Hyperbaric oxygen treatment ameliorates gentamicin-induced nephrotoxicity and expression of kidney injury molecule 1 in the rat model

Özlem Öztopuz 1, Hakan Türkön 2, Müşerref Hilal Şehitoğlu 2, Başak Büyük 3, Metehan Uzun 4, Mehmet Akif Ovalı 4, Ufuk Demir 4

1 Çanakkale Onsekiz Mart University, Faculty of Medicine, Department of Biophysic, Çanakkale Turkey
2 Çanakkale Onsekiz Mart University, Faculty of Medicine, Department of Biochemistry, Çanakkale Turkey
3 Çanakkale Onsekiz Mart University, Faculty of Medicine, Department of Histology and Embryology, Çanakkale Turkey
4 Çanakkale Onsekiz Mart University, Faculty of Medicine, Department of Physiology, Çanakkale Turkey

CORRESPONDING AUTHOR: Özlem Öztopuz – ozlemoztopuz@yahoo.com.tr

ABSTRACT

In recent years, hyperbaric oxygen (HBO₂) therapy has been considered as an effective method for the treatment of gentamicin (GM)-induced renal toxicity. However, the findings related to the use of HBO₂ for GM toxicity are limited and contradictory. The aim of this study is to investigate the protective role of HBO₂ on GM-induced nephrotoxicity. For this purpose, Wistar albino rats (n=28) were randomly divided into four equal groups: C, HBO₂, GM and GM+HBO₂. GM (100 mg/kg, ip) and HBO₂ were applied over seven days. On the eighth day blood and kidney tissue samples were harvested. The albumin, creatinine, and urea levels were determined from serum samples. Superoxide dismutase (SOD), glutathion peroxidase (GSH-Px) activities, malondialdehyde (MDA), total antioxidant status (TAS) and total oxidant status (TOS) values were analyzed spectrophotometrically. The relative expression level of TNF-α, IL-1β and Kim-1 gene were determined by qRT-PCR assays; histopathologic investigation was completed in kidney tissue samples. Serum urea, albumin and creatinine levels significantly increased in the GM group compared to the GM+HBO₂ group. For antioxidant parameters the GM+HBO₂ group was not statistically different from the C group but was significantly different compared with the GM group. TNF-α, IL-1β and Kim-1 gene expression levels in the GM group were statistically increased compared to the GM+HBO₂ group (p=0.015, p=0.024, p=0.004) respectively. Severe tubular necrosis, epithelial desquamation and mild peritubular hemorrhage were observed in the GM-administrated group, while HBO₂ exposure ameliorated these alterations. In conclusion, HBO₂ exposure may be defined as a potential method for the prevention of GM-induced renal toxicity.

INTRODUCTION

Gentamicin (GM) is currently used as a bactericidal aminoglycoside against gram-negative infections, despite the development of nephrotoxicity [1,2]. The side effects of GM such as nephrotoxicity and ototoxicity may limit its use and cause an interruption of antibiotic treatment. Renal insufficiency has been observed in 30% of patients who used GM over seven days [3]. GM usually accumulates in tubular cells by protein transport [3]. As a result of nephrotoxicity, vascular, glomerular and tubular modifications as well as cellular membrane functions and structures may be changed [4,5]. The underlying mechanism of GM toxicity has not been fully explained. However, according to a previous study oxidative stress plays an essential role in the mechanism of nephrotoxicity linked to GM [1]. GM inhibits oxidative phosphorylation and reduces ATP levels in renal tubular cells [6]. GM also promotes the production of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radicals in kidneys [1]. Nephrotoxicity of GM involves complex mechanisms because GM toxicity occurs after increases in urea and

KEYWORDS: hyperbaric oxygen therapy; gentamicin; nephrotoxicity; rat
creatinine concentrations [7,8]. In recent years, gene expression of the kidney injury molecule 1 (Kim-1) has been investigated in GM nephrotoxicity [7,9]. This molecule, expressed in renal tubules, is one of the markers of necrotic and apoptotic processes [8,9]. Tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) are known as other considerably inflammatory molecules in renal injury [10].

