Oxidative Stress Induced by Ureteral Obstruction and Tadalafil

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ABSTRACT

Objective: It has been reported that tadalafil, a specific phosphodiesterase-5 (PDE5) inhibitor, has an antioxidant effect besides its well-known vasoactive properties. This study aimed to determine the possible protective effects of tadalafil in managing renal oxidative stress induced by unilateral ureteral obstruction (UUO).

Methods: Male Sprague-Dawley rats aged 2.5-3 months were randomly divided into 5 groups (n=8) as sham (S), partial obstruction (P), P+tadalafil (PT), complete obstruction (C), and C+tadalafil (CT). Animals in the PT and CT groups were administered with tadalafil (10 mg/kg, ig) just before the anesthesia. Twenty-four hours after the obstruction surgery, the animals were sacrificed. In the kidney homogenates, MDA and AOPP levels were examined in addition to SOD and CAT activities. Serum creatinine levels were also measured.

Results: Both partial and complete obstruction caused a significant increase in the serum creatinine, tissue MDA and tissue AOPP levels. Although unchanged SOD activity, renal CAT activity decreased significantly in P and C groups. In tadalafil treated animals, serum creatinine, tissue MDA and AOPP levels, or renal SOD activity did not change compared to obstructed groups. However, the renal CAT activity increased in tadalafil-treated groups. A single-dose tadalafil treatment significantly restored renal CAT activity to the range of controls in the PT group.

Conclusion: Although tadalafil did not affect renal oxidative stress induced by partial or complete UUO, elevation of CAT activity to control values in the PT group suggests that increasing doses or duration of tadalafil treatment might be protective for kidneys in UUO.

Keywords: PDE5, tadalafil, ureter, obstruction, oxidative stress

INTRODUCTION

Obstructive uropathy occurs with the blockage of urine flow at any point in the urinary system and may cause impaired renal blood flow and functional loss due to an increase in intrarenal pressure. The most common cause of unilateral ureteral obstruction (UUO) is urolithiasis, and sudden urethral blockage and acute obstruction are frequently seen due to the presence of calcium oxalate stones (1, 2). Increased backward hydrostatic pressure caused by obstruction causes interstitial inflammation, death of tubular cells due to apoptosis and necrosis, and decrease in capillary density, and it triggers progressive fibrosis (1-4).

Although molecular mechanisms involved in the pathogenesis of UUO are not fully known, the role of oxidative stress has been demonstrated in many studies (5-8). Ischemia and hypoxia, which develop due to reduced blood flow during obstruction, trigger the production of reactive oxygen species (ROS) in many cells in the kidney. In addition, granulocytes that infiltrate the renal parenchyma and inflammatory cells, such as monocytes and macrophages, play a major role in increasing oxidative stress by producing large amounts of ROS (7-9). Increased ROS production leads to lipid peroxidation and oxidative damage in important cellular macromolecules such as proteins and even DNA. The expression of heat shock protein-70, heat shock protein-27, and heme oxygenase-1, which are among the oxidative stress response molecules, increases due to obstruction. In addition to increased ROS production, decreased activity of cellular antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase, leads to greater damage in UUO depending on oxidative stress (7-10).

Phosphodiesterase-5 (PDE5) inhibitors are drugs that are clinically used to treat erectile dysfunction and pulmonary hypertension (11, 12), and there are studies that have shown its cardioprotective effects (13). Further, there are studies that have shown the antioxidant effects of this group of drugs that inhibit cyclic guanosine monophosphate (cGMP) degradation in the cell and that show its effect by increasing the concentration of cytoplasmic cGMP (14-22). PDE5 inhibitors administered at different doses and different times have been shown to reduce oxidative stress and provide functional recovery in heart, kidney, ovary, and cavernous tissues in experimental models such as models of diabetes (14, 15), ischemia–reperfusion (16-18), pulmonary hypertension (19, 20), and cardiomyopathy (21). In addition, PDE5 inhibitors acutely decrease serum oxidative stress levels in patients with erectile dysfunction (22).

