The effect of trimetazidine on renal oxidative stress in partial and complete ureteral obstructions in a rat model

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1. Introduction
Obstructive uropathy results from the blockage of urinary flow at any point in the urinary system. If the obstruction is not relieved in time, it leads to loss of kidney function. The most widespread cause of unilateral ureteral obstruction (UOO) in adult humans is sudden blockage leading to acute obstruction in a ureter. It generally appears with ureteral stones. An obstruction-related increase in retrograde hydrostatic pressure triggers tubular cell death due to apoptosis and necrosis, interstitial inflammation, decreased capillary numbers, and progressive fibrosis (1).

Oxidative stress causes injury and functional losses in many tissues, including the kidney. Clinical and experimental studies have shown that oxidative stress causes glomerular, tubulointerstitial, and endothelial changes in the kidneys and mediates the development of several kidney diseases, such as acute kidney failure, rhabdomyolysis, obstructive nephropathy, glomerular injury, and chronic kidney failure (2).

Reactive oxygen species (ROS) are highly reactive molecules derived from the mitochondrial electron transport chain (3). Overproduction of ROS and/or insufficiency in the antioxidant mechanism leads to compromise of the oxidant/antioxidant balance and thus to oxidative stress (4–6). The most important ROS include superoxide (O$_2^-$), hydroxyl (OH), hydrogen peroxide, singlet oxygen, and nitric oxygen radicals (6). Since reactive oxygen products have a rather unstable structure due to their unpaired electron, they can damage these structures by reacting with all elements in the environment in order to achieve stability (3–6). Excessive ROS formation in the body can lead to changes in the structure of DNA, resulting in activation of transcription factors induced by various stresses, production of pro- and anti-inflammatory cytokines, and modification of lipids (4).

Background/aim: Renal oxidative stress occurs in ureteral obstructions. The purpose of this study was to investigate the effect of the antioxidant and antiischemic agent trimetazidine (TMZ) on oxidative stress following ureteral obstruction.

Materials and methods: Ten groups were established. Sham groups were checked as controls after 1 and 3 weeks. The other 8 groups had partial or complete ureteral obstruction while receiving or not receiving trimetazidine (TMZ) at 5 mg/kg daily and were evaluated after either 1 week or 3 weeks. Creatinine and cystatin C measurements were performed in the serum. Malondialdehyde, myeloperoxidase, catalase, and glutathione peroxidase activity were measured in renal tissue and serum.

Results: In the 1-week groups, tissue malondialdehyde, serum myeloperoxidase, and glutathione peroxidase activity increased significantly with obstruction and TMZ use compared to the control group (P < 0.005). In the 3-week TMZ group, cystatin C, tissue malondialdehyde, serum and tissue myeloperoxidase, and tissue glutathione peroxidase differed significantly (P < 0.05). There was no significant difference in all parameters after 3 weeks of partial obstruction (P > 0.05), with only serum malondialdehyde being significantly elevated (P < 0.05).

Conclusion: TMZ did not exhibit a renal oxidative stress-lowering effect in obstruction. It causes mild impairment of renal functions in obstruction. Patients using TMZ must be closely monitored in terms of kidney function in the event of any ureteral obstruction.

Key words: Ureteral obstruction, renal oxidative stress, trimetazidine, renal function

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Research Article
Protection of the cell against free oxygen species occurs by means of enzymatic systems such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), thioredoxin, peroxiredoxin, and glutathione transferase (GST) and of nonenzymatic systems such as vitamins E and C, and glutathione (GSH). The activity of antioxidant enzymes such as SOD, CAT, and GPx is of considerable importance in protecting against oxidative stress injury (3,4,6). In addition to an increase in ROS in kidneys developing obstruction in an UUO model, significant decreases also occur in the activity of the important antioxidants SOD, CAT, and GPx. This decrease in antioxidant defense contributes to the development of oxidative stress (7–9).

Trimetazidine (TMZ) [1–(2,3,4–trimethoxybenzyl)piperazine dihydrochloride] is an antianginal, antiischemic drug (10). It inhibits the beta-oxidation pathway of fatty acid metabolism (10,11), prevents overproduction of free radicals (11), and protects cellular integrity by eliminating ROS (12). Clinically, TMZ is used in heart disorders, cerebral ischemia, and vertigo.

