THE PROTECTIVE EFFECT OF LYCOPENE ON KIDNEY AGAINST EXPERIMENTALLY INDUCED UNILATERAL URETERAL OBSTRUCTION

HASAN IKBAL ATILGAN[*], ARIF AYDIN[**], MURAT SADIC[***], MELIHA KORKMAZ[***], TOLGA KARAKAN[****], ELMAS OGUS[*****], PELIN BORCEK[******], GOKHAN KOCA[***]

[*]Kahramanmaras Necip Fazil City Hospital, Department of Nuclear Medicine, Kahramanmaras, Turkey - [**]Sanliurfa Mehmet Akif Inan Training and Research Hospital, Clinic of Urology, Sanliurfa, Turkey - [***]Ankara Training and Research Hospital, Clinic of Nuclear Medicine, Ankara, Turkey - [****]Ankara Training and Research Hospital, Clinic of Urology, Ankara, Turkey - [*****]Ankara Training and Research Hospital, Department of Biochemistry, Ankara, Turkey - [******]Baskent University Medical School, Department of Pathology, Ankara, Turkey

ABSTRACT

***Introduction**: To investigate the possible protective effect of lycopene on kidney after unilateral ureteral obstruction.*

***Materials and methods**: Twenty-one Wistar albino rats were divided into three groups in experimental study. In lycopene group, lycopene was administered two days before left ureteral ligation and was continued for 15 days. In control group, left ureteral ligation was applied with no medication. In sham group, midline incision was made to abdomen and then closed. Histopathological, biochemical and scintigraphic evaluations were made.*

***Results**: Microscopic score was 50.56 ± 34.32 in lycopene, 97.22 ± 39.14 in control and 28.33 ± 12.58 in sham group. Microscopic score was lower in lycopene group than control group (p=0.033). In biochemical analysis, mean value of myeloperoxidase (MPO), malondialdehyde (MDA) and nitrite levels were 0.05 ± 0.03, 4.64 ± 1.49 and 0.06 ± 0.01 respectively in lycopene group, 0.10 ± 0.03, 8.37 ± 3.31 and 0.14± 0.09 in control group and 0.04 ± 0.01, 18.76 ± 9.30 and 0.05 ± 0.01 in sham group. MPO, MDA and nitrite levels were lower in lycopene group than in control group (p=0.033, p=0.007 and p < 0.001, respectively). Postoperative mean Technetium-99m dimercaptosuccinic acid (Tc-99m DMSA) uptake values of left kidneys were 6.26 ± 5.17 in lycopene, 2.09 ± 1.03 in control and 49.17 ± 1.37 in sham group. There was no statistically significant difference between lycopene and control groups in respect of postoperative DMSA uptake values (p=0.063).*

***Conclusion**: Lycopene is effective for the protection of kidney after ureteral obstruction as shown by microscopic parameters and decreased reactive oxygen species.*

***Keywords**: Antioxidants, DMSA; lycopene; ureteral obstruction.*

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Introduction

Ureteral stones usually cause ureter obstruction and kidney functions may be disturbed during the period from presentation at hospital and examination until the operation. Obstruction causes tubular atrophy and cell death. Renal tubular apoptosis has been reported to start within four days and increase to a maximum level in 15 days in rat kidneys[(1)]. Kidneys also have high oxygen consumption and metabolic activity, with infiltrative cells and the kidney's own cells producing reactive oxygen species.

Reactive oxygen species produced in excess of the number required for the kidney's antioxidant protection mechanism, may cause tissue damage[(2, 3)]. In addition, reactive oxygen species have a role in the synthesis and degradation of the extracellular matrix and may cause renal fibrosis. Despite timely surgical management of the obstruction, irreversible damage may occur. Relief of the obstruction may not totally reverse the function and histopathological changes.

Some pharmacological agents may be needed for the protection of kidney until the time of surgery and to reverse the functions after the operation.

Free radical scavengers have protective effects on renal injury caused by reactive oxygen species induced ureteral obstruction[4]. Carotenoids are divided into two groups as carotenes (α-carotene, β-carotene and lycopene) and xanthophylls (lutein and zeaxanthin). Lycopene is an antioxidant agent, which has been used for protection in many types of cancer such as prostate, skin and breast[5]. It is a red carotenoid pigment and found in red vegetables and fruits such as tomato and watermelon[6]. Lycopene has the strongest singlet oxygen-quenching ability among the carotenoids and only lycopene is related with reduced prostate cancer risk[7].