In the present study, the effect of tadalafil, a specific PDE5 inhibitor, on renal oxidative stress was examined in a model of UUO in rats.

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METHODS

Groups

Forty male, adult (2.5–3 months old) Spraque Dawley albino rats were used in the study, and five groups were formed (n=8 in each group): Sham control (S), Partial ureteral obstruction (P), Partial ureteral obstruction+Tadalafil therapy (PT), Complete ureteral obstruction (C), and Complete ureteral obstruction+Tadalafil therapy (CT). Ethics committee approval was received for this study from the ethics committee of Ondokuz Mayıs University (B. 30.2.ODM.0.20.09.00-050.04-88).

Surgical procedures

Laparotomy was performed in rats under general anesthesia (ketamine, 50 mg/kg, intramuscular), and the left ureter was isolated. In the S group, the incision site was closed without narrowing the ureters. In the P and PT groups, a catheter (24 G) was placed in the ureter lumen and was tied with 4/0 silk; thus, standard narrowing and partial obstruction were created in the ureters of these rats in these groups. In the rats in the C and CT groups in which complete obstruction was performed, the left ureter was cut after being tied with 4/0 silk at two points; thus, complete obstruction was created. Following the surgical procedures, the abdominal incision was closed appropriately, and each rat was placed in a cage alone. Rats were sacrificed under general anesthesia (ketamine, 100 mg/kg, intraperitoneal) 24 h after the obstruction procedure, and blood samples and left kidneys were taken and preserved appropriately (−80°C).

Drug application

Immediately before the obstruction procedure, a single dose of the drug (10 mg/kg) was administered to the rats in the PT and CT groups. The drug that was suspended in drinking water was administered through intragastric gavage.

Biochemical measurements

The creatinine level in serum samples was examined in an autoanalyzer (Abbott Laboratory, Architect C8000) using an enzymatic method for the purpose of evaluating renal function.

Tissues were homogenized in PBS buffer and centrifuged at 1500 ´*g* for 15 min for the measurements of the levels of malondialdehyde (MDA) and advanced oxidation protein products (AOPP) in the kidney tissue. Suitable ELISA kits were used in the obtained supernatants for determining the levels of MDA (BlueGene: E02M0023) and AOPP (BlueGene: E02A0832).

For SOD activity, the tissues were homogenized in HEPES buffer (20 mM HEPES, 1 mM EGTA, 210 mM mannitol, 70 mM sucrose; pH 7.2). After 5-min centrifugation at 1500 ´*g*, SOD activity was enzymatically measured in the obtained supernatants (Cayman, 706002).

Kidney tissues were homogenized in a different buffer (50 mM potassium phosphate, 1 mM EDTA; pH 7.0) for determining CAT activity and were centrifuged at 10,000 ´*g* for 15 min. Activity measurement was spectrophotometrically performed using a suitable commercial kit (Cayman; 707002).

The levels of MDA and AOPP in the tissue as well as activities of SOD and CAT were standardized according to the protein level. Protein level determination in appropriately prepared tissue homogenates was spectrophotometrically performed according to the Bradford method (Thermo Scientific; 23200).

Statistical Analysis

The results are presented as mean±standard deviation. One-way analysis of variance was used in the evaluation of the results, and Newman–Keuls test was used for intergroup evaluation (GraphPad Prism 6). $p<0.05$ was accepted to be statistically significant.

RESULTS

The mean body weight of the 2.5- to 3-month-old Sprague Dawley rats that were included in the study was 272.6±28.30, and there was no difference between the groups.

Serum Creatinine

Serum creatinine level was found to be 0.51±0.05 mg/dL in the S group in which no obstruction was created. The application of partial and complete obstruction caused statistically significant augmentations at this value (0.63±0.06 in the P group and 0.64±0.08 mg/dL in the C group). The difference was $p<0.001$ according to the S group. A single dose of tadalafil therapy in the groups in which obstruction was created did not cause a significant change in serum creatinine level. The values that were found to be 0.63±0.03 mg/dL in the PT group and 0.61±0.03 mg/dL in the CT group were not different from the untreated group but statistically significantly higher in comparison to the S group ($p<0.001$).