Although many studies support the protective effect of TMZ on the heart, there is a lack of data about the effects of this pharmaceutical on the kidney. This study investigated the potential protective effect of TMZ on increased oxidative stress in the kidney caused by partial and complete UUO.

2. Materials and methods
Approval for this study, performed at the Ondokuz Mayis University Experimental Animals Practice and Research Center, Turkey, was granted by the Animal Experiments Local Ethical Committee (B.30.2.O DM.020.09.00-050.04-91). Sixty-eight male, adult (2.5–3 months old), albino Sprague Dawley rats were used, divided into 10 groups (see Table 1 for definitions of groups G1–G10).

2.1. Surgical procedures
Laparotomy was performed under general anesthesia (ketamine, 50 mg/kg, intramuscular), and the left ureter was isolated. The incisions in groups G1 and G2 were closed without narrowing being performed. An Angiocath catheter (24 G) was placed in the ureteric lumen in the G3, G4, G5, and G6 groups. The ureter was then sutured over this catheter using 4/0 silk. Standard narrowing was thus established over the catheter using 4/0 silk. Standard narrowing was thus established in the ureters of the animals in these groups undergoing partial obstruction. In groups G7, G8, G9, and G10, undergoing complete obstruction, the left ureter was tied off from two points with 4/0 silk. It was then severed, and complete obstruction was thus established. Following the surgical procedures, the abdominal incisions were closed appropriately. The experimental groups are shown in Table 1.

2.2. Drug application
A single daily dose of TMZ (5 mg/kg) was administered by intragastric lavage for 1 week after the surgical procedure to the rats in groups G5 and G9 and for 3 weeks after the surgical procedure to the rats in groups G6 and G10. A minimum dose of 5 mg/kg was used in this study in order to prevent rat losses, bearing in mind the expulsion of the drug from the kidney.

All biological specimens in this study were obtained at the Ondokuz Mayis University Experimental Animals Practice and Research Center, stored at –80 °C, and transferred on dry ice and under appropriate conditions to Ordu University once all groups had been established. Specimens were kept in deep freeze at our faculty at –80 °C until the day of the study.

Malondialdehyde (MDA) levels, myeloperoxidase (MPO) activity, and CAT and GPx activity were measured in tissue and serum specimens. As the increase of creatinine and cystatin C measurements represents renal insufficiency, creatinine and cystatin C measurements were performed in serum specimens.

2.3. Creatinine measurement
This was performed in rat serum using commercial creatinine kits (Creatinine Lot No: 78067UN14), on an

| Group 1 (G1) | Sham, 1-week control (n = 6) |
| Group 2 (G2) | Sham, 3-week control (n = 6) |
| Group 3 (G3) | PUO, 1 week (n = 6) |
| Group 4 (G4) | PUO, 3 weeks (n = 6) |
| Group 5 (G5) | PUO + TMZ, 1 week (n = 8) |
| Group 6 (G6) | PUO + TMZ, 3 weeks (n = 8) |
| Group 7 (G7) | CUO, 1 week (n = 6) |
| Group 8 (G8) | CUO, 3 weeks (n = 6) |
| Group 9 (G9) | CUO + TMZ, 1 week (n = 8) |
| Group 10 (G10) | CUO + TMZ, 3 weeks (n = 8) |

Experimental animals in groups G1, G3, G5, G7, and G9 were sacrificed under general anesthesia (ketamine, 100 mg/kg, intraperitoneally) 1 week after obstruction, and those in groups G2, G4, G6, G8, and G10 were sacrificed under general anesthesia 3 weeks after obstruction. Blood and tissue samples were collected. Blood specimens were placed into gel-coated tubes, kept at room temperature for 20–30 min, and then centrifuged for 15 min at 3000 × g. Serum specimens were kept as aliquots at –80 °C. Left kidney tissues for use in the analysis were obtained by total nephrectomy and stored at –80 °C.
Abbott Architect C8000 autoanalyzer, at the Ministry of Health Ordu University Education and Research Hospital biochemistry laboratory.

