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ORIGINAL ARTICLE

The effect of carvedilol on serum and tissue oxidative stress parameters in partial ureteral obstruction induced rat model

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Abstract Although the pathological mechanism underlying kidney damage is not completely understood, it has been reported that reactive oxygen species (ROS) formed during ureteral obstruction may play an important role in this process. Carvedilol has been used in a limited number of studies examining oxidative injury. The aim of this study was to investigate the effect of carvedilol on serum and tissue oxidative stress parameters in the partial unilateral ureteral obstruction (PUUO)-induced rat model. To our knowledge, the protective effects of carvedilol in the PUUO-induced rat model have not been reported. Twenty-six male Wistar albino rats, age 5.5 to 6 months and weighing 250 to 300 g, were used in this study. The rats were randomly divided into three groups. In Group 1 ($n = 9$), the control group, a sham operation was performed. In Group 2 ($n = 8$), the PUUO group, the left ureter was embedded into the psoas muscle to create PUUO and maintained for 7 days. In Group 3 ($n = 9$), carvedilol was orally administered to the rats (2 mg/kg). After the establishment of PUUO, carvedilol was given for the following 7 days. After partial unilateral ureteral obstruction, a nephrectomy was performed to determine the blood and tissue levels of superoxide dismutase (SOD), malondialdehyde (MDA), protein carbonyl (PC), and nitric oxide (NO). The median SOD, MDA, PC, and NO levels in the tissues were 0.006 U/mg protein, 5.11 nmol/g protein, 4.31 nmol/mg protein, and 0.337 μmol/g protein in the control group, respectively. There was a significant increase in tissue SOD ($p = 0.014$), MDA ($p = 0.002$), and NO ($p = 0.004$) levels in Group 2. However, a statistically significant difference was not observed in PC ($p = 0.847$) enzymatic activity in Group 2. When compared with Group 2, carvedilol treatment caused a reduction in NO ($p = 0.003$), and PC ($p = 0.001$) activities in Group 3. The serum SOD ($p = 0.004$),

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MDA ($p = 0.043$), PC ($p = 0.043$), and NO ($p = 0.001$) levels were significantly different in Group 3 compared with Group 2. Administration of carvedilol also reduced the detrimental histopathologic effects caused by PUUO. According to histopathological examination of the renal tissues, the inflammation rates were 22.2%, 87.5% and 33.3% in Groups 1, 2, and 3, respectively ($p < 0.05$). The results of the present study show that partial unilateral ureteral obstruction caused oxidative stress in the serum and kidney tissues of rats, and treatment with carvedilol reduced the harmful effects of ureteral obstruction.

Introduction

Ureteral obstruction may result from various intraluminal and extraluminal causes including retroperitoneal fibrosis, trauma, blood clots, tumors, and foreign materials. In addition, ureteral obstruction can be observed due to intra-abdominal surgical operations such as caesarean section, hysterectomy and colonic surgery. Ureteral obstruction has also been implicated in renal parenchymal damage [1—4]. Although partial unilateral ureteral obstruction (PUUO) is one of the most common urologic problems, the pathophysiologic changes in renal tissue due to PUUO are not understood. Known factors in the pathophysiology of renal obstructive parenchymal injury include renal blood flow impairment, intrapelvic pressure elevation, vasoactive and inflammatory mediators such as activation of renin angiotensin system, expression of transforming growth factor-α (TGF-α), clusterin, Fas ligand, kaspase and catalase activity, N-acetyl-β-glucosaminidase, β2 microglobulin, γ-glutamyl transferase, α-glycosidase, endothelial growth factor (EGF), and prostaglandins [5—10]. Recently, it has been suggested that reactive oxygen species (ROS), which are formed during ureteral obstruction, may play a role in this process. Antioxidant agents have been used to prevent the negative effects of ROS on serum and tissue oxidative parameters [11,12].