Hyperbaric oxygen (HBO₂) therapy is a relatively safe and adjunct method in which patients are exposed continuously to high oxygen pressure over a short period [11,12]. Administration of HBO₂ causes an increase in arterial and tissue oxygen levels. It has been reported that HBO₂ exposure may be beneficial for renal injury related to both sepsis and ischemia-reperfusion injury [13,14]. Atasoyu, et al. [15] reported that, in rats, HBO₂ has a protective effect on the kidney injury caused by cisplatin. However, the findings related to the use of HBO₂ in renal injury are limited and contradictory. For instance, a study using rats reported that HBO₂ exposure intensified vancomycin-induced nephrotoxicity [16]. Moreover, it has been suggested that HBO₂ administration increases the renal damage in vancomycin-induced kidney toxicity and that HBO₂ has no protective effect on gentamicin-induced kidney toxicity [16].

Although the protective effect of HBO₂ exposure was reported in recent studies, limited and contradictory results indicate that further studies are required in this area. For that purpose, this study aimed to determine if HBO₂ exposure(s) provided protection(s) from GM-induced renal toxicity using histochemical, genetics, and biochemical methods in rats.

**MATERIAL AND METHODS**

**Animal model**

Male Wistar rats weighing 200±20 grams (g) were used in the study. The animals were maintained under standard housing conditions (22°C room temperature and 12-hour light/dark cycle) and supplied with standard rodent chow and tap water ad libitum. All animal procedures were approved by the Çanakkale 18 Mart University Institutional Animal Care and Use Committee (Protocol number: 2017/04-15).

GM sulfate was obtained from Fujikan Fukang Pharmaceutical Co. Ltd. The daily dose of GM (100 mg/kg/day) given to each rat as a single injection. HBO₂ was administered using a locally manufactured hyperbaric chamber designed to contain 20 rats. HBO₂ exposure consisted of administering 100% oxygen at 2.5 atmospheres absolute (ATA) for 90 minutes, including 15 minutes for compression and five minutes for decompression.

On the eighth day of the study rats were anesthetized with 5 mg/kg xylazine (Rompun®, Bayer, Istanbul, Turkey) and 50 mg/kg ketamine hydrochloride (Ketalar®, Eczacıbasi, Istanbul, Turkey).

**Experimental design**

Twenty-eight rats were randomly assigned to four groups.

- **Group 1 (C):** Rats were injected intraperitoneally (ip) with isotonic saline solution for seven days (n=7).
- **Group 2 (HBO₂):** HBO₂ exposure was administered to the rats for seven days (100% oxygen at 90-minute intervals every 24 hours under 2.5-ATA pressure; n=7).
- **Group 3 (GM):** Rats were injected intraperitoneally (ip) with GM (100 mg/kg) for seven days (n=7).
- **Group 4 (GM+HBO₂):** Rats were treated with GM (100 mg/kg) and HBO₂ (100% oxygen at 90-minute intervals every 24 hours under 2.5-ATA pressure; n=7) for seven days.

On day 8, blood samples were drawn by cardiac puncture under anesthesia conditions. After sacrifice, rat kidneys were immediately harvested for genetic, biochemical and histological examination in all groups.

**Biochemical assays**

Kidney tissues and blood samples were harvested for biochemical analysis after the experimental procedure. The blood samples, taken in tubes without anticoagulants, were centrifuged at 4,000 rpm for 10 minutes. Resultant serum samples were stored at -80 °C until analysis. Albumin, urea and creatinine levels were determined using a colorimetric method.

Kidney samples were weighed, cut into small pieces and homogenized. Assays were performed on the supernatant of the homogenate that was prepared by centrifugation at 4,000 rpm for five minutes at +4 °C. The protein contents of the tissues were determined according to the Bradford assay method.