Tissue MDA

Tissue MDA level, which is an indicator of oxidative stress-induced lipid peroxidation, was found to be 4.70±1.06 mg/mg prot in the S group. The values increased to 8.02±2.29 ($p<0.01$) in the P group and to 7.10±2.32 mg/mg prot ($p<0.05$) in the C group. Tadalafil therapy did not cause a statistically significant change in the tissue MDA values of these groups (Figure 1).

Tissue AOPP

This parameter that was studied as an indicator of oxidative stress-induced protein oxidation was determined to be 74.01±17.08 mg/mg prot in the S group rats. Levels of AOPP that increased to 129.7±38.36 mg/mg prot ($p<0.001$) in the P group and 141.5±33.60 mg/mg prot ($p<0.01$) in the C group were not affected by tadalafil therapy (Figure 2).

Tissue SOD activity

SOD activity in the kidney was found to be 1.61±0.22 mU/mg prot in the S group rats and did not show any statistically significant change due to obstruction and/or tadalafil treatment (Figure 3).

Tissue CAT activity

The tissue CAT activity determined to be 1.54±0.27 nmol/min/mg prot in the S group rats decreased statistically significantly due to both partial and complete obstruction. The values were

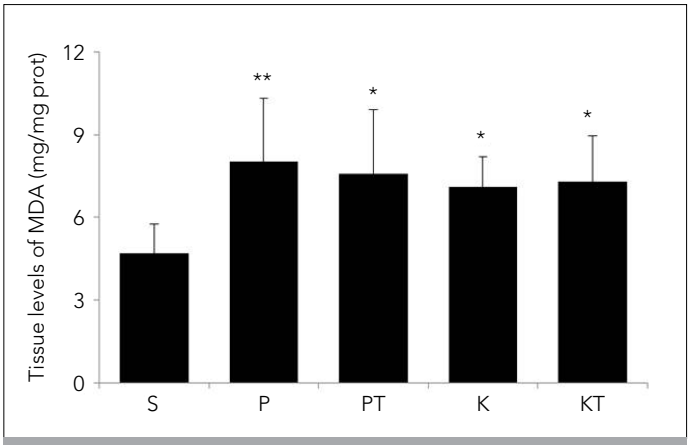

Figure 1. Levels of malondialdehyde (MDA) in kidney tissue. Sham control (S), Partial urethral obstruction (P), Partial urethral obstruction+Tadalafil therapy (PT), Complete urethral obstruction (C), and Complete urethral obstruction+Tadalafil therapy (CT). The statistical difference according to the S group: *p<0.05, **p<0.01.

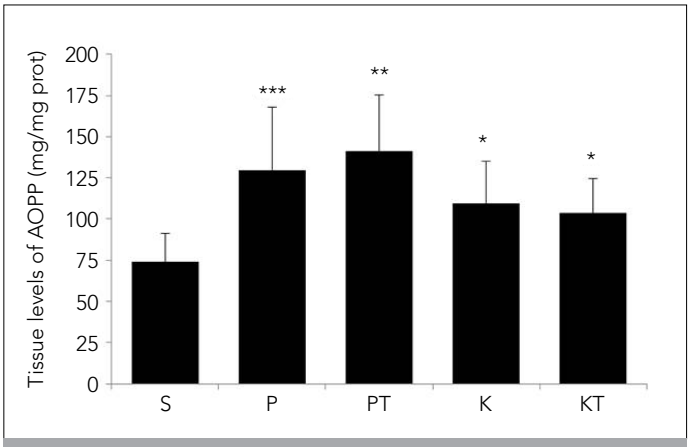

Figure 2. Levels of advanced oxidation protein products (AOPP) in kidney tissue. Sham control (S), Partial urethral obstruction (P), Partial urethral obstruction+Tadalafil therapy (PT), Complete urethral obstruction (C), and Complete urethral obstruction+Tadalafil therapy (CT). The statistical difference according to the S group: *p<0.05, **p<0.01, ***p<0.001.