Carvedilol is a nonselective β-blocker with additional α-blocker properties. Carvedilol competitively blocks α-1, β-1, and β-2 adrenergic receptors and also has vasodilatory properties. It has been commonly used for the clinical treatment of congestive heart failure, hypertension and myocardial infarction [13—15]. In addition to its anti-adrenergic effects, carvedilol also has antioxidant properties. Due to its antioxidant properties, carvedilol can treat many pathologic conditions that are associated with enhanced cellular oxidative stress [13—16]. Currently, the relationship between the oxidative stress in the kidneys and treatment with antioxidants in the PUUO-induced rat model has been shown in a very limited number of studies. To our knowledge, the protective effects of carvedilol in the PUUO-induced rat model has not been studied.

The aim of this study was to evaluate the effects of carvedilol on antioxidant enzyme levels and histopathologic changes in the PUUO-induced rat kidney.

Materials and methods

After obtaining approval from the local ethics committee (2010-HADYEK-042), a total of twenty-six male Wistar albino rats, age 5.5 to 6 months and weighing 250 to 300 g, were used in the study. The rats were handled in the laboratory according to the *Guide for Care and Use of Laboratory Animals* of the National Research Council as well as institutional guidelines. The rats were kept in a temperature-controlled room (20°C to 23°C) on a 12-hour light/dark cycle with food (commercial rat chow) and fresh water available *ad libitum*. All surgical procedures were performed under xylazine/ketamine anesthesia under sterile conditions. All rats were sacrificed after the experimental procedures. The rats were randomly divided into three groups. Group 1 ($n = 9$) was designated as the control group. These sham-operated rats had undergone laparotomy through the abdominal midline incision, and their ureters were manipulated but not ligated. The rats in this group were used to determine basal values for biochemical and tissue evaluation. Group 2 ($n = 8$) was designed to see the effects of PUUO on serum and renal tissue oxidative stress parameters. In this group, PUUO was performed with two-thirds of the left ureter embedded in the *psoas* muscle with 4-0 silk through a midline abdominal incision, as previously described in the literature [17] (Fig. 1). Group 3 ($n = 9$) was designed to determine the effect of carvedilol on serum and tissue oxidative stress parameters after PUUO. In this group, after PUUO was performed, carvedilol was given to the rats orally (2 mg/kg). Carvedilol was administered to the rats for 7 days after the establishment of PUUO. At the end of the experiment, left-sided nephrectomies were performed for all the rats in Groups 2 and 3 for histopathologic and biochemical analysis. The tissue levels of oxidative stress parameters, such as

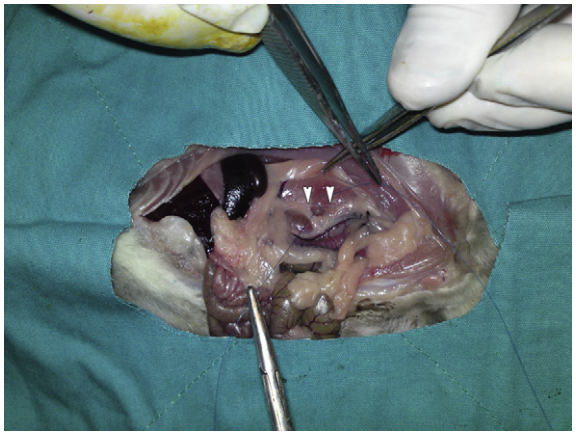

Figure 1. Partial unilateral ureteral obstruction-induced rat model (arrowheads).

malondialdehyde (MDA), superoxide dismutase (SOD), protein carbonyl (PC), and nitric oxide (NO), were studied in all groups. The serum levels of the same parameters were also studied in the blood, 5 ml of which was drawn from the *vena cava inferior* of all the rats immediately prior to sacrifice.

The data were analyzed using SPSS for Windows software. A p-value <0.05 was considered statistically significant. For statistical analysis, Mann-Whitney U test and Chi-square tests were performed.