2.4. Cystatin C measurement
Cystatin C measurements in serum specimens were performed using an ELISA kit specific to rats (Boster, EK1109). Specimens were studied in 1:50 dilution, and the results were read at 450 nm wavelength on an ELISA reader (BioTek, ELx800).

2.5. MDA assay
A 2-thiobarbituric acid reactive substances (TBARS) analysis kit (Cayman Cat No: 10009055, Lot No: 0459122) was used for the measurement of MDA levels in tissue and serum specimens. Tissues were washed in PBS buffer (0.02 mol/L, pH 7.0–7.2) to remove all blood, and homogenization was subsequently performed (100 mg tissue/1 mL PBS). The tissue homogenates obtained were subjected to freezing and thawing procedures, which were repeated twice, and then centrifuged for 15 min at 1500 × g. The kit prospectus was applied to supernatants at a 1:10 dilution, and the results were read at 450 nm wavelength. Results obtained for tissue were standardized with the protein level (mg) in the homogenate.

2.6. MPO activity measurement
A rat-specific commercial ELISA kit (BlueGene: E02M0032, Lot No: 20140825) was used for the measurement of MPO in tissue and serum. Prior to homogenization, tissues were washed in PBS buffer (0.02 mol/L, pH 7.0–7.2) for blood removal. Homogenization was then performed (100 mg tissue/1 mL PBS). The tissue homogenates obtained were twice frozen and thawed, and then centrifuged for 15 min at 1500 × g. The kit protocol was applied to supernatants at a 1:10 dilution, and the results were read at 450 nm wavelength. Results obtained for tissue were standardized with the protein level (mg) in the homogenate.

2.7. CAT activity measurement
A commercial CAYMAN analysis kit (Cayman Chemical 707002) was used in the measurement of CAT activity in tissue and serum. Tissues were placed in homogenization buffer (50 mM potassium phosphate, 1 mM EDTA, pH 7.0) (5 mL buffer/g tissue) and homogenized on ice, after which they were centrifuged for 15 min at 10,000 × g. The supernatants obtained were diluted 250-fold and used in enzyme activity measurement. Results were read at 450 nm wavelength.

2.8. GPx activity measurement
Measurement of GPx in tissue specimens was performed using spectrophotometry with a commercial Cayman analysis kit (Cayman 703102). Prior to homogenization, tissues were washed in PBS buffer (0.02 mol/L, pH 7.0–7.2) for blood removal. Homogenization was then performed. The tissue was washed in 5 mL of cold buffer (in 50 mM Tris-HCL, pH 7.5, 5 mM EDTA, and 1 mM DTT) per gram of tissue and centrifuged for 15 min at 10,000 × g. GPx measurement in serum specimens was performed using a BlueGene commercial rat-specific ELISA kit (BlueGene E02G0369).

2.9. Tissue protein measurement
Protein assay of tissue homogenates prepared as described above was performed spectrophotometrically, using the Bradford method (Thermo Scientific; 23200). Measurements in specimens diluted at a level of 1:20 were performed at 595 nm, and the protein level in the specimen was determined on the basis of a standard chart.

2.10. Statistical analysis
Statistical analysis was performed using the one-way ANOVA post hoc multiple comparisons Tukey test with SPSS 16 software at a significance level of P < 0.05.

3. Results
Comparisons were performed in the 1- and 3-week groups against the control groups. Group G1 was compared with groups G3, G5, G7, and G9, while groups G2 and G4, G6, G8, and G10 were compared among themselves. Values for all parameters in the study are shown in Table 2.

3.1. Serum creatinine
Mean serum creatinine values for all groups are shown in Table 2. Values were at the lowest level in the control groups and increased in the study groups. Although a statistically significant difference was observed between G1 and G3 (P = 0.011), no significant differences were determined among the other groups (P > 0.05) (Table 3). No significant difference was observed between G2 and the other 3-week groups (P > 0.05) (Table 4).