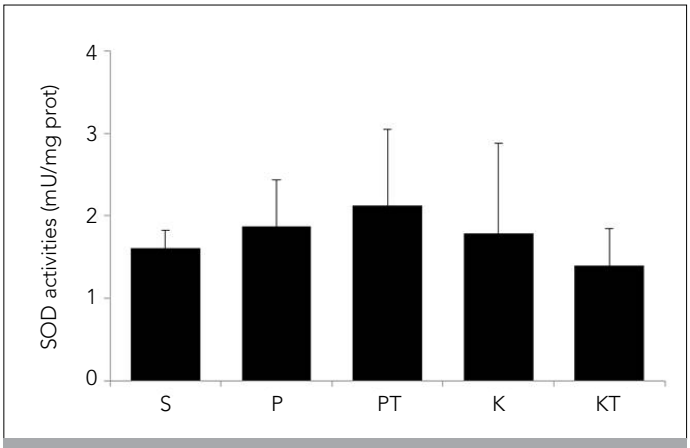

Figure 3. Superoxide dismutase (SOD) activity in kidney tissue. Sham control (S), Partial urethral obstruction (P), Partial urethral obstruction+Tadalafil therapy (PT), Complete urethral obstruction (C), and Complete urethral obstruction+Tadalafil therapy (CT)

determined to be 0.99±0.50 nmol/min/mg prot (p<0.01) in the P group and as 0.78±0.06 nmol/min/mg prot (p<0.001) in the C group. CAT activity increased to S group values (1.36±0.37 nmol/min/mg prot) in the PT group receiving tadalafil treatment, but no statistically significant change was observed in the C group (0.85±0.13 nmol/min/mg prot).

DISCUSSION

Urinary system obstruction is a serious and common problem causing renal parenchymal damage and loss of function because of an increase in the intraluminal pressure increase in the ureter and renal tubules and because of its direct and/or indirect effects (1-3). In addition to locally produced cytokines and various growth factors, reactive oxygen and nitrogen species are known to play an important role in the pathogenesis of UUO. It has been shown that increased reactive products can lead to lipid and protein oxidation as well as DNA damage. The decrease in antioxidant capacity in the cell due to obstruction appears to be an important factor causing growth in the damage (5-10).

In our study, lipid and protein oxidation levels in kidney tissue were examined in rats sacrificed 24 h after the unilateral partial and complete obstruction procedures were performed, and oxidative stress was evaluated. In both obstructive models, high levels of AOPP, in addition to levels of MDA, which is an indicator of lipid peroxidation, indicate increased oxidative stress due to obstruction in the kidney (Figures 1, 2). Obstruction-related changes in the activities of SOD and CAT, which are antioxidant enzymes, were also investigated in our study, and it was found that 24-h obstruction did not change SOD activity in kidney tissue; however, CAT activity was found to significantly decrease in both the P and C groups (Figures 3, 4). Although our findings do not show the expected decrease in SOD activity, they are consistent with the results of studies in the literature showing increased oxidative stress parameters and decreased CAT activity (8, 23); thus, they indicate that oxidative stress increases in the kidney due to ureteral obstruction.

Ureteral obstruction is associated with a decrease in renal blood flow and glomerular filtration rate due to renal vasoconstriction. The elevation in serum creatinine levels in the groups in which obstruction was created in our study indicates a functional decline due to obstruction and is consistent with results in similar literature studies (24, 25).

Specific PDE5 inhibitors whose vasoactive effects are beneficial in clinics are often used in the treatment of erectile dysfunction as well as pulmonary hypertension (11, 12). Besides the known effects, new studies have suggested that PDE5 inhibitors have antioxidant effects. For example, Küçük et al. (17) examined the effects of two different PDE5 inhibitors (tadalafil and sildenafil) on oxidative stress induced by ischemia–reperfusion injury in the kidney. In the aforementioned study, the researchers evaluated oxidative stress via MDA level and myeloperoxidase (MPO) activity and showed that MDA level and MPO activity that increased with ischemia–reperfusion in the kidney decreased after tadalafil and sildenafil treatment and that sildenafil treatment was more effective in this model. In conclusion, this study demonstrated that both