Histopathologic examination

Approximately half of the nephrectomy specimens were embedded into formaldehyde solution for pathologic examinations, while the biochemical studies were performed with the other half. All histologic analysis were performed in routinely processed formalin-fixed, paraffin-embedded tissue sections (4 μm thick), which were stained with hematoxylin-eosin; the slides were examined with a light microscope under 20x magnification. Randomly selected fields were evaluated for cellular and tubular structures. Using pathologic examination, interstitial inflammation was evaluated. A semi-quantitative grading system was used for each parameter. The inflammation process was graded as mild (+1), moderate (+2) or severe (+3). Interstitial mononuclear cell infiltration with one small focus was accepted as mild inflammation (Fig. 2). If the mononuclear cell infiltration was detected in more than one focus in the interstitia, it was accepted as moderate inflammation (Fig. 3). Finally, severe inflammation was evident when mononuclear inflammatory cell infiltration with large aggregates was observed (Fig. 4).

Biochemical analyses

Blood samples were drawn into heparin-free tubes for biochemical analyses. After centrifugation (3000 × ***g*** for 15 min at +4°C), serum samples were stored frozen at −70°C. Determinations of the following parameters were made in the serum samples using commercial chemicals supplied by Sigma (St Louis, MO, USA) (17).

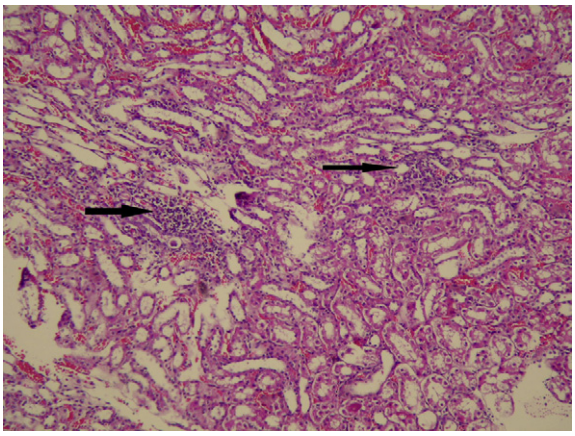

Figure 3. Multifocal lymphocytic infiltration observed as two very small foci in the interstitium of the rat kidney (arrow; HE, ×15).

Serum antioxidant enzyme analysis

Determination of SOD activity

Total (Cu−Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method described by Sun et al. [18]. The method is based on inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine−xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1.0 ml of an ethanol−chloroform mixture (5:3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the amount causing 50% inhibition in the NBT reduction rate. The SOD activity was expressed as U/mL in the tissue and U/mg protein in the serum.

Determination of MDA levels

The tissue thiobarbituric acid-reactive substance (TBARS) level was determined by a method [19] based on reaction with thiobarbituric acid (TBA) at 90°C to 100°C. In the TBA

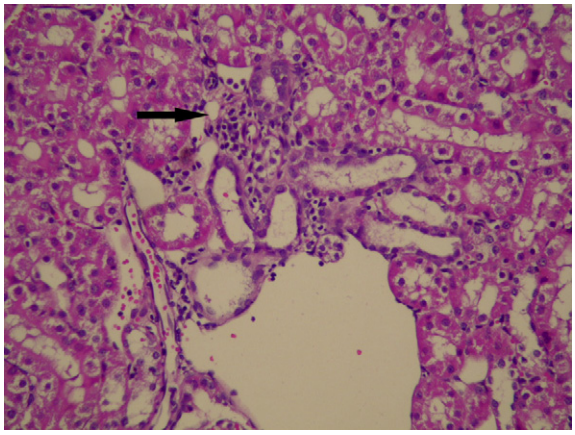

Figure 2. Pericapillary lymphocytic infiltration observed as a very small focus in the interstitium of the rat kidney (arrow; HE, ×30).

