Surg Today (2005) 35:653–661
DOI 10.1007/s00595-004-3000-5

SURGERY TODAY

The Effect of Hyperbaric Oxygen Treatment on the Renal Functions in Septic Rats: Relation to Oxidative Damage

MUSTAFA EDREMITLIOĞLU[1], DILEK KILIÇ[2], ŞÜKRÜ ÖTER[4], ÜÇLER KISA[3], AHMET KORKMAZ[4], ÖMER COŞKUN[6], and ORHAN BEDIR[5]

Department of [1] Physiology, [2] Clinical Microbiology and Infectious Disease, and [3] Biochemistry, Kirikkale University School of Medicine, 71100 Kirikkale, Turkey
Departments of [4] Physiology and [5] Microbiology, Gülhane Military Medical Academy, Etlik-Ankara, Turkey
[6] Department of Medical Histology and Embryology, Zonguldak Karaelmas University, Zonguldak, Turkey

Abstract

Purpose. To investigate the effects of hyperbaric oxygen (HBO) treatment on renal functions and damage in septic rats.

Methods. The animals were divided into four groups, each containing ten animals: control, hyperbaric oxygen, sepsis, and sepsis/hyperbaric oxygen. One milliliter of saline containing live *Escherichia coli* cells (2.1×10^9) was injected intraperitoneally to induce sepsis. The groups treated with HBO were given five sessions of 2 atmospheres absolute of 100% oxygen at intervals of 6 h. Blood, urine, and tissue samples were then collected, and the functional renal parameters, malondialdehyde (MDA) levels, and superoxide dismutase (SOD) and catalase activities were examined.

Results. The reduced glomerular filtration rate and urine flow returned to normal levels after HBO treatment; however, the increase in fractionated sodium excretion continued. The increased MDA levels in the renal cortex and medulla also decreased to the level of the control group. In the sepsis group, both the SOD and catalase activities decreased in the renal cortex, while a reduction was observed only in the catalase activity in the medulla. The reduced enzyme activities significantly increased in the sepsis/hyperbaric oxygen group.

Conclusion. HBO treatment has a beneficial effect on renal dysfunction in sepsis. The probable reason for this effect is the reduction in oxidative damage because of the increase in antioxidative capacity.

Key words Hyperbaric oxygen · Kidney function · Sepsis · Oxidative damage · Rat

Reprint requests to: M. Edremitlioğlu
Received: April 21, 2004 / Accepted: November 16, 2004

Introduction

Despite developments in surgical techniques and supportive care, sepsis and its sequelae continue to be one of the main causes of morbidity and mortality in the intensive care unit. Mortality rates range from 16%–64%, depending on the status of host factors, the origin of the sepsis, the degree of infection, and the conditions of intervention.[1–3] Sepsis, doubtlessly, shows a severely poor prognosis and high mortality rates when it is associated with multiorgan dysfunction. Kidneys are one of the organs most affected by sepsis,[4,5] and the corruption of renal function, which is essential for maintaining homeostasis, causes additional sepsis. One reason for the occurrence of renal dysfunction in sepsis is oxidative strees.[6,7] The overproduction of reactive oxygen species (ROS) or an antioxidative defense deficit, for example, in superoxide dismutase (SOD) and catalase (CAT) activities, can cause oxidative stress. Both may occur in sepsis.[8]

High mortality rates have stimulated the search for new approaches to sepsis treatment to supplement the conventional treatments. Among these, an interesting method is hyperbaric oxygen (HBO) treatment, which is based on 100% oxygen exposure at a level of pressure higher than normal atmospheric pressure. HBO treatment is very beneficial in diseases with infectious processes, including refractory osteomyelitis, problem wounds, and necrotizing fasciitis,[9] and its effects on sepsis in some experimental models are being investigated. HBO treatment has been shown to have a beneficial effect in sepsis[10] and sepsis-like zymosan-induced shock models.[11] Thom et al.[10] observed that HBO treatment resulted in declining mortality rates in sepsis cases. Luongo et al.[11] applied HBO to zymosan-induced shock cases and observed no mortality at the end of a 72-h period, while also noting a significant decrease in the peritoneal leukocyte count and the amount of peritoneal exudation.

The key point of HBO treatment is the elevated pO_2 levels reached in the plasma and tissues. The normal alveolar pO_2 level is reached under 1 atmosphere absolute (ATA; 1 ATA = 760 mmHg, which is the normal atmosphere pressure at sea level), and higher pressures cause an increase in the level of oxygen dissolved in the plasma. It is possible to increase the level of oxygen dissolved in plasma from 0.3% ml to 6% ml by exposure to 3 ATA with 100% oxygen, the maximum limit for HBO treatment.[9] This level leads to a 10- to 15-fold increase in tissue oxygenation. The inhalation of oxygen in greater than typical concentrations can stimulate ROS generation in several cell types; however, the results of studies are conflicting. Some studies of HBO treatments show that oxidative damage in several cell types increases,[12–15] while others conclude the opposite.[16–19]

The effect of HBO treatment on renal function in sepsis is unknown. In this study, we investigated whether HBO improved functional renal parameters such as the glomerular filtration rate (GFR), urinary sodium excretion ($U_{Na}V$), fractional excretion of sodium (FE_{Na}), tubular sodium reabsorption (T_{Na}), and urine flow in sepsis-induced rats. Furthermore, we also investigated the effect of HBO treatment on the levels of oxidative damage and antioxidative defense mechanisms in the kidneys of septic rats.