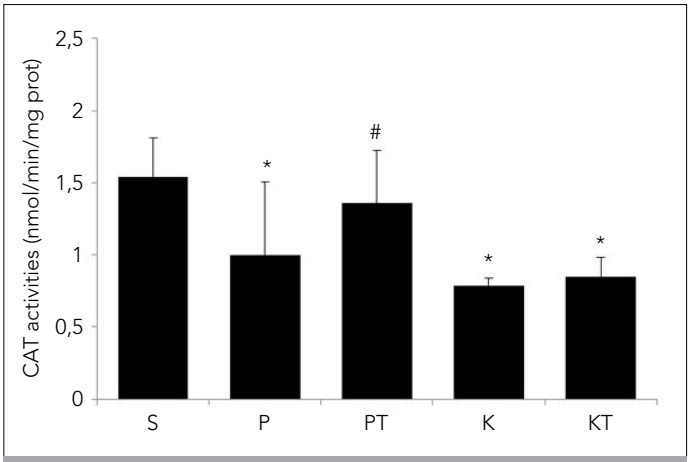

Figure 4. Catalase (CAT) activity in kidney tissue. Sham control (S), Partial urethral obstruction (P), Partial urethral obstruction+Tadalafil therapy (PT), Complete urethral obstruction (C), and Complete urethral obstruction+Tadalafil therapy (CT). The statistical difference according to the S group: *p<0.01, **p<0.001. The statistical difference according to the P group: #p<0.05.

drugs reduce oxidative stress and alleviate kidney damage (17). Koka et al. (16) showed in their studies with diabetic rats that 28-day low-dose tadalafil treatment (1 mg/kg, i.p.) reduces oxidative stress in the heart, and Chen et al. (14) showed that 8-week tadalafil treatment decreases oxidative stress in the cavernous tissue in diabetic rats. Mostafa et al. (15) also showed that oxidative stress decreases in the cavernous tissue with 12-week sildenafil, tadalafil, and sildenafil/tadalafil therapy (low dose, 12 weeks) in elderly diabetic rats (15). These and similar studies (14-21) have demonstrated the antioxidant effects of tadalafil in different tissues, such as kidney, heart, ovaries, and cavernous tissue, under different oxidative stress conditions. In our study, there was an increase in oxidative stress in the kidney due to both partial and complete obstruction, but single-dose tadalafil therapy did not have a significant corrective effect on oxidative stress parameters. In groups receiving tadalafil before obstruction, levels of MDA, which is a marker of lipid peroxidation, and of AOPP, which is a marker of protein oxidation, did not show a significant change when compared with untreated groups (Figures 1, 2). In our study, activities of SOD and CAT, which are among the cellular antioxidant enzymes, were also examined, and no change was observed in SOD activity depending on obstruction or treatment (Figure 3). Contrary to this, the response of tadalafil therapy to CAT whose activity decreased in both partial and complete urethral obstruction was different. While the mentioned drug did not have an effect in the C group, it was observed that the enzyme activity returned to normal in the P group (Figure 4).

In our study, changes in renal function and serum creatinine levels were followed, but it was seen that tadalafil therapy did not provide a functional correction as in oxidative stress. When the literature studies related to rats are examined, it is seen that PDE5 inhibitors are effective during long-term treatment periods such as 4, 8, or 12 weeks and decrease oxidative stress and provide functional improvements (14-21). The fact that the expected antioxidant effects of tadalafil were not observed in the present study is thought to have been caused by short-term and single-dose administration. The fact that the treatment in the P group corrected CAT activity suggests that our opinion is true and shows that a more effective treatment method is required to determine the increase in activity, the decrease in oxidative stress, and functional improvement in the other antioxidant enzyme systems.

CONCLUSION

In this study in which the effects of tadalafil, a specific PDE5 inhibitor, on the increase in oxidative stress due to UUO in the kidney were examined, it was determined that the administration of the drug in a single dose (10 mg/kg) had no apparent effect. However, the increase in CAT activity along with the use of the drug in the P group suggests that the administration of tadalafil at high doses and over a long period of time provides more significant results.

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