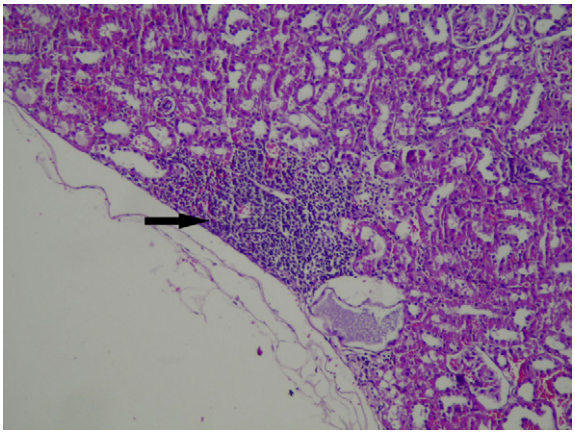

Figure 4. Lymphocytic infiltration observed as a large aggregate in the interstitium of the rat kidney (arrow; HE, ×20).

test reaction, MDA or MDA-like substances and TBA react to produce a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2 to 3 and 90°C for 15 minutes. The sample was mixed with two volumes of cold 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation, and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water-bath for 10 min. After cooling, the absorbance was read at 532 nm. The results were expressed as μmol/L in the tissue and nmol/g protein in the serum, according to the standard graphic prepared from measurements with a standard solution (1,1,3,3 tetramethoxypropane) [19].

Determination of tissue PC content

The carbonyl contents were determined spectrophotometrically, based on the reaction of the carbonyl group with 2, 4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone. 2,4-Dinitrophenylhydrazine was the reagent originally used for proteins subjected to metal-catalyzed oxidation. The results were given as nmol/ml in the tissue and nmol/mg protein in the serum [20,21].

NO determination

NO measurement is very difficult in biological specimens; therefore, tissue nitrite (NO_2^-) and nitrate (NO_3^-) were estimated as an index of NO production. The samples were initially deproteinized with Somogyi reagent. Total nitrite (nitrite + nitrate) was measured after conversion of nitrate to nitrite by copperized cadmium granules using a spectrophotometer at 545 nm. A standard curve was established with a set of serial dilutions (10^{-8} to 10^{-3} mol/L) of sodium nitrite. Linear regression was carried out using the peak area from the nitrite standard. The resulting equation was then used to calculate the unknown sample concentrations. The results were expressed as mmol/L in the tissue and μmol/L in the serum [22].

Results

The median serum levels of MDA, NO, and PC were 2.327 μmol/L, 155.6 mmol/L, and 2860 nmol/ml in Group 1, respectively. The serum levels of MDA, NO, and PC increased in the PUUO group compared with the control group; however, only the difference between MDA and NO was statistically significant ($p < 0.05$). Carvedilol treatment ameliorated serum MDA, NO and PC levels in Group 3. Similarly, antioxidant enzyme activity (SOD) increased in the PUUO group. Carvedilol treatment caused decreased SOD activity compared with the sham-operated control group ($p < 0.05$). The results of blood SOD, MDA, NO, and PC values in all three groups are shown in Table 2. The renal tissue levels of MDA, NO, and PC were detected as 5.11 nmol/g protein, 0.337 μmol/g protein and 4.31 nmol/mg protein, in Group 1, respectively. The renal tissue levels of MDA and NO increased in the PUUO group compared with the sham-operated group ($p < 0.05$). However, a statistically significant difference was observed in PC and NO enzyme activities in Group 3. Antioxidant enzyme activities (SOD) were increased in the PUUO group. In Group 3, carvedilol treatment caused decreased SOD, MDA, NO, and PC levels, compared with the sham-operated control group. The results of renal tissue SOD, MDA, NO and PC values in all groups are shown in Table 1. The renal inflammation rates were 22.2%, 87.5%, and 33.3% in Groups 1, 2, and 3, respectively ($p < 0.05$). The inflammation severity was mild (+1) in two rats (22.2%) in Group 1. In this group, moderate or severe inflammation was not detected. However, mild (+1), moderate (+2) and severe (+3) inflammation rates were 37.5% ($n = 3$), 37.5% ($n = 3$), and 12.5% ($n = 1$) in Group 2, respectively. In Group 3, only three (33.3%) mild inflammation rates were detected. Inflammation rates were increased in the PUUO group compared with Group 1, and carvedilol treatment caused decreased inflammation rates ($p < 0.05$).