Superoxide dismutase (SOD; EC 1.15.1.1) activities and IC₅₀ (50% inhibition of SOD activity) values were determined colorimetrically at 450 nanometers (nm) using the SOD assay kit (Sigma-Aldrich-19160, St. Louis, Missouri U.S.). The results are expressed as U/mL (U/mg protein. mL) per mg of protein. Glutathione peroxidase (GSH-Px; EC 1.6.4.2) of kidney tissues was determined using a ELISA kit (ADI-900-158, Glutathione Peroxidase Activity
The results are expressed as U/mL (U/mg protein.mL) per milligram of protein. Malondialdehyde (MDA) levels (STA-330) were determined using the Cell Biolabs’ OxiSelect™ TBARS Assay Kit (MDA Quantitation). Total antioxidant (TAS) and total oxidant status (TOS) levels were analyzed with spectrophotometric kits (Rel Assay Diagnostics, Gaziantep, Turkey). Results for TAS and TOS are expressed as mmol Trolox Equiv./mg protein and µmol H₂O₂ Equiv./mg protein, respectively. The ratio percentage of TOS to TAS was used to calculate the oxidative stress index (OSI): specifically, OSI (arbitrary unit) 1/4 \[(TOS, \text{mmol Trolox Equiv./mg protein})/(TAS,\text{µmol H}_2\text{O}_2 \text{ Equiv./mg protein})\].

**Gene expression**

Total ribonucleic acid (RNA) was isolated from 10-30 mg kidney tissue using a QIAamp RNA spin column (PureLink RNA MiniKit, Ambion) according to manufacturer recommended protocol. The quality and amount of the RNA was examined by determining 260/280 absorbance ratio using a NanoDrop ND-1000 Spectrophotometer.

Reverse transcription was performed using a kit (High Capacity cDNA Revere Transcription Kit). All samples were amplified using Taqmanprob PCR master mix (Applied Biosystems).

Synthesized complementary (cDNA) samples were used for quantitative real-time polymerase chain reaction (PCR, ABI Stepone). PCR condition was one cycle of two minutes at 50°C and 10 minutes at 9°C. This was followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing and extension at 60°C for one minute. Gene expression levels were analyzed by qRT-PCR using Applied Biosystems™ TaqMan® Gene Expression Assays (Thermo Fisher Scientific, U.S.).

Beta-actin was used for normalization of the genes. Primer ID numbers for TNF-α, IL-1β, Kim-1 and β-actin are Rn01525859_gram per liter (gL), Rn00580432_mL, Rn00597703-mL, and Rn00667869_mL, respectively (Thermo Fisher).

**Histopathological analysis**

Kidney tissue samples were fixed in 10% neutral buffered formalin for 48 hours. After fixation, each tissue sample was processed routinely and embedded in paraffin. After embedding, 5-µm sections were taken from the paraffin blocks and stained with hematoxylin and eosin (H&E). Digital images of the kidney tissues were obtained using a light microscope (Zeiss Axio Scope.A1) at a magnification of x400. Histopathological evaluation was conducted based on tubular necrosis, tubular epithelial desquamation and peritubular hemorrhage criteria and scored as follows:

- 0 no injury
- + mild injury
- ++ moderate injury
- +++ severe injury.

**Statistical analysis**

Biochemical and genetic values were evaluated with IBM SPSS Statistics for Windows, Version 18.0 (Armonk, New York, U.S., IBM Corp.) Groups were compared by using the one-way analysis of variance (ANOVA) followed by the Tukey test; p <0.05 was accepted as statistically significant. To evaluate the expression levels of the genes, the 2^{-\Delta\Delta Ct} method was used \[2^{-\Delta\Delta Ct} = (\text{Ct target gene} - \text{Ct reference gene})\].

**RESULTS**

In this study, the protective effects of HBO₂ administration on GM-induced renal toxicity were investigated using genetic, biochemical and histopathological methods.