3.2. Serum cystatin C
Intergroup comparison revealed no statistically significant difference between the 1-week groups (P > 0.05), while in the 3-week groups significant differences were only observed between G2 and G10 (P = 0.002) (Tables 3 and 4).

3.3. Serum and tissue MDA
No difference was observed among the 1-week groups in terms of serum MDA levels (P > 0.05) (Table 3). Comparison of the 3-week serum MDA levels revealed a significant difference between G2 and G8 (P = 0.031), but none among the other groups (P > 0.05) (Table 4). In terms of tissue MDA levels, significant differences were only determined between G1 and G3, G1 and G5, G1 and G7, and G1 and G9 (P = 0.000; P = 0.000; P = 0.002; P = 0.002, respectively) (Table 3). Comparison of the 3-week tissue MDA groups and the control groups revealed a statistically significant difference only between G2 and G6 (P = 0.047), and none between the other groups (P > 0.05) (Table 4).
3.4. Serum and tissue MPO

Comparison of the 1-week groups in terms of serum MPO values revealed significant differences between G1 and G3, G1 and G5, G1 and G7, and G1 and G9 (P = 0.000; P = 0.000; P = 0.000, respectively). There was no difference between the 1-week groups in terms of tissue MPO levels (P > 0.05) (Table 3). Comparison of the 3-week groups revealed statistical significance only between G2

Table 2. Mean values ± SEM for all the parameters in the experimental study.

<table>
<thead>
<tr>
<th></th>
<th>S. creatinine (mg/dL)</th>
<th>S. cystatin-C (ng/mL)</th>
<th>S. MDA (µmol TMABRS/mg protein)</th>
<th>T. MDA (µmol TMABRS/mg protein)</th>
<th>S. MPO (ng/dL)</th>
<th>T. MPO (ng/mg protein)</th>
<th>S. CAT activity (µmol/min/mg protein)</th>
<th>T. CAT activity (µmol/min/mg protein)</th>
<th>S. GPx (µmol/mL)</th>
<th>T. GPx activity (µmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0.437 ± 0.007</td>
<td>571.4 ± 19.61</td>
<td>25.78 ± 5.07</td>
<td>4.497 ± 0.309</td>
<td>45.73 ± 5.03</td>
<td>51.82 ± 7.51</td>
<td>0.360 ± 0.094</td>
<td>0.806 ± 0.136</td>
<td>135.5 ± 13.5</td>
<td>0.090 ± 0.020</td>
</tr>
<tr>
<td>G2</td>
<td>0.482 ± 0.008</td>
<td>572.4 ± 21.39</td>
<td>29.11 ± 5.83</td>
<td>3.763 ± 0.807</td>
<td>28.61 ± 4.41</td>
<td>45.66 ± 3.69</td>
<td>0.109 ± 0.017</td>
<td>0.636 ± 0.059</td>
<td>87.48 ± 17.35</td>
<td>0.086 ± 0.021</td>
</tr>
<tr>
<td>G3</td>
<td>0.530 ± 0.010</td>
<td>574.7 ± 14.98</td>
<td>35.47 ± 9.68</td>
<td>1.675 ± 0.491</td>
<td>22.51 ± 3.38</td>
<td>62.58 ± 6.33</td>
<td>0.120 ± 0.015</td>
<td>0.403 ± 0.025</td>
<td>95.69 ± 10.93</td>
<td>0.164 ± 0.011</td>
</tr>
<tr>
<td>G4</td>
<td>0.513 ± 0.026</td>
<td>559.9 ± 34.05</td>
<td>36.70 ± 3.71</td>
<td>2.390 ± 0.393</td>
<td>16.33 ± 0.72</td>
<td>41.38 ± 6.52</td>
<td>0.382 ± 0.148</td>
<td>0.957 ± 0.071</td>
<td>40.57 ± 9.62</td>
<td>0.090 ± 0.030</td>
</tr>
<tr>
<td>G5</td>
<td>0.501 ± 0.016</td>
<td>547.3 ± 47.01</td>
<td>60.59 ± 8.12</td>
<td>1.966 ± 0.424</td>
<td>19.18 ± 4.05</td>
<td>56.16 ± 4.81</td>
<td>0.166 ± 0.048</td>
<td>0.497 ± 0.026</td>
<td>47.37 ± 2.83</td>
<td>0.121 ± 0.018</td>
</tr>
<tr>
<td>G6</td>
<td>0.474 ± 0.014</td>
<td>607.8 ± 18.90</td>
<td>63.36 ± 9.46</td>
<td>2.067 ± 0.298</td>
<td>10.66 ± 1.38</td>
<td>61.96 ± 3.82</td>
<td>0.233 ± 0.065</td>
<td>0.805 ± 0.135</td>
<td>39.53 ± 3.44</td>
<td>0.213 ± 0.024</td>
</tr>
<tr>
<td>G7</td>
<td>0.508 ± 0.022</td>
<td>620.4 ± 11.04</td>
<td>75.28 ± 11.49</td>
<td>1.205 ± 0.085</td>
<td>20.01 ± 2.44</td>
<td>68.18 ± 2.99</td>
<td>0.357 ± 0.095</td>
<td>0.466 ± 0.032</td>
<td>78.15 ± 2.45</td>
<td>0.141 ± 0.027</td>
</tr>
<tr>
<td>G8</td>
<td>0.497 ± 0.018</td>
<td>612.8 ± 15.55</td>
<td>84.05 ± 16.42</td>
<td>2.045 ± 0.160</td>
<td>19.59 ± 2.79</td>
<td>66.57 ± 5.62</td>
<td>0.271 ± 0.084</td>
<td>0.406 ± 0.054</td>
<td>71.71 ± 2.45</td>
<td>0.151 ± 0.013</td>
</tr>
<tr>
<td>G9</td>
<td>0.506 ± 0.017</td>
<td>617.7 ± 46.39</td>
<td>76.33 ± 12.36</td>
<td>2.245 ± 0.154</td>
<td>14.66 ± 1.52</td>
<td>75.36 ± 3.63</td>
<td>0.585 ± 0.052</td>
<td>0.523 ± 0.022</td>
<td>35.58 ± 11.06</td>
<td>0.194 ± 0.020</td>
</tr>
</tbody>
</table>
| G10      | 0.526 ± 0.015         | 819.4 ± 72.79         | 72.03 ± 12.96                    | 2.533 ± 0.100                    | 17.64 ± 2.96  | 77.08 ± 9.03           | 0.309 ± 0.062                       | 0.459 ± 0.043                       | 52.31 ± 8.18  | 0.148 ± 0.035          