Discussion

Urinary tract obstruction is a common problem in the practice of urology and can occur at any location along the urinary tract. The occurance of urinary tract obstruction is most often secondary to calculi, tumors, strictures, ureteropelvic junction obstruction or ureterovesical junction obstruction, ectopic ureter, ureterocele, megaureter, and posterior urethral valves. Ureteral obstruction frequently occurs unilaterally and is usually a reversible condition [1,2,23]. Although ureteral obstructions may cause renal parenchymal damage due to several mechanisms, the exact pathophysiologic mechanism of the kidney damage is complex and has not been fully understood. Obstructive uropathy leads to functional and morphological changes in the kidney. If the ureteral obstruction persists, tubular atrophy, interstitial fibrosis, ischemia, and necrosis may occur. Although pressure increases and ischemic atrophy have been implicated in the pathogenesis of urinary tract obstructions in recent studies, vasoconstriction and

Table 1 The median levels of the reactive oxygene species are seen in renal tissue.

Groups	SOD U/mg protein	MDA nmol/g protein	NO μmol/g protein	PC nmol/mg protein
Group 1	0.006	5.11	0.337	4.31
Group 2	0.0095	9.05	0.433	3.89
p	0.014	0.002	0.004	0.847
Group 2	0.0095	9.05	0.433	3.89
Group 3	0.008	6.56	0.312	2.54
p	0.585	0.083	0.003	0.001

MDA = malondialdehyde; NO = nitric oxide; PC = protein carbonyl; SOD = superoxide dismutase.

Table 2 The comparison of the serum reactive oxygen levels in all groups.

Groups	SOD U/mL	MDA μmol/L	NO mmol/L	PC nmol/ml
Group 1	1.475	2.327	155.6	2860
Group 2	1.803	2.745	204.3	3320
P	0.144	0.038	0.001	0.149
Group 2	1.803	2.745	204.3	3320
Group 3	1.311	2.353	152.5	2950
P	0.004	0.043	0.001	0.043

MDA = malondialdehyde; NO = nitric oxide; PC = protein carbonyl; SOD = superoxide dismutase.

decreased renal blood flow are other important factors in the pathogenesis of kidney damage in ureteral obstructions [3,7,23]. Associated with this situation, it has been reported that many molecular and biochemical factors including oxidative stress may also play a role in the pathophysiology of the parenchymal kidney damage in ureteral obstruction [3,7—10,23]. Oxidative stress is a condition related to an increased rate of cellular damage induced by oxygen and oxygen-derived oxidants commonly known as ROS [24]. ROS are intermediary metabolites that are normally produced in the course of oxygen metabolism. Under normal conditions, ROS play a critical role as signaling molecules. However, the overexpression of ROS can avidly react with and denature proteins, lipids, nucleic acids, carbohydrates and other molecules, as well as cause inflammation, apoptosis, fibrosis, and cell proliferation [25]. ROS can disrupt the functional state and integrity of the cell membrane by lipid peroxidation reaction. In the literature it is reported that, in various conditions such as ischemia—reperfusion injury, arthritis, cancer, diabetes mellitus, and central nervous system disorders, ROS may lead to severe injury in the cell membrane by lipid peroxidation reactions [26]. In addition to the above disorders, ROS are thought to play a role in the tubulointerstitial inflammation process in PUUO-induced rat models [27].

Indeed, in recent studies it has been demonstrated that antioxidant enzyme activities were diminished and ROS production was increased during ureteral obstruction [28]. All of these changes occur secondary to PUUO and may contribute to tubulointerstitial damage and fibrosis. In the present study, serum and tissue levels of MDA and NO significantly increased in PUUO-induced rat group compared with the control group. A study by Demirbilek et al. showed that oxidative stress products, antioxidants and lipid peroxidation levels in the PUUO group were significantly different from the levels in the control group [29]. Similar findings have been reported by other authors in the PUUO-induced rat model [30,31].