Materials and Methods

Male albino Wistar rats weighing 220–300 g were used. The animals were kept in stainless steel cages and maintained on laboratory food pellets and water ad libitum. The rats were randomly divided into four groups. One group of animals was treated with 1 ml physiologic saline solution (0.9% sodium chloride) by an intraperitoneal (IP) route and served as the control group ($n = 10$). HBO and saline were administered to the second group of rats (HBO group) ($n = 10$). In the third group, sepsis was induced by IP *Escherichia coli* injection (SEP group) ($n = 17$). In the fourth group, HBO was administered following sepsis induction (SEP/HBO group) ($n = 11$). Seven rats in the SEP group and one rat in the SEP/HBO group died during the experiment after *E. coli* inoculation. The surviving ten rats in each group were included in this study. All experiments were performed in accordance with the guidelines for animal experimentation of Kirikkale University School of Medicine.

Experimental Sepsis Model

The rats in the SEP and SEP/HBO groups received an IP inoculum of 1 ml of saline containing viable *E. coli* cells (2.1×10^9). The *E. coli* was isolated from the blood of a septic patient who was hospitalized in the Gülhane Military Medical Academy Hospital.

HBO Treatment Procedure

Hyperbaric treatments were done in a steel animal hyperbaric oxygen chamber. It was flushed with 100% oxygen at the beginning of the treatment, and chamber pressure was increased to 2 ATA in 10 min. For all groups receiving HBO, five sessions of HBO treatment were performed in total. The interval between sessions was 6 h, and each session lasted 90 min. The first session was held 45 min after either the physiologic saline or *E. coli* injection. Gradual decompression to normobaric air at the end of the each session was completed in 5 min. A temperature of 22°–26°C and an airflow of 15 l/min were maintained in the chamber for a period of 90 min.

Surgical Procedure

All animals were subjected to the same surgical procedure. At 45 min after the last HBO session, the rats were anesthetized with a mixture of ketamine and xylazine (100 and 10 mg/kg intramuscularly, respectively). The trachea was exposed and cannulated to ensure an open airway. The right femoral artery, right femoral vein, and bladder were catheterized for arterial blood pressure monitoring, blood infusion, and urine sampling, respectively. The right femoral artery cannula was connected to a pressure transducer (P23 XL, Statham; Statham Instruments, Oxnard, CA, USA) for blood pressure monitoring with a Nec Sanei (Nec-Sanei, Tokyo, Japan) polygraph. The right femoral vein cannula was connected to an infusion pump (May 9601 infusion pump, Commat, Ankara, Turkey) set at 0.060 ml/min, and infusion was started immediately. After 30 min of stabilization following the completion of all surgical procedures, urine samples were collected for 30 min. Next, the kidneys were removed and blood samples were collected. The kidneys were frozen in liquid nitrogen, and blood samples were centrifuged immediately for plasma separation. All samples were stored at –68°C until they were used.

Biochemical Analysis

Proteins in tissue homogenates and plasma and urine creatinine and sodium were determined by an Olympus A800 (Olympus Optical, Tokyo, Japan) autoanalyzer using kits from Olympus.

Malondialdehyde levels (MDA) were measured as a thiobarbituric acid-reactive material. The renal cortex and medulla were carefully separated. All tissue specimens were homogenized in ice cold physiologic

saline. The MDA levels in homogenates were measured spectrophotometrically as described previously.[20] Tetramethoxypropane solution was used as the standard. The MDA values determined in this way were expressed as nanomoles per gram protein in the renal cortex and medulla.

The CAT activity was determined using the method of Aebi.[21] Briefly, slices of renal tissues obtained as before were transferred to tubes containing 50 mM phosphate buffer and homogenized. The homogenates were centrifuged at 700 *g* for 10 min. The supernatant was diluted 50-fold with phosphate buffer, and 200 µl of the diluted supernatant was added to 2.8 ml of 30 mM H_2O_2. The change in absorbance was read at 240 nm. The rate constant of a first-order reaction (k) was used: $k = (2.3/\Delta t) \times \log (A1/A2)$, where Δt is a measured time interval (30 s) and A1 and A2 are the absorbances at the initial and final measurement times, respectively. The CAT activity was expressed as k/mg protein.

The SOD activity was determined using the method of Paoletti et al.[22] Briefly, the renal tissues were homogenized in triethanolamine-diethanolamine (25 mM/25 mM) buffer. The homogenates were centrifuged at 108 800 g for 60 min at 4°C. After 800 µl triethanolamine-diethanolamine (100 mM/100 mM) buffer, 40 µl nicotinamide adenine dinucleotide (NADH) solution (7.5 mM), 25 µl ethylenediaminetetraacetate/$MnCl_2$ solution (100 mM/50 mM), and 100 µl supernatant were mixed thoroughly and read against air at 340 nm for a stable baseline, 100 µl mercaptoethanol solution (10 mM) was added and mixed. The decrease in absorbance was recorded for 20 min. The curves thus obtained were compared with the SOD standard curves. For this purpose, a least-squares linear regression analysis was used. The SOD activity was reported as U/mg protein.

Functional Kidney Parameters

Urine flow, FE_{Na}, $U_{Na}V$, T_{Na}, and GFR were calculated using appropriate formulas.[23] Creatinine clearance was used as an indicator of GFR.

Histopathological Procedures

Kidney tissue specimens were harvested from the dead animals, and fragments from the tissues were fixed in 10% neutral formalin solution, embedded in paraffin, and then stained with hematoxylin and eosin. The preparations were evaluated by a bright field microscope and photographed (Nikon Optiphot 2, Tokyo, Japan).

Statistics

A statistical analysis was performed using SPSS statistical software (version 8.0). The results are expressed as means ± SE. The data were not normally distributed, so differences among multiple groups were assessed using the Kruskal-Wallis test and those between two groups with the Mann-Whitney U test. A value of $P < 0.05$ was considered to be significant.