**Physical and biochemical parameters**

Initial body weights were similar in all groups at the beginning of study, although, the mean body weight (217.14±5.21 g) decreased (199.257±7.86) in the group receiving HBO₂ (p=0.006). GM caused an obvious alteration in renal functions. Serum urea and creatinine levels were determined as 49±2.39 mg/dL and 0.35±0.01 mg/dL in the C group and 65.76±1.10 mg/dL and 0.44±0.01 mg/dL in the GM group, respectively. When all groups were compared there were statistically significant differences identified for serum albumin (p=0.003), creatinine and urea (p=0.000). In terms of oxidative stress parameters, there were significant variations determined for MDA, TAS, TOS and OSI values. The SOD level was greatly reduced in the GM group (10.76±1.67 (U/mg protein.mL) compared to the C group (25.52±4.11U/mg protein.mL), with an increase observed in the GM+HBO₂ group (18.43±1.27 U/mg protein.mL). The MDA levels were increased in the GM (16.14±0.92 nmole/mL) compared to the C group (7.83±0.39 nanomole/mL). In the GM+HBO₂ group (11.86±0.71 nmole/mL), MDA was reduced compared to the GM group. The TAS value was 1.92±0.12 in the C group and 1.44±0.11 in the HBO₂ group, with statistical significance determined between
the organ (Table 2; Figure 1). Generally, no hemorrhagic areas were encountered in but the majority of tubular structures were normal. Ocasional desquamation of tubular epithelium continued, levels of amelioration of tubular necrosis areas. Occa-
tissue belonging to the GM+HBO 2 group found significant hemorrhage areas. Histopathological assessment of kidney desquamation of tubular epithelium and peritubular amelioration due to HBO 2 administration in this study. Improvements were recorded on histopathological

<table>
<thead>
<tr>
<th></th>
<th>C (n = 7) (mean±SE)</th>
<th>HBO 2 (n = 7) (mean±SE)</th>
<th>GM (n = 7) (mean±SE)</th>
<th>GM+HBO 2 (n = 7) (mean±SE)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>body weight (g) day 1</td>
<td>222.00±7.74</td>
<td>217.14±5.21</td>
<td>219.86±7.90</td>
<td>214±3.03</td>
<td>0.775</td>
</tr>
<tr>
<td>body weight (g) day 8</td>
<td>233.85±5.14</td>
<td>199.25±7.86</td>
<td>212.57±7.60</td>
<td>209.14±3.71</td>
<td>0.006*</td>
</tr>
<tr>
<td>absolute kidney weight (g)</td>
<td>0.91±0.05</td>
<td>0.77±0.05</td>
<td>0.811±0.04</td>
<td>0.84±0.03</td>
<td>0.219</td>
</tr>
<tr>
<td>kidney/body weight ratio (g)</td>
<td>3.88±0.18</td>
<td>3.87±0.20</td>
<td>3.82±0.21</td>
<td>4.016±0.13</td>
<td>0.899</td>
</tr>
<tr>
<td>albumin (g/dL)</td>
<td>4.22±0.05</td>
<td>4.13±0.10</td>
<td>4.48±0.06</td>
<td>4.06±0.57</td>
<td>0.003*</td>
</tr>
<tr>
<td>creatinine (mg/dL)</td>
<td>0.35±0.01</td>
<td>0.37±0.03</td>
<td>0.44±0.01</td>
<td>0.40±0.01</td>
<td>0.000*</td>
</tr>
<tr>
<td>urea (mg/dL)</td>
<td>49±2.39</td>
<td>39±1.34</td>
<td>65.76±1.10</td>
<td>48.6±2.39</td>
<td>0.000*</td>
</tr>
<tr>
<td>SOD (U/mg protein.mL)</td>
<td>25.52±4.11</td>
<td>18.83±1.03</td>
<td>10.76±1.67</td>
<td>18.43±1.27</td>
<td>0.002*</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>7.83±0.39</td>
<td>6.89±0.78</td>
<td>16.14±0.92</td>
<td>11.86±0.71</td>
<td>0.000*</td>
</tr>
<tr>
<td>GSH-Px (U/mg protein.mL)</td>
<td>21.27±3.26</td>
<td>20.13±2.75</td>
<td>15.72±1.55</td>
<td>18.24±2.06</td>
<td>0.434</td>
</tr>
<tr>
<td>TAS mmol Trolox Equiv./ mg protein</td>
<td>1.92±0.12</td>
<td>1.44±0.11</td>
<td>1.39±0.08</td>
<td>1.52±0.03</td>
<td>0.002*</td>
</tr>
<tr>
<td>TOS µmol H2O2 Equiv./ mg protein</td>
<td>22.41±0.91</td>
<td>20.37±1.21</td>
<td>25.56±0.76</td>
<td>22.81±0.4</td>
<td>0.001*</td>
</tr>
<tr>
<td>OSI ([TOS µmol H2O2 equiv/ (mg protein)/(TAS, mmol Trolox equiv/ mg protein)]</td>
<td>11.90±0.59</td>
<td>14.84±1.90</td>
<td>18.75±1.31</td>
<td>15.01±0.43</td>
<td>0.006*</td>
</tr>
<tr>
<td>NO µM/protein</td>
<td>4.71±0.16</td>
<td>4.30±0.30</td>
<td>5.11±0.24</td>
<td>4.64±0.29</td>
<td>0.187</td>
</tr>
</tbody>
</table>