S: Serum; T: tissue; MDA: malondialdehyde; MPO: myeloperoxidase; GPx: glutathione peroxidase.

Table 3. Comparison of the 1-week groups.

<table>
<thead>
<tr>
<th></th>
<th>S. creatinine (mg/dL)</th>
<th>S. cystatin-C (ng/mL)</th>
<th>S. MDA (µmol TMABRS/mg protein)</th>
<th>T. MDA (µmol TMABRS/mg protein)</th>
<th>S. MPO (ng/dL)</th>
<th>T. MPO (ng/mg protein)</th>
<th>S. CAT activity (µmol/min/mg protein)</th>
<th>T. CAT activity (µmol/min/mg protein)</th>
<th>S. GPx (µmol/mL)</th>
<th>T. GPx activity (µmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1–G3</td>
<td>0.011*</td>
<td>NS</td>
<td>NS</td>
<td>0.000*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>G1–G5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.000*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>G1–G7</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.000*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>G1–G9</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.002*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
</tr>
</tbody>
</table>

*One-way ANOVA post hoc multiple comparisons Tukey test, P < 0.05; NS: nonsignificant, P > 0.05; S: serum; T: tissue; MDA: malondialdehyde; MPO: myeloperoxidase; GPx: glutathione peroxidase.

Table 4. Comparison of the 3-week groups.