Results

Blood Pressure

Mean arterial blood pressure (MAP) was 115.8 ± 2.8 mmHg in the control group. MAP values of the other groups were significantly lower than those of the control animals ($P < 0.05$; Table 1). Although the mean blood pressure of the SEP/HBO group was lower than that of the control group, it was significantly higher than that of the SEP group ($P < 0.05$). The mean blood pressure of the HBO group was also higher than that of the SEP group; however, the difference was not statistically significant ($P = 0.052$).

Renal Functions

We observed a decline in urine output, which is widely seen in sepsis. The urine flow was significantly lower in the SEP and HBO groups as compared with the control group (Table 2; $P < 0.05$). In contrast, the urine flow in the SEP/HBO group was significantly higher than those in the HBO and SEP groups.

Table 1. Mean arterial pressure (MAP) and plasma sodium and creatinine levels (means ± SE) in all experimental groups

	Control	HBO	SEP	SEP/HBO
MAP mmHg	115.8 ± 2.8	93.7 ± 2.8[a]	80.0 ± 5.2[a]	95.1 ± 2.7[a,c]
Plasma sodium mmol/l	145.7 ± 0.8	146.4 ± 1.1	151.7 ± 1.0[a,b]	153.1 ± 2.6[a,b]
Plasma creatinine mg/dl	0.35 ± 0.01	0.38 ± 0.02	0.82 ± 0.16[a,b]	0.36 ± 0.03[c]

HBO, hyperbaric treatment group; SEP, sepsis-induced group; SEP/HBO, sepsis-induced and hyperbaric treatment group; MAP, mean arterial blood pressure

[a] $P < 0.05$ vs. control group

[b] $P < 0.05$ vs. HBO group

[c] $P < 0.05$ vs. SEP group

Table 2. Functional kidney parameters (means ± SE) in all experimental groups

	Control	HBO	SEP	SEP/HBO
Urine flow µl/min	11.98 ± 1.77	7.55 ± 0.99[a]	5.31 ± 0.51[a]	13.05 ± 1.81[b,c]
GFR µl/min per 100 g body wt	589.49 ± 31.40	393.61 ± 26.02[a]	240.26 ± 35.09[a,b]	671.17 ± 69.81[b,c]
$U_{Na}V$ µmol/min	0.762 ± 0.117	0.795 ± 0.298	0.429 ± 0.053[a]	0.993 ± 0.125[c]
FE_{Na} %	0.40 ± 0.09	0.56 ± 0.20	0.61 ± 0.12[a]	0.52 ± 0.05[a]
T_{Na} mmol/min	0.207 ± 0.017	0.146 ± 0.013[a]	0.083 ± 0.012[a,b]	0.184 ± 0.019[c]

GFR, glomelular filtration rate; $U_{Na}V$, urinary sodium excretion; FE_{Na}, fractional excretion of sodium; T_{Na}, tubular sodium reabsorption
[a] $P < 0.05$ vs. control group
[b] $P < 0.05$ vs. HBO group
[c] $P < 0.05$ vs. SEP group

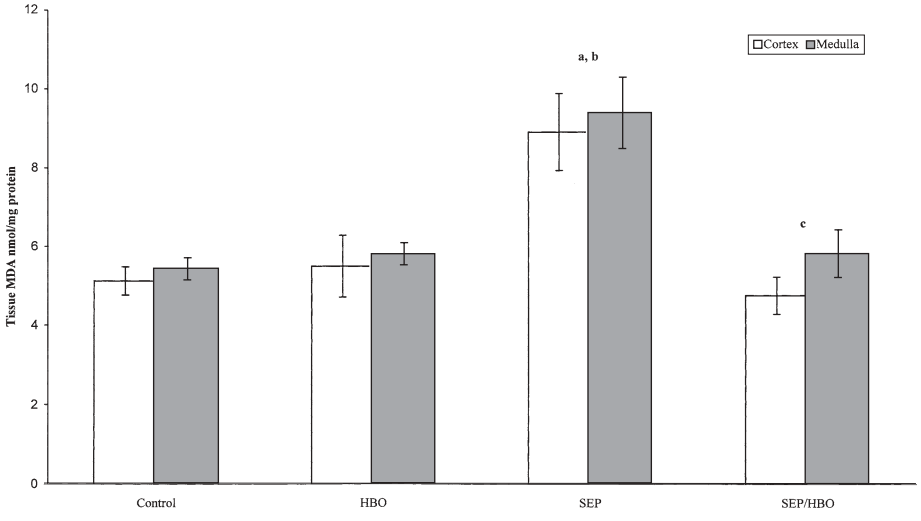

Fig. 1. Kidney cortex and medulla malondialdehyde (*MDA*) levels in all experimental groups. *Bar heights* are the means; *error bars* indicate ± SEM. *HBO*, hyperbaric oxygen group; *SEP*, sepsis induction group; *SEP/HBO*, sepsis induction and hyperbaric oxygen group; *a*, $P < 0.05$ vs. control group; *b*, $P < 0.05$ vs. HBO group; *c*, $P < 0.05$ vs. SEP group

Similar to the urine flow, GFR was also found to be lower in the SEP group ($P < 0.05$). Similarly, GFR in the HBO group was lower than in the control group ($P < 0.05$). Interestingly, while the GFR values in the SEP and HBO groups were lower, the GFR value in the SEP/HBO group was higher than in those in the HBO and SEP groups ($P < 0.05$).

When we examined the $U_{Na}V$ values, we observed a significant decrease in the SEP group versus the control group. In addition, the $U_{Na}V$ values in the SEP/HBO group were significantly higher than those in the SEP group ($P < 0.05$).

FE_{Na}, another functional renal parameter, was found to be higher in the SEP and SEP/HBO groups than in the control group ($P < 0.05$), but the high level of FE_{Na} in the HBO group was not statistically significant.