*One-way ANOVA followed by Tukey test

Histopathologic evaluation
Histopathological investigation of renal tissue from the C group observed that normal tissue architecture was preserved, with no pathology encountered in tubular and glomerular structures.

Histopathological appearance of renal tissue from the HBO 2 group was similar to the C group, with a natural appearance of glomerular and tubular structures. Histopathological assessment of kidney tissues belonging to the GM group encountered widespread tubular necrosis, desquamation of tubular epithelium and peritubular hemorrhage areas. Histopathological assessment of kidney tissue belonging to the GM+HBO 2 group found significant levels of amelioriation of tubular necrosis areas. Ocasional desquamation of tubular epithelium continued, but the majority of tubular structures were normal. Generally, no hemorrhagic areas were encountered in the organ (Table 2; Figure 1).

Gene expression
Gene expression levels of TNF-α, IL-1β and Kim-1 were analyzed from renal tissues. The significant differences were observed in the GM group TNF-α, IL-1β and Kim-1 levels compared to the C group. Comparison of the C group with the GM group showed nearly a two-time increase in TNF-α, IL-1β and Kim-1 expression levels (p=0.01, p=0.004, p=0.006) respectively. When the GM group was compared with the GM+HBO 2 group, results close to the C group were obtained; in other words, all expression levels were reduced (p=0.015, p=0.024, p=0.006) respectively (Figures 2, 3 and 4).

DISCUSSION
We observed that GM caused increases in oxidative stress and necrosis-related nephrotoxicity. Variations in albumin, urea and creatinine levels were significantly ameliorated due to HBO 2 administration in this study.

HBO 2 administration also reduced the gene expression levels of inflammatory markers such as TNF-α and IL-1β, and renal injury markers such as Kim-1 in renal tissue. Improvements were recorded on histopathological
Table 2. Effect of treatment with HBO₂ on histopathological findings of kidney tissue in GM-treated rats

<table>
<thead>
<tr>
<th>histological findings</th>
<th>C</th>
<th>HBO₂</th>
<th>GM</th>
<th>GM+HBO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>tubular necrosis</td>
<td>0</td>
<td>0</td>
<td>+++</td>
<td>0</td>
</tr>
<tr>
<td>tubular epithelial desquamation</td>
<td>0</td>
<td>0</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>peritubular hemorrhage</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1. Microscopic comparison of all groups (H&E. x400 magnification).

<table>
<thead>
<tr>
<th>Images</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal tissue architecture</td>
</tr>
<tr>
<td>B</td>
<td>Tubular necrosis, peritubular hemorrhage (arrow) and tubular epithelial desquamation and degeneration (star) seen in group GM. Tubules were revealed to be normal in Group GM+HBO₂ except for desquamation of epithelial cells (star) in a few tubules.</td>
</tr>
</tbody>
</table>

One of the mechanisms described for the pathogenesis of GM-induced nephrotoxicity is related to oxidative stress [14,17]. GM increases the production of ROS like hydroxyl radicals, superoxide anions and hydrogen peroxide, causing reductions in antioxidant enzyme capacity [1,18]. Many studies have confirmed the relationship between nephrotoxicity and oxidative stress, with the role of ROS including hydroxyl radicals described in nephrotoxicity induced by GM [8,18]. The occurrence of these nephrotoxic effects of aminoglycosides make it necessary to research protective agents for the kidney against these effects. With this aim, different molecules including curcumin, n-acetyl cysteine and olive leaf extract have been investigated [1,8,19]. Those molecules were investigated as to whether they have a protective role on nephrotoxicity due to antioxidative properties. In recent years HBO₂ has been shown to be a remarkable protective method for this purpose. However, the protective effects of HBO₂ against antibiotic-induced nephrotoxicity have not been fully revealed. As a consequence, in this study we investigated the efficacy of HBO₂ exposure against GM-induced nephrotoxicity.