To the best of our knowledge, there has been no study using lycopene for protection of the kidneys after unilateral ureteral obstruction. The aim of this study was to investigate the possible protective effect of lycopene on the kidney after unilateral ureteral obstruction through the use of histopathological and biochemical analysis and Technetium-99m dimercaptosuccinic acid (Tc-99m DMSA) scintigraphy uptake.

Materials and methods

This prospective and empirical study was carThe Local Ethics Committee of Animal Experiments in Ankara Training and Research Hospital granted approval for the study. A total of 21 Wistar albino male rats aged 3-5 months and weighing 250-300 gr were used in this experimental study. The rats were acclimatized for one week before the study. The animals were fed with standard chow and water ad libitum in the animal experiment laboratory. The rats were housed under a constant temperature of 21 ± 2 °C with relative humidity of 50-60%.

The animals were divided into three groups. Group 1 (n=9) was administered lycopene [5 mg/kg body weight/day (lyc-o-mato lycopene®, GNC product)] starting two days before left ureteral obstruction and this was continued for 15 days. Static renal scintigraphy images with Tc-99m DMSA were obtained preoperatively and on postoperative day 15 and then the rats were sacrified. Group 2 was the control group (n=9) to which left ureteral obstruction was applied and static renal scintigraphy as in the Group 1 protocol but without any medication. Group 3 (n=3) was the sham group to which no ureter ligation or no medication was applied.

Surgery and Tc-99m DMSA scintigraphy were applied under anesthesia of 40 mg/kg ketamine hydrochloride (Ketalar; Parke Davis Eczacibasi, Istanbul, Turkey) and 5 mg/kg xylazine (Rompun; Bayer, Istanbul, Turkey) applied intramuscularly.

Surgical method

In the lycopene and control groups, a laparotomy was made with a midline incision, and the left kidney was reached. The left renal pedicle was isolated and the left ureter was visualized at the level of the inferior pole of the kidney after retraction of the intestine. The left ureter was sutured with 4/0 Vicryl (Coated Vicryl Plus®, Ethicon, New Jersey, USA) while preserving the surrounding tissue. After suturing, the laparotomy incision was closed in anatomical planes. In the sham group, a midline incision was made to the abdomen and then closed in anatomical planes.

Macroscopic evaluation

Kidneys were evaluated for hydronephrosis, the pelvis and ureter for dilatation and the parenchyma for edema.

Histopathological evaluation

Kidney tissues obtained from the sacrified rats were fixed in 10% formalin solution and macroscopic sections were taken to include the renal cortex and pelvis. Sections of 5 μm thickness were stained with hematoxylin-eosin dye. For histopathological evaluation the following criteria were used:

Normal histology: 0 point

Swelling of tubule cells, loss of brush border and nuclear condensation of ≤1/3: 1 point

In addition to the changes in 1 point, between 1/3-2/3 tubule changes: 2 points

Tubular changes more than 2/3: 3 points

All kidneys were examined in 100 areas with a maximum score of 300.

Biochemical evaluation

Tissue samples were stored at -80°C until analysis. Kidney samples were homogenized with 0.15M KCl at a rate of 1/10 (weight per volume). Malondialdehyde (MDA), myeloperoxidase (MPO) and total nitrite levels were measured in the tissue samples.

Determination of MPO level

MPO activity was assayed spectrophotometrically by determining the decomposition of hydrogen peroxide using o-dianisidine as the hydrogen donor. Tissue samples of approximately 50 mg were taken, weighed and homogenized 3 times for 30 secs at 4oC

in 1 ml of ice-cold 0.5% hexadecyltrimethylammonium bromide in 50 mmol/L phosphate buffer (pH 6). The homogenate was subjected to 3 freeze / thaw cycles and centrifuged for 15 min at 40,000xg. MPO activity was determined by the addition of 0.1 ml of the supernatant to 2.9 ml of 50 mmol/L phosphate buffer containing 0.167 mg/ml o-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm over a 5 min period was measured at 25°C. The data are expressed as the change in absorbance/min/g tissue[8].