The T_{Na} values in the HBO group were lower than in the control group. The T_{Na} value in the SEP group was considerably lower than in all of the other groups ($P < 0.05$).

Plasma Sodium and Creatinine Levels

The plasma sodium values were higher in both septic groups ($P < 0.05$). There was an increase in the plasma creatinine levels in the SEP group compared with the control and HBO groups ($P < 0.05$). However, the plasma creatinine levels in the SEP/HBO group were significantly lower than those in the SEP group (Table 1).

Oxidative Damage in the Kidney

The MDA levels, an indicator of oxidative damage, increased in the renal cortex and medulla in the SEP group (Fig. 1). However, this increase did not occur in the SEP/HBO group.

CAT and SOD Activities in the Kidney

The CAT activities of the control, HBO, SEP, and SEP/HBO groups were, respectively, 0.123 ± 0.013, 0.108 ± 0.006, 0.064 ± 0.006, and 0.120 ± 0.014 *k*/mg protein in the cortex, and 0.161 ± 0.010, 0.167 ± 0.022, 0.085 ± 0.006

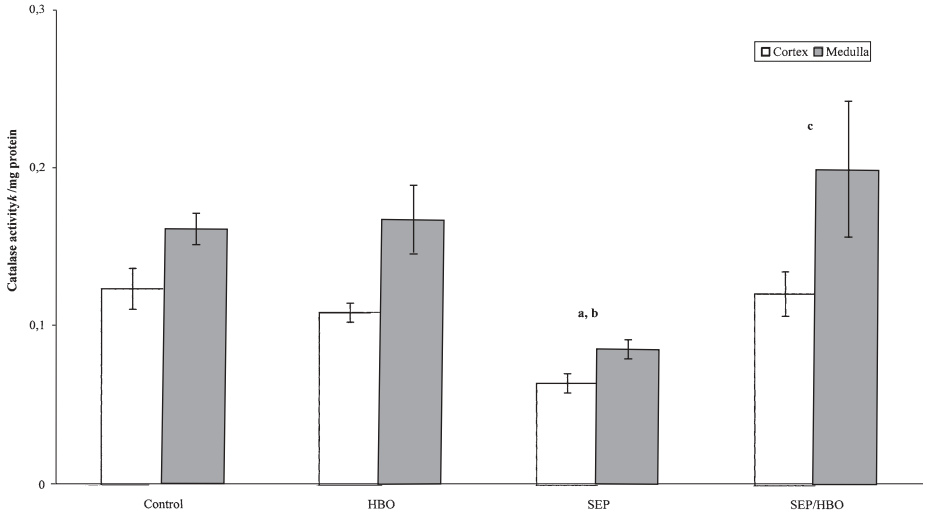

Fig. 2. Kidney cortex and medulla catalase activities in all experimental groups. *Bar heights* are the means; *error bars* indicate ± SEM. *a*, $P < 0.05$ vs. control group; *b*, $P < 0.05$ vs. HBO group; *c*, $P < 0.05$ vs. SEP group

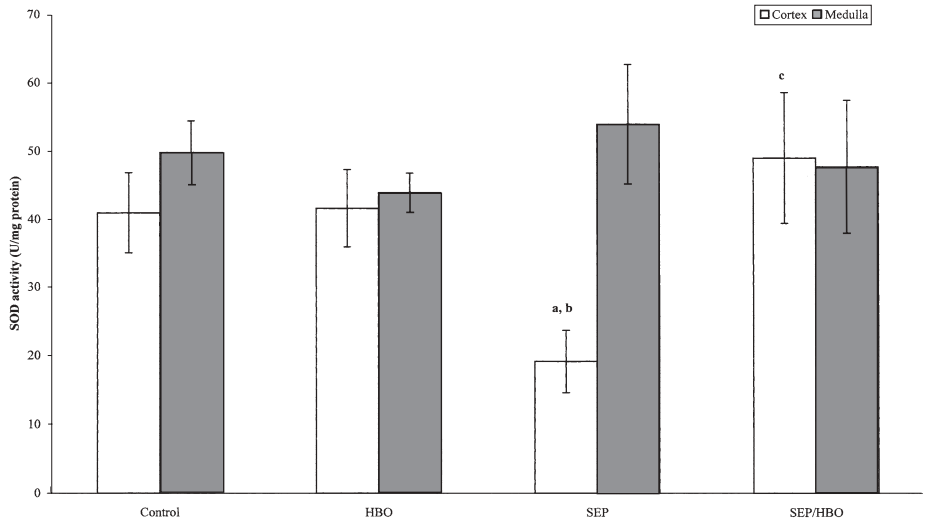

Fig. 3. Kidney cortex and medulla superoxide dismutase (*SOD*) activities in all experimental groups. *Bar heights* are the means; *error bars* indicate ± SEM. *a*, $P < 0.05$ vs. control group; *b*, $P < 0.05$ vs. HBO group; *c*, $P < 0.05$ vs. SEP group

and 0.199 ± 0.043 *k*/mg protein in the medulla (Fig. 2). As mentioned before, the MDA levels, a sign of oxidative damage, clearly increased in both the cortex and medulla in the SEP group (Fig. 1), and were accompanied by a concurrent decrease in CAT activity, which is an important component of antioxidative capacity. However, the CAT activity in the SEP/HBO group was significantly higher than that in the SEP group.

Similarly, a considerable decrease was observed in the renal cortex SOD activity in the SEP group (Fig. 3). Although the renal cortex SOD activities in the control, HBO, and SEP/HBO groups (respectively, 40.93 ± 5.89, 41.62 ± 5.68, and 48.96 ± 9.56 U/mg protein) were similar, the SOD activity in the SEP group (19.16 ± 4.53 U/mg protein) was significantly lower than in the other groups. However, we could not find such a difference in the medulla SOD activities. The SOD activities in the medulla (as U/mg protein) were 49.77 ± 4.63 in the control group, 43.92 ± 2.86 in the HBO group, 53.91 ± 8.67 in the SEP group, and 47.68 ± 9.72 in the SEP/HBO group.