The most important strategy to reduce ROS is antioxidant use. Both biological and chemical antioxidants are compounds that scavenge ROS and/or suppress their production and actions. The beneficial effects of antioxidants on oxidative stress parameters and different tissues in oxidative stress conditions have been reported in numerous previous studies. In this context, to prevent the harmful effects of oxidative stress, many antioxidant agents such as vitamin E, melatonin, retinol, β-carotene, omega-3, resveratrol, vitamin C, coenzyme Q10, and acetylcysteine have been used with different success rates [6,8,32—34]. Additionally, the beneficial effects of antioxidants have been shown in the prevention and management of coronary heart disease, cancer, hypertension, type 2 diabetes mellitus, renal diseases, rheumatoid arthritis, ulcerative colitis, Crohn's disease, and chronic obstructive pulmonary disease [6,8,26]. Moreover, many experimental animal studies have confirmed the efficacy of antioxidants in reducing the short-term detrimental effects of obstruction on the kidney [12,35]. In a PUUO-induced rat model, Fitzgerald et al. found that the administration of atorvastatin decreased the grade of kidney injury histologically [12]. Similarly, in another study, Ozbek et al. investigated the effects of melatonin on PUUO-induced kidney injury and reported that melatonin exerted a preventive effect on oxidative stress with an eventual decrease in PUUO-induced kidney injury. According to these results, the authors claimed that melatonin diminished the destructive effect of ROS and can be used to protect kidney from the harmful effects of PUUO [31]. The beneficial effects of some other antioxidants, such as L-carnitine and tocopherol, on PUUO-induced rat kidney had also been shown by authors in previous studies [12,36].

Carvedilol is a lipophilic, third-generation, nonselective β-adrenoceptor and selective α-1-adrenoceptor blocker [13,14]. In addition to its β-blocker and α-blocker activities, carvedilol and some of its metabolites, such as SB 211475 and SB 209995, are potent antioxidants. This activity has been attributed to the carbazole moiety of this drug. For this reason, several experimental studies have demonstrated that the administration of carvedilol can attenuate the negative effects of oxidative stress under different conditions. The inhibitory effects of carvedilol on lipid peroxidation have been shown in various systems and cell groups [13,16]. For example, in a recent study, chronic administration of carvedilol resulted in attenuation of oxidative damage in the streptozotocin-induced model of dementia in rats [37]. In addition, it has been suggested that carvedilol may play a potential role in the treatment of neurodegenerative diseases. Moreover, it has been shown that carvedilol inhibits the release of superoxide ions from activated neutrophils. Due to the recognized role of superoxide anions in postischemic tissue injury, it has been suggested that carvedilol and SB-211475 could prevent the formation of more toxic oxidants and thus reduce oxidative tissue injury [38] through their scavenging activity against superoxide anions. In another study, Hayashi et al. investigated the effects of carvedilol on ischemia reperfusion injury in rats. In this study, the authors detected that serum creatinine levels were higher in the control group compared with the carvedilol treatment group on postoperative Days 2 and 4. According to these results, the authors emphasized that increased antioxidant modulation by carvedilol

attenuated renal ischemia reperfusion injury [39]. In another similar study using the renal ischemia reperfusion-induced rat model, the pretreatment of animals with carvedilol attenuated renal dysfunction led to decreased morphological alterations and restored the depleted renal antioxidant enzymes. In that study, the authors also detected that the renal function and morphological damage significantly improved by carvedilol administration [40]. Similarly, in our study, the administration of carvedilol attenuated ROS levels and minimized oxidative stress induced by PUUO in the kidney. The administration of carvedilol also reduced the detrimental histopathologic effects caused by PUUO. As a consequence, according to the results of these studies, the antioxidant effects of carvedilol was explained by three mechanisms: (1) inhibition of direct cytotoxic actions of free radicals; (2) prevention of oxygen-free radicals from activated transcription factors such as NF-κB; and (3) protection and replenishment of the endogeneous antioxidant defense mechanisms, GSH-PX, and vitamin E [41].

As a result, our study was the first study to evaluate the potential protective effects of carvedilol against oxidative stress in PUUO-induced rat kidneys. According to the results of this study, we suggest that the administration of carvedilol may attenuate the effects of oxidative stress in the PUUO-induced rat kidney. In addition, although this is an animal study, it can be speculated that carvedilol may be used clinically as an antioxidant agent in ureteral obstruction to minimize or prevent paranchymal damage in the kidney.

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