<table>
<thead>
<tr>
<th></th>
<th>S. creatinine (mg/dL)</th>
<th>S. cystatin-C (ng/mL)</th>
<th>S. MDA (µmol TMABRS/mg protein)</th>
<th>T. MDA (µmol TMABRS/mg protein)</th>
<th>S. MPO (ng/dL)</th>
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<th>T. CAT activity (µmol/min/mg protein)</th>
<th>S. GPx (µmol/mL)</th>
<th>T. GPx activity (µmol/mL)</th>
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<tbody>
<tr>
<td>G2–G4</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>G2–G6</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.047*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.012*</td>
</tr>
<tr>
<td>G2–G8</td>
<td>NS</td>
<td>NS</td>
<td>0.031*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>G2–G10</td>
<td>0.002*</td>
<td>NS</td>
<td>NS</td>
<td>0.010*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*One-way ANOVA post hoc multiple comparisons Tukey test, P < 0.05; NS: nonsignificant, P > 0.05; S: serum; T: tissue; MDA: malondialdehyde; MPO: myeloperoxidase, GPx: glutathione peroxidase.
Research is still taking place in a shorter time when the obstruction is complete (7–9).

Large numbers of locally produced cytokines, various growth factors, ROS, and reactive nitrogen species are involved in the pathogenesis of UUO. In addition, it has been shown that an increase in reactive products may give rise to DNA injury during and as a result of lipid peroxidation (19). MDA in serum and tissue occurs as a result of oxidative lipid peroxidation (19). MDA is formed from polyunsaturated fatty acids. Its major precursors are generally regarded as arachidonic acid and docosahexaenoic acid (20). MDA reacts with nucleic acid bases in DNA and ribonucleic acid, similarly to those in proteins, to form various products (19). Although studies have reported that TMZ

and G6 (P = 0.004), and none between the other groups (P > 0.05). In the tissue MPO levels, the only significant differences in the 3-week groups were between G2 and G10 (P = 0.01) (Table 4).

4. Discussion

Ureteral obstruction in clinical practice may be partial (PUO) or complete (CUO). The severity of the obstruction and the damage it causes depend on its duration, form, and location. Reflections of severity of injury in the obstructed kidney also vary, depending on whether or not the contralateral kidney is normal (1).

In UUOs, cellular and molecular mechanisms in the ipsilateral kidney are affected. This leads to varying degrees of inflammation, apoptosis, increased oxidative stress, fibrosis, and kidney loss, depending on whether the obstruction is partial or complete. These processes take place in a shorter time when the obstruction is complete (7–9).

In our study, 1-week rat groups were established to investigate the short-term results after PUO and CUO, and 3-week groups were formed to investigate the long-term results. MDA, MPO, CAT, and GPx were investigated in serum and in tissue to show increases in oxidative stress, whether that stress would decrease, and whether antioxidant capacity would increase in the drug groups. In addition, serum creatinine and cystatin C levels were investigated in all groups. Increases were observed in serum creatinine and cystatin C levels in all groups using TMZ. Maximum serum creatinine and cystatin C levels were observed in 3-week CUO. Serum creatinine values decreased in 1-week and 3-week PUO with TMZ, but it increased in all the CUO + TMZ groups. Cystatin C levels were largely similar to creatinine. This shows that TMZ compromises kidney function in both the long and the short term in partial obstructions. In complete obstruction, TMZ significantly impairs kidney functions. Indeed, TMZ is eliminated from the body through the kidneys (20). An antioxidant, antiischemic agent eliminated from the body through the kidneys might be expected to have a better healing effect in kidney tissues and to act as a corrective factor. However, this was not observed in our study. To our knowledge, there are no data about the nephrotoxicity of TMZ in the literature.

The oxidative damage markers of serum and tissue MDA and MPO, serum GPx levels, tissue GPx activity, and serum and tissue CAT activity were investigated in this study.

Free oxygen radicals play important roles in the pathophysiology of ureteral obstruction. Accumulation of MDA in serum and tissue occurs as a result of oxidative lipid peroxidation (19). MDA is formed from polyunsaturated fatty acids. Its major precursors are generally regarded as arachidonic acid and docosahexaenoic acid (20). MDA reacts with nucleic acid bases in DNA and ribonucleic acid, similarly to those in proteins, to form various products (19). Although studies have reported that TMZ
reduces MDA levels in ischemia-reperfusion injury (16), no difference was observed in serum MDA levels in the 1-week groups in our study. Serum MDA levels were highest in the 3-week CUO group. A slight increase occurred in the partial obstruction groups. MDA levels continued to increase in the 1- and 3-week complete and partial obstruction groups that were administered TMZ. In terms of tissue MDA levels, change was either minimal or nonexistent in the groups receiving the drug compared to the partial and complete obstruction groups not receiving the drug.