Histopathological Findings

The control and HBO groups showed normal histological results (Fig. 4A,B). The most consistent findings in the histological sections of renal tissues of rats in the SEP group stained with hematoxylin and eosin were severe degenerative changes, with shrunken tubules and glomerules of the renal cortex. There was also severe mononuclear cell infiltration with congestion among glomerules and tubules in the renal cortex (Fig.

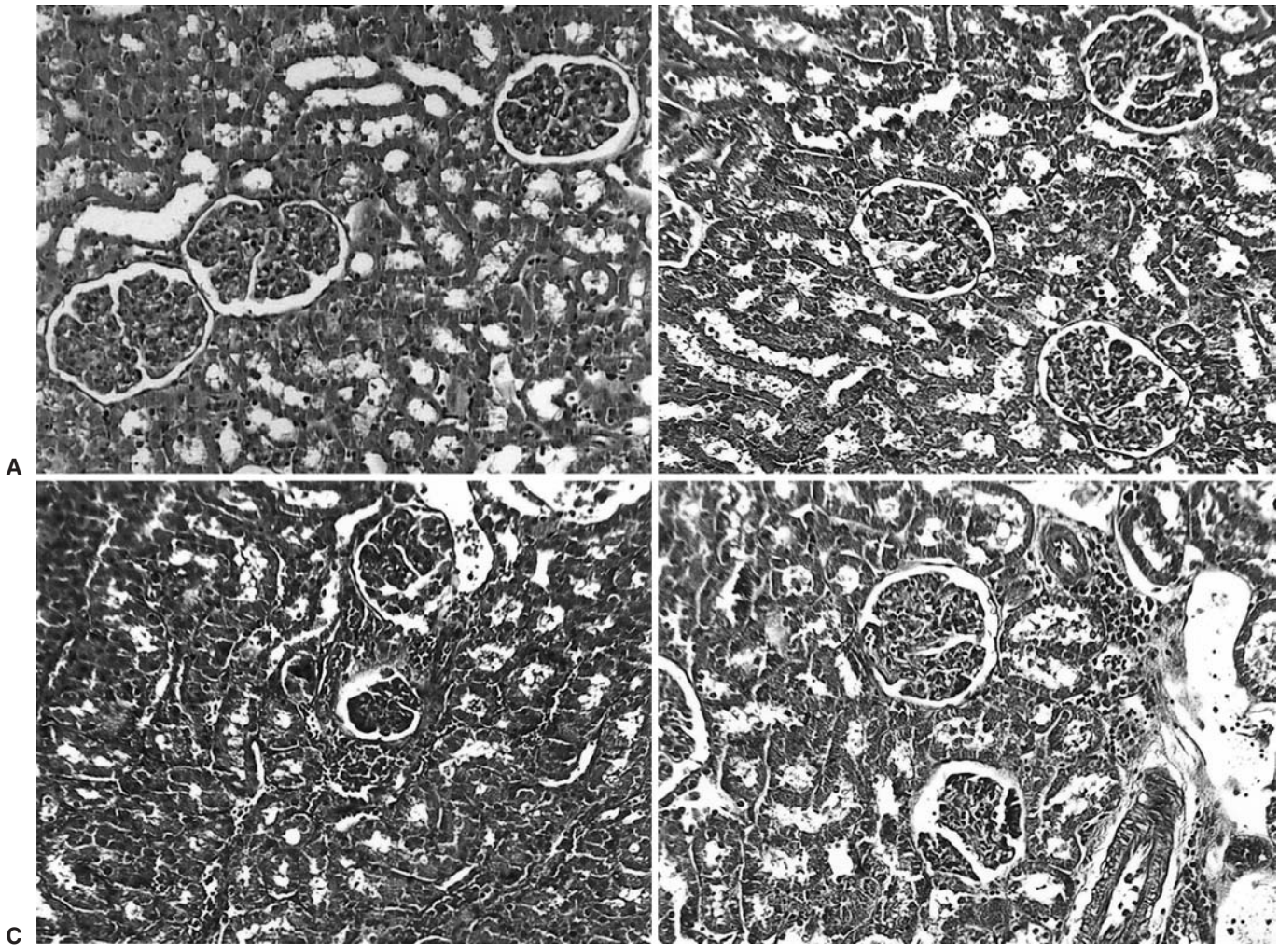

Fig. 4A–D. Kidney histology in all experimental groups (hematoxylin and eosin; 360×). **A** Control group; **B** HBO group. **C** SEP group: focal, intense interstitial mononuclear cell infiltration, severe degenerative changes, and shrunken tubules and glomerules of the renal cortex are observed. Congestion of the vessels is dominant. **D** SEP/HBO group: the severity of degenerative changes in the glomerules and especially in the tubules of the renal cortex was less than that in the SEP group. Although the active hyperemic fields slightly decreased, mononuclear cell infiltration was still severe

4C). In the SEP/HBO group, the histology of the kidneys of three rats was completely normal. The severity of degenerative changes in the glomerules and tubules of the renal cortex of the kidneys of the other rats in SEP/HBO group were considerably lower than those in the SEP group. Although the degree of congestion (active hyperemic fields) slightly decreased, mononuclear cell infiltration was still severe (Fig. 4D).

Discussion

The results of this study showed that renal dysfunction in sepsis was attenuated by the use of HBO, and that this improvement was accompanied by a concurrent decrease in the MDA level in renal tissue, an increase in the SOD and CAT activities in the renal cortex, and an increase in the CAT activity in the renal medulla. The corruption of organ functions by sepsis makes treatment difficult and increases the mortality rate. Because the kidneys play an essential role in maintaining homeostasis, they are among the most often affected organs in sepsis. All of the functional renal parameters included in this study were corrupted in septic rats.

