cell injury. Kidney Int 1997;51:1341-51.
5. Zamboni WA, Roth AC, Russell RC, Graham B, Suchy H, Kucan JO. Morphologic analysis of the microcirculation during reperfusion of ischemic skeletal muscle and the effect of hyperbaric oxygen. Plastic Reconstr Surg 1993;91:1110-23.
6. Tjarnstrom J, Wikstrom T, Bagge U, Risberg B, Braide M. Effects of hyperbaric oxygen treatment on neutrophil activation and pulmonary sequestration in intestinal ischemia- reperfusion in rats. Eur Surg Res 1999;31:147-54.
7. Chen MF, Chen HM, Ueng SWN, Shyr MH. Hyperbaric oxygen pretreatment attenuates hepatic reperfusion injury. Liver 1998;18:110-6.
8. Vargas AV, Krishnamurthi V, Masih R, Robinson AV, Schulak JA. Prostaglandin E1 attenuation of ischemic renal reperfusion injury in the rat. J Am Coll Surg 1995;180:713-7.
9. Simpson PJ, Mitsos SE, Ventura A, Gallagher KP, Fantone JC, Abrams GD *et al.* Proctacyclin protects ischemic reperfused myocardium in the dog by inhibition of neutrophil activation. Am Heart J 1987;113:129-37.
10. Natori S, Fujii Y, Kurosawa H, Nakano A, Shimada H. Prostaglandin E1 protects against ischemia-reperfusion injury of the liver by inhibition of neutrophil adherence to endothelial cells. Transplantation 1997;64:1514-20.
11. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978;86:271.
12. Spitz DR, Oberley LW. An assay for superoxide dismutase activity in mammalian tissue homogenates. Anal Biochem 1989;179:8-18.
13. Woolliams JA, Wiener G, Anderson PH, McMurray CH. Variation in the activities of glutathione peroxidase and superoxide dismutase and in the concentration of copper in the blood in various breed crosses of sheep. Res Vet Sci 1983;34:253-6.
14. Tullis MJ, Brown S, Gewertz BL. Hepatic influence on pulmonary neutrophil sequestration following intestinal ischemiareperfusion. J Surg Res 1996;66:143-6.
15. Onal A, Astarcioglu H, Ormen M, Atila K, Sarioglu S. The beneficial effect of L-carnitine in rat renal ischemiareperfusion injury]. Ulus Travma Derg 2004;10:160-7
16. Solmazgul E, Uzun G, Cermik H, Atasoyu EM, Aydinoz S, Yildiz S. Hyperbaric oxygen therapy attenuates renal ischemia/reperfusion injury in rats. Urol Int 2007;78:82-5.
17. Carden DL, Granger DL. Pathophysiology of ischemia–reperfusion injury. J Pathol 2000;190:255-66.
18. Mizutani A, Okajima K, Uchiba M, Noguchi T. Activated protein C reduces ischemiayreperfusion-induced renal injury in rats by inhibiting leukocyte activation. Blood 2000;95:3781-7.
19. Hung TK, Pai MP. The effect of varying ambient oxygen tensions on wound metabolism and collagen synthesis. Surg Gynecol Obstet 1972;135:561-7.
20. Rossman JE, Caty MG, Zheng S, Karamanoukian HL, Thusu K, Azizkhan RG *et al.* Mucosal protection from intestinal ischemia-reperfusion reduces oxidant injury to the lung. J Surg Res 1997;73:41-46.
21. Ueno S, Tanabe G, Kihara K, Aoki D, Arikawa K, Dogomori H *et al.* Early post-operative hyperbaric oxygen therapy modifies neutrophil activation. Hepatogastroenterology 1999;46:1798-9.

22. Katircioglu SF, Ulus AT, Gokce P, Surucu S. Iloprost protects the spinal cord during aortic cross-clamping in a canine model. J Cardiovasc Surg (Torino) 2000;1:89-93.
23. Pablos MI, Reiter RJ, Chuang JI, Ortiz GG,Guerrero JM, Sewerynek E *et al*. Acutely administered melatonin reduces oxidative damage in lung and brain induced by hyperbaric oxygen. J Appl Physiol 1997;83:354-8.
24. Boadi WY, Thaire L, Kerem D, Yannai S. Effects of dietary factors on antioxidant enzymes in rats exposed to hyperbaric oxygen. Vet Hum Toxicol 1991;33:105-9.
25. Boadi WY, Thaire L, Kerem D, Yannai S. Effects of supplementation with vitamin E, riboflavin and selenium on central nervous system oxygen toxicity. Pharmacol Toxicol 1991;68:77-82.
26. Puglia CD, Loeb GA. Influence of rat brain superoxide dismutase inhibition by diethyldithiocarbamate upon the rate of development of central nervous system oxygen toxicity. Toxicol Appl Pharmacol 1984;75:258-64.
27. Gurer A, Ozdogan M, Gomceli I, Demirag A, Gulbahar O, Arikok T *et al*. Hyperbaric oxygenation attenuates renal ischemia-reperfusion injury in rats. Transplant Proc 2006;38:3337-40.
28. Jamieson D, Chance B, Cadenas E, Boveris A. The relation of free radical production to hyperoxia. Ann Rev Physiol 1986;8:703-19.
29. Jamieson DD. Lipid peroxidation in brain and lungs from mice exposed to hyperoxia. Biochem Pharmacol 1991;41:749-56.

Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Received on October 12, 2012.
Accepted for publication on April 17, 2013.