Cermik, et al. [19] revealed the protective effect of HBO₂ exposure against oxidative stress and tissue injury for two days before renal ischemia-reperfusion injury (45 minute ischemia and 24 hours reperfusion) in rats. Similarly, Rubenstein, et al. treated rats with HBO₂ twice per day after a 45-minute interval of renal ischemia and identified an increase in lipid peroxidation and renal SOD activity [14]. Another study investigating the effects of different doses of HBO₂ on cisplatin-induced nephrotoxicity identified that lipid peroxidation decreased and SOD and GSH-Px activities increased in the group with a single administration per day of HBO₂ [15].
In our study SOD and GPx activity significantly reduced in the GM group compared to the C group, with a significant increase observed in the HBO2 group. MDA levels in the GM group were found to be high compared to the C and HBO2 groups. These results indicate that lipid peroxidation occurred at high levels in kidney related with GM toxicity. The lower MDA levels occurring in the GM+HBO2 group compared to the GM group shows that lipid peroxidation was reduced significantly in this group. As a result, HBO2 treatment may be said to be very effective in eliminating ROS forming as a result of renal toxicity.

Vancomycin is yet another antibiotic with nephrotoxic effects. When Sabler, et al. [16] exposed vancomycin-treated rats to HBO2, they determined that HBO2 increased toxicity. These findings are not compatible with our results. They suggested that this effect was related to high oxygen levels which in turn caused high levels of free radicals. However, in our study, high free radical levels did not occur in the group receiving HBO2. We also saw that HBO2 caused an increase in antioxidant enzyme levels.

These outcomes may be due to the two different antibiotics causing renal injury by different mechanisms. Moreover, Berkovitch, et al. [11] determined that HBO2 showed no effect against GM nephrotoxicity. Our findings contradict these results, demonstrating that HBO2 exhibited an ameliorating effect on renal injury. The differences may be related to the dosage of GM. While Berkovitch, et al. [11] administered the GM in 150 mg/kg dosage for five days, our administration was 100 mg/kg for seven days.

Despite conflicting reports on the effects of HBO2 on the oxidative system, it is generally accepted as beneficial. Cuzzocrea, et al. [20] observed that HBO2 administration after zymosan could protect tissues. Yasar, et al. [21] found that HBO2 treatment has beneficial effects on the course of acute pancreatitis. Rubenstein, et al. [14] observed that HBO2 ameliorates endothelial-dependent vasorelaxation on kidney injury. Our results are in parallel with these findings.

An increase of serum creatinine and urea levels and a decrease of glomerular filtration rate are commonly observed in GM-induced nephrotoxicity [22,23]. In our study, similar to previous reports, urea and creatinine levels increased in the GM group compared to that of the C group. The creatinine level did not change in the GM+HBO2 group, but the urea levels decreased. High urea level indicates severe nephrotoxicity. We observed extensive cortical tissue degeneration and widespread tubular necrosis in renal tissue samples with high urea levels in the GM group. Obviously, HBO2 exposure ameliorated the histopathological alterations and decreased the urea levels caused by GM treatment in the GM+HBO2 group. HBO2 exposure also decreased serum creatinine levels.

Studies on GM-induced nephrotoxicity determined histopathologic damage occurred in kidney. Vyshak, et al. [7] observed high levels of tubular necrosis, hyaline casts in tubular lumen, tubular degeneration and cellular inflammatory infiltration after the GM administration. When Rotula aquatica extracts were applied, its anti-inflammatory capabilities ameliorated these pathological changes. Valipour, et al. [22] observed that mononuclear cell infiltration, hemorrhage and tubular degeneration in the kidney after GM administration were partially ameliorated after use of Ferulago angulate extract. In our study, tubular necrosis, tubular epithelial desquamation and peritubular hemorrhage were determined at moderate levels in the GM group. When HBO2 was applied after GM administration, tubular necrosis and peritubular hemorrhage were not observed. However, mild tubular epithelial desquamation was noted in the kidney.