The substantial increase in the plasma creatinine level and the decrease in GFR indicate that renal failure was generated in the SEP group. FE_{Na}, $U_{Na}V$, and T_{Na} were indicators of tubular function in this study. Increased FE_{Na} and decreased T_{Na} in the SEP group as compared with the control group indicated tubular damage. The histological sections also clearly illustrated tubular damage (Fig. 4).

It is known that cellular oxidative damage plays a crucial role in multiple organ dysfunction in sepsis.[24] Renal dysfunction in rats in septic shock has been reported to improve in the presence of free radical scavengers.[6,7] ROS show their pathophysiological effects by directly attacking lipids[25] and proteins[26] in biological membranes, causing their dysfunction in cases of ROS overproduction or ineffective ROS scavenging. MDA is produced by lipid peroxidation caused by ROS in biological membranes, and it is a reliable indicator of oxidative damage. Renal dysfuction occurs together with increased oxidative damage in experimental models of cyclosporin nephrotoxicity[27] and glycerol-induced acute renal failure.[28] The administration of antioxidative agents in these studies ameliorated renal dysfunction. In our study, too, corrupted renal functions were accompanied by a significant increase in MDA levels in the renal cortex and medulla in the SEP group. In sepsis, it is well known that oxidative stress increases, causing antioxidative defense mechanisms, including SOD and CAT activities to become inadequate because of the overproduction of ROS.[8] We also observed a decrease in SOD and CAT activities, indicating a reduction of antioxidative capacity. As a result, the oxidative damage increased in the SEP group. Although SOD activities in the renal cortex of rats in the SEP group did not decrease, there was a decrease in CAT activities; therefore, the increase in the oxidative damage may be attributable to the relative inadequacy of the break-down process of the hydrogen peroxide generated by the action of SOD.

However, in the SEP/HBO group, we observed that the level of MDA stabilized, and indicators of renal function improved, except for FE_{Na}. High FE_{Na} values occur in the late stages of endotoxemia and are considered an indicator of tubular damage.[29] In this study, we found that, although the $U_{Na}V$ and T_{Na} values of the SEP/HBO group were not significantly different from those of the control group, FE_{Na} values were higher, suggesting that tubular function was not completely restored by the HBO protocol we used. Additional studies using a longer treatment protocol need to be carried out in order to find out whether HBO can restore tubular functions impaired by sepsis.

Although there was no improvement in tubular function, we observed complete recovery of plasma creatinine, GFR, and urine output levels, indicating the restoration of renal function. This improvement was accompanied by the development of degenerative changes in the histological sections (Fig. 4D). ROS directly cause vasoconstriction in kidney microcirculation. Furthermore, they are indirectly affect vascular tone by affecting the action or production of other agents that modulate vascular tone. They also affect medullary blood flow and the liquid and electrolyte balance.[30] When the balance between ROS formation and the defense mechanisms changes, kidney function is inevitably negatively affected. In fact, Wang et al.[31] determined that the reduction of GFR in endotoxemia recovered considerably after administration of an SOD mimetic. In another study, Leach et al.[6] concluded that kidney damage in endotoxemia was a result of an increase in the formation of superoxides and other ROS and the inadequacy of defense mechanisms. The level of oxidative damage in the kidneys is closely related to SOD and CAT activities.[32] Because the SOD and CAT activities in the renal cortex of the SEP/HBO group were significantly higher than those of the SEP group, the improvement in renal function was apparently related to an increase in antioxidative capacity and a corresponding decrease in oxidative damage.

It is known that hyperbaric oxygen treatment can change SOD and CAT activities. It has been shown that SOD activities are increased by HBO treatment in rat extensor digitorum longus muscle,[33] in pancreas and erythrocytes of rats with induced acute necrotizing pancreatitis,[16] and in the lungs of rats and guinea pigs[37] and in the erythrocytes of human patients with multiple sclerosis.[35] Similarly, CAT activities increase in rat heart[36] and in the erythrocytes of human patients with multiple sclerosis.[35] The increase in the myocardial CAT activity was determined to be caused by HBO, which increases CAT mRNA by a pretranslation effect. Furthermore, a 200% increase in mRNA by three sessions of HBO treatment reached a level of 600% after five sessions.[36] However, some studies have shown that CAT activities in rat soleus muscle[33] and in brain and lungs[34] decrease after HBO treatment. However, different HBO protocols were used in these studies. In addition, regarding the renal cortex and medulla SOD activities in the SEP group in our study, it is reasonable that SOD expression differs depending on tissue type. No previous study has reported changes in renal SOD and CAT activities from hyperbaric oxygen treatment, so we could not compare our results with those of other studies.

One conclusion of this study is that the effects of HBO treatment were not limited by the length of the HBO sessions. This can be clearly seen by looking at the mean arterial pressure (Table 1) and functional renal parameters (Table 2) of the HBO group. Although we began recording these parameters 45 min after the last HBO session, there were clear differences compared with the control group. Nitric oxide synthesis, which is very effective in regulating renal hemodynamic variables and arterial pressure, has recently been shown to increase as a result of HBO treatment. Thom et al.[37] showed such an increase in the perivascular part of the rat aorta, and Elayan et al.[38] showed it in the rat brain. Furthermore, No levels remained high for several minutes after the end of HBO treatment in both studies.

However, further investigations are needed to clarify the relationship between HBO treatment and nitric oxide.