Similarly to our own study, Tirani et al. reported a significant increase in tissue MDA levels after 24 h in rats with bilateral induced ureteral obstruction (21). While increases were observed in our study in terms of serum MDA levels compared to the sham group, the exact opposite was observed in tissue MDA. This may be due to the contralateral kidney contributing to MDA values as a result of obstruction being induced unilaterally. In addition, the decrease in tissue MDA shows that lipid peroxidation does not occur in obstructed kidneys, in a manner incompatible with findings in ischemia-reperfusion injury. Further varied and detailed studies are needed on this subject.

Analysis of serum and tissue MPO levels in this study revealed a decrease in serum levels and an increase in tissue levels. MPO is a proinflammatory enzyme stored in the azurophilic granules of neutrophils (22). It catalyzes the formation of hypochlorous acid from hydrogen peroxide. Gene polymorphism has been reported (23,24). Enzymatic activity can vary significantly between individuals. Increases and decreases in activity have therefore both been reported (23,24). A significant difference was observed in our study in terms of serum values between the sham group and the other groups. Serum MPO values decreased in CUO and PUO and in the drug groups, while tissue MPO levels increased. Similar findings were observed in the 3-week group. While TMZ increased antioxidant activity in renal tissue, serum MPO levels decreased. However, in a study of thermal injury-related intestinal mucosa and kidney damage, Yalçın et al. (18) reported that TMZ reduced MDA levels in cardiac ischemia-reperfusion injury (16). They also observed, in contrast to our study, that it reduced intestinal MPO levels.

CAT converts hydrogen peroxide into water and molecular oxygen. The cells are thus protected against damage by hydrogen peroxide. In addition, alcohols such as methanol and ethanol oxidize into formaldehyde and acetaldehyde under the effect of peroxidase in the presence of hydrogen peroxide (5). Serum CAT activities increased in our CUO + TMZ groups (1 week and 3 weeks, G9 and G10) compared to the CUO groups without TMZ. The same increases also occurred in renal tissue CAT activities in the same groups. Tissue CAT activities increased with length of partial obstruction. The effect of TMZ on tissue CAT activity in the partial obstruction groups was slight. GPx represents a broad enzyme family that uses glutathione. They reduce hydrogen peroxide or organic hydroperoxides to water or alcohol, respectively. The GPx family metabolizes lipid hydroperoxides, but CAT serves no such function. Therefore, the absence of an increase in CAT activity despite a rise in GPx activity shows that lipid hydroperoxides are implicated in oxidative stress response in ureteral obstruction (14). Serum GPx levels and tissue GPx activity were also measured in this study. Serum GPx levels decreased in both the 1- and 3-week CUO groups receiving TMZ. The same decrease occurred in the 1-week PUO group receiving TMZ, but it was minimal in the 3-week TMZ group. Tissue GPx activity rose to the highest level in the 3-week PUO + TMZ group. Tissue GPx activity increased in almost all groups compared to the sham groups. Kinter et al. showed that increased sodium wasting in rats in which they induced unilateral CUO did not change antioxidant enzyme CAT levels but did cause a slight increase in GPx (14).

No renal oxidative stress-lowering effect was observed in UUO in rats when TMZ was used at a dose of 5 mg/kg daily. Additionally, it had a minimal adverse effect on renal function. New studies are planned for different doses. Future studies involving oxidant and antioxidant systems in both the ipsilateral and contralateral kidneys in UUO may provide more detailed information concerning serum values. Patients using TMZ, an antiischemic and antioxidant agent, must be closely monitored in terms of kidney function. The possibility that the drug may compromise kidney functions should be considered and appropriate measures should be taken.

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