These findings suggest that HBO2 therapy has a powerful protective effect against the degenerative effects of antioxidants on kidneys. HBO2 has been used for various diseases, with successful results obtained in numerous studies. However, effect mechanisms outside the oxidant/antioxidant system are not fully understood [15,16,17,19]. It is generally accepted that HBO2 has a positive effect on the inflammatory response [19] as well as activating the immune system. Injury is known to be due to the initiation of the inflammatory process in many tissues. Variations in proinflammatory cytokine levels such as TNF-α and IL-6 play important roles in tissue injury [10]. The inflammatory and immune response triggered by GM is a process initially mediated by release of TNF-α [22-24]. It was reported that HBO2 treatment suppressed TNF-α and IL-1β [25]. Bai, et al. reported that HBO2 exposure reduced IL-2 and INF-γ expression in acute pancreatitis compared to a control group [26].

Our study showed significant increases in TNF-α and IL-1β gene expression in the GM-administered group (Figures 2 and 3). HBO2 exposure reduced the gene expression levels in renal tissue in both markers. These results suggest that local and systemic inflammation may have a role in GM-induced nephrotoxicity while HBO2 decreases this inflammatory response. However,
Values are presented as relative expression of $2^{\text{ΔΔCT}}$ after normalization of expression levels against β-actin mRNA level.

*a* Statistical difference compared to other groups using One-Way ANOVA followed by the Tukey test (p<0.05). Results are mean ± standard error (SE) of three independent determinations (n = 7).

our study did not focus on the underlying mechanisms of HBO2 effects. For that reason, we cannot say whether this effect of HBO2 is direct or indirect.

Preclinical study has demonstrated that Kim-1 may be a useful biomarker for an early diagnosis or for monitoring acute renal tubular necrosis [9]. Kim-1 gene expression levels increased in a dose-dependent manner were reported in a toxicity study [9]. Recent studies have indicated the correlation between Kim-1 and GM administration, showing increases in Kim-1 expression levels in kidney tissue after GM treatment [27,28]. However, we have found no study demonstrating the effect of HBO2 exposure on Kim-1 gene expression in GM-induced nephrotoxicity. The effect of HBO2 exposure on GM-induced renal injury as dependent on Kim-1 gene expression alteration was revealed for the first time in our study. We observed an increase in renal Kim-1 gene expression levels after GM treatment, but HBO2 exposure reduced these levels (Figure 4). This result is another finding suggesting protective properties of HBO2 against GM-induced nephrotoxicity.

**CONCLUSION**

Our results revealed that renal injury occurred in rats as a result of 100 mg/kg/day administration of gentamicin over seven days. When the GM group was exposed to HBO2:

1. histopathologic vacuolization, necrosis, tubular degeneration and inflammation resolved;
2. development of oxidative stress and apoptosis in the kidney was prevented;
3. improvements in kidney functions occurred;
4. the increase in TNF-α and IL-1β gene expression levels, markers of inflammation in the kidney, was decreased;
5. the increase in Kim-1 gene expression levels, a marker of kidney injury, was reduced.
Despite the limited and contradictory results in previous studies, we observed that HBO₂ exposure has ameliorative effects on GM-induced kidney injury. This study investigated the correlation between Kim-1 and HBO₂ therapy for the first time and revealed that HBO₂ prevents the increase in Kim-1 expression levels related to GM toxicity in the kidney. In addition, our results suggested that HBO₂ exposure may be defined as a potential therapeutic method for the treatment of renal injuries. Further studies are needed in this area.

**Acknowledgments**

The authors declare that no conflict of interest exists with this submission.

This study was supported by Çanakkale Onsekiz Mart University Scientific Research Projects with project number THD-2017-1257.

**REFERENCES**