In our research, we included a group that was treated only by HBO (HBO group) in order to examine the effects of HBO treatment on not only septic kidneys but also healthy ones. $U_{Na}V$ values were observed to be higher in the control, HBO, and SEP/HBO groups than in the SEP group (Table 2). The reason for this seems to be the high urine flow values in the control and SEP/HBO groups. Even though, the $U_{Na}V$ level of the group treated with HBO alone is close to that of control group, the urine flow rate of the HBO group was significantly lower than that of the control group (Table 2). In this case, HBO seems to have a natriuretic effect. In fact, the FE_{Na} and T_{Na} values of the HBO group suggest natriuresis as well. The effects of HBO treatment on healthy kidneys are unknown. Detailed studies should thus be carried out to elucidate how tubular function and renal hemodynamics are affected by HBO.

The most surprising result of this study is the mean GFR value in septic animals treated with hyperbaric oxygen. Even though HBO treatment (HBO group) or sepsis (SEP group) separately showed a decreased GFR when the two treatments were applied together they showed an increased GFR. Therefore, hyperbaric oxygen treatment seems to have different effects on healthy and damaged kidneys. We speculate that the decreased GFR in the HBO group resulted from hemodynamic changes generated by the decrease in blood pressure, because the status of the HBO group was not complex and did not involve mediators such as sepsis. In addition, oxidative damage was not different from that in the control group. However, in the SEP/HBO group, numerous sepsis-caused cytokines, which probably affect renal function were present. In the SEP group, the oxidative stress faced by the kidneys also causes deterioration of their status. In the SEP/HBO group, we could show that there was less oxidative damage. However, it is also known that HBO treatment affects cytokines.[39] Therefore, the additional effect on GFR in the SEP/HBO group is a complex problem to explain.

The glomerular filtration determinants, which are damaged in sepsis, were improved by HBO treatment. The GFR, urine output, and plasma creatinine values in the SEP/HBO group also indicate recovery from renal failure. Kidney inefficiency considerably increases mortality rates in diseases such as sepsis, which cause serious corruption of the patient's general health.[40] Therefore, the use of HBO treatment in septic patients who develop renal failure appears to be useful. However, it HBO does not reduce tubular damage in sepsis. In spite of the wide use of HBO treatment for many diseases, the effective mechanisms are still not well understood. In our study, the prevention of renal oxidative damage by HBO treatment may have ameliorated renal dysfunction in sepsis. The increase in the antioxidative capacity (SOD and CAT activities) by HBO treatment may have played a role in decreasing oxidative damage. The effects of HBO should be clarified in complicated conditions such as sepsis, in which various physiologic regulatory mechanisms are involved. By taking into consideration the findings of previous studies, it may be stated that HBO treatment considerably improves survival from sepsis and sepsis-like zymosan-induced shock.[9,10] Although, HBO demonstrated a beneficial effect on renal dysfunction according to our findings, numerous studies much still be performed before HBO treatment can be recommended as an effective treatment modality for sepsis.

Acknowledgment. This work was supported by Kırıkkale University Research Foundation Grant 01/08-01-06. We are very grateful to Esen Özdemir for helpful discussions and her review of the manuscript.

References

1. Knaus WA, Sun X, Nystrom O, Wagner DP. Evaluation of definitions for sepsis. Chest 1992;101:1656–62.
2. Pittet D, Thievent B, Wenzel RP, Li N, Auckenthaler R, Suter PM. Bedside prediction of mortality from bacteremic sepsis. A dynamic analysis of ICU patients. Am J Respir Crit Care Med 1996;153:684–93.
3. Rangel-Frausto MS, Pittet D, Costigan M, Hwang T, Davis CS, Wenzel RP. The natural history of the systemic inflammatory response syndrome (SIRS). A prospective study. JAMA 1995; 273:117–23.
4. Doğanay M. Sepsis. In: Topçu AW, Söyletir G, Doğanay M, editors. İnfeksiyon Hastalıkları ve Mikrobiyolojisi. İstanbul: Nobel Tıp Kitabevleri; 2002. p. 621–36.
5. Parrillo JE. Pathogenetic mechanisms of septic shock. N Engl J Med 1993;328:1471–7.
6. Leach M, Frank S, Olbrich A, Pfeilschifter J, Thiemermann C. Decline in the expression of copper/zinc superoxide dismutase in the kidney of rats with endotoxic shock: effects of the superoxide anion radical scavenger, tempol, on organ injury. Br J Pharmacol 1998;125:817–25.
7. Krysztopik RJ, Bentley FR, Spain DA, Wilson MA, Garrison RN. Free radical scavenging by lazaroids improves renal blood flow during sepsis. Surgery 1996;120:657–62.
8. Macdonald J, Galley HF, Webster NR. Oxidative stress and gene expression in sepsis. Br J Anaesth 2003;90:221–32.
9. Tibbles PM, Edelsberg JS. Hyperbaric oxygen therapy. N Engl J Med 1996;334:1642–8.
10. Thom SR, Lauermann MW, Hart GB. Intermittent hyperbaric oxygen therapy for reduction of mortality in experimental polymicrobial sepsis. J Infect Dis 1986;154:504–10.
11. Luongo C, Imperatore F, Cuzzocrea S, Filippelli A, Scafuro MA, Mangoni G, et al. Effects of hyperbaric oxygen exposure on a zymosan-induced shock model. Crit Care Med 1998;26:1972–6.
12. Etlik O, Tomur A, Dundar K, Erdem A, Gundogan NU. The effect of antioxidant vitamins E and C on lipoperoxidation of erythrocyte membranes during hyperbaric oxygenations. J Basic Clin Physiol Pharmacol 1997;8:269–77.
13. Monstrey SJ, Mullick P, Narayanan K, Ramasastry SS. Hyperbaric oxygen therapy and free radical production: an experimen-

tal study in doxorubicin (Adriamycin) extravasation injuries. Ann Plast Surg 1997;38:163–8.
14. Shang F, Gong X, Egtesadi S, Meydani M, Smith D, Perrone G, et al. Vitamin C prevents hyperbaric oxygen-induced growth retardation and lipid peroxidation and attenuates the oxidation-induced up-regulation of glutathione in guinea pigs. J Nutr Biochem 2002;13:307–13.
15. Pablos MI, Reiter RJ, Chuang J-I, 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.
16. Yasar M, Yildiz S, Mas R, Dundar K, Yildirim A, Korkmaz A, et al. The effect of hyperbaric oxygen treatment on oxidative stress in experimental acute necrotizing pancratitis. Physiol Res 2003;52:111–6.
17. Chen MF, Chen HM, Ueng SW, Shyr MH. Hyperbaric oxygen pretreatment attenuates hepatic reperfusion injury. Liver 1998;18: 110–6.
18. Kudchodkar BJ, Wilson J, Lacko A, Dory L. Hyperbaric oxygen reduces the progression and accelerates the regression of atherosclerosis in rabbits. Arterioscler Thromb Vasc Biol 2000;20:1637–43.
19. Cuzzocrea S, Imperatore F, Costantino G, Luongo C, Mazzon E, Scafuro MA, et al. Role of hyperbaric oxygen exposure in reduction on lipid peroxidation and in multiple organ failure induced by zymosan administration in the rat. Shock 2000;13:197–203.
20. Yagi K. Lipid peroxides in hepatic, gastrointestinal, and pancreatic diseases. In: Armstrong D, editor. Free radicals in diagnostic medicine. New York: Plenum Press; 1994. p. 165–9.
21. Aebi H. Catalase. In: Bergmeyer HU, editor. Methods of enzymatic analysis. 2nd English ed., volume 2. New York and London: Academic Press; 1974. p. 673–84.
22. Paoletti F, Aldinucci D, Mocali A, Caparrini A. A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. Anal Biochem 1986;154:536–41.
23. Laiken ND, Fanestil DD. Body fluids and renal function. In: West JB, editor. Best and Taylor's physiological basis of medical practise. Baltimore: Williams and Wilkins; 1990. p. 406–515.
24. Tanjoh K, Shima A, Aida M, Tomita R, Kurosu Y. Nitric oxide and active oxygen species in severe sepsis and surgically stressed patients. Surg Today 1995;25:774–7.
25. Yamamoto Y. Oxidation of biological membranes and its inhibition. Free radical chain oxidation of erythrocyte ghost membranes by oxygen. Biochim Biophys Acta 1985;819:29–36.
26. Koster JF, Slee RG. Lipid peroxidation of human erythrocyte ghosts induced by organic hydroperoxides. Biochim Biophys Acta 1983;752:233–9.
27. Wang C, Salahudeen AK. Lipid peroxidation accompanies cyclosporine nephrotoxicity: effects of vitamin E. Kidney Int 1995;47:927–34.
28. Ferraz FF, Kos AG, Janino P, Homsi E. Effects of melatonin administration to rats with glycerol-induced acute renal failure. Ren Fail 2002;24:735–46.
29. Khan RZ, Badr KF. Endotoxin and renal function: perspectives to the understanding of septic acute renal failure and toxic shock. Nephrol Dial Transplant 1999;14:814–8.
30. Schnackenberg CG. Physiological and pathophysiological roles of oxygen radicals in the renal microvasculature. Am J Physiol Regul Integr Comp Physiol 2002;282:R335–42.
31. Wang W. Jittikanont S, Falk SA, Li P, Feng L, Gengaro PE, et al. Interaction among nitric oxide, reactive oxygen species, and antioxidants during endotoxemia-related acute renal failure. Am J Physiol Renal Physiol 2003;284:F532–7.
32. Hamano K, Tsuboi H, Seyama A, Esato K. Shock-reinfusion injury to the central organs and the effect of free radical scavengers in the rat. Surg Today 1993;23:891–6.
33. Gregorevic P, Lynch GS, Williams DA. Hyperbaric oxygen modulates antioxidant enzyme activity in rat skeletal muscles. Eur J Appl Physiol 2001;86:24–7.
34. Harabin AL, Braisted JC, Flynn ET. Response of antioxidant enzymes to intermittent and continuous hyperbaric oxygen. J Appl Physiol 1990;69:328–35.
35. Ansari KA, Wilson M, Slater GE, Haglin JJ, Kaplan E. Hyperbaric oxygenation and erythrocyte antioxidant enzymes in multiple sclerosis patients. Acta Neurol Scand 1986;74:156–60.
36. Kim CH, Chol H, Chun YS, Kim GT, Park JW, Kim MS. Hyperbaric oxygenation pretreatment induces catalase and reduces infarct size in ischemic rat myocardium. Pflugers Arch 2001;442: 519–25.
37. Thom SR, Fisher D, Zhang J, Bhopale VM, Ohnishi ST, Kotake Y, et al. Stimulation of perivascular nitric oxide synthesis by oxygen. Am J Physiol Heart Circ Physiol 2003;284:H1230–9.
38. Elayan IM, Axley MJ, Prasad PV, Ahlers ST, Auker CR. Effect of hyperbaric oxygen treatment on nitric oxide and oxygen free radicals in rat brain. J Neurophysiol 2000;83:2022–9.
39. Yamashita M, Yamashita M. Hyperbaric oxygen treatment attenuates cytokine induction after massive hemorrhage. Am J Physiol Endocrinol Metab 2000;278:E811–6.
40. Shinkawa H, Yasuhara H, Naka S, Yanagie H, Nojiri T, Furuya Y, et al. Factors affecting the early mortality of patients with montraumatic colorectal perforation. Surg Today 2003;33: 13–7.