Determination of MDA level

MDA levels were calculated by the fluorometric method, developed by Wasowicz et al[9]. After the reaction of thiobarbituric acid with MDA, the reaction product was extracted in butanol and was measured spectrofluorometrically at wavelengths of 525 nm for excitation and 547 nm for emission. 0-5 µmol2/L 1,1',3,3'-tetraethoxypropane solutions were used as standard. For the measurement of tissue MDA, 50 µL of homogenate was added and introduced into 10 mL glass tubes containing 1 ml of distilled water. After the addition of 1 ml of the solution containing 29 mmol/L TBA in acetic acid and mixing, the samples were placed in a water bath and heated for 1 hr at 95-100 oC. After the samples cooled, 25 µL of 5 mol/L HCL was added and the reaction mixture was extracted by agitation for 5 min with 3,5 ml n-butanol. After separation of the butanol phase by centrifugation at 1500 x g for 10 min, the fluorescence of the butanol extract was measured with a fluorometer (HITACHI F-2500) at wavelengths of 525 nm for excitation and 547 nm for emission. 0-5 µmol/L 1,1',3,3'-tetraethoxypropane solutions were used as standard. MDA levels are given as μmol / g wet tissue.

Determination of total nitrite level

300 μl homogenate was added to 300 μl of KH2PO4 / K2HPO4 buffer (pH 7.5), 50 μl of 2 mmol/L NADPH, 50μl of 50 μmol/L FAD and 50 μl of 1 unit/mL aspergillus nitrate reductase. This was incubated at room temperature for one hour followed by the addition of 500 μl of 0.2 mol/L ZnSO4 and 70 μl 2 mol/L NaOH to deproteinate the sample. After centrifugation, 0.75 mL of the supernatant was added to 1 mL of 1% sulphanilic acid (in 4 mol/L HCL). After 10 minutes at room temperature 0.75 mL of freshly prepared 1% N-naphtyl ethylenediamine was also added. The final color change was measured at 548 nm using a spectrophotometer.

Nitrite concentration was calculated from 5, 12.5, 25, 50 μmol/L sodium nitrite standards[10].

Tc-99m DMSA scintigraphy evaluation

Two hours after injection of 37 MBq (1 mCi) Tc-99m DMSA to the tail vein, static renal images were taken under anesthesia. Images were taken with a single-head gamma camera (Siemens, Ecam, Hoffman Estates, IL, USA) equipped with pinhole collimator, energy peak adjusted to 140 keV ± 20% with 256x256 matrix and 2.67 zoom factor for two minutes in anterior and posterior positions. ROIs (regions of interest) were drawn on the anterior and posterior images and the geometric mean of these values was accepted as the relative uptake percentage of the kidneys.

Statistical analysis

Data classified for statistical analysis were input to the Statistical Package for Social Sciences program (SPSS for Windows 16.0). The Kruskal Wallis and Mann Whitney U tests were used and a value of $p< 0.05$ was accepted as statistically significant.

Results

In the macroscopic evaluation, with the exception of the sham group, marked hydronephrosis was seen in all kidneys, marked dilatation in all pelvises and ureters and edema in all parenchyma.

In the microscopic evaluation, obstructive dilatation findings were seen in the lycopene and control groups but not in the sham group. The microscopic score was 50.56 ± 34.32 in the lycopene group, 97.22 ± 39.14 in the control group and 28.33 ± 12.58 in the sham group (Table 1).

	Minimum score	Maximum score	Mean score ± SD
Lycopene Group	10	100	50.56 ± 34.32
Control Group	75	200	97.22 ± 39.14
Sham Group	15	40	28.33 ± 12.58

Table 1: Mean ± SD of microscopic scores of the groups. The mean microscopic score of the control group is the highest. SD: standard deviation.

The microscopic score was lower in the lycopene group than in the control group (p=0.033) and higher in the control group than in the sham group (p=0.012). The microscopic score of the lycopene group was higher than that of the sham group, but it was not statistically significant (p=0.458) (Table 2).

	MPO level	MDA level	Nitrit level	Microscopic Score
Lycopene Group vs Control Group	*0.001*	*0.007*	*0*	*0.033*
Lycopene Group vs Sham Group	0.643	*0.013*	0.266	0.458
Control Group vs Sham Group	*0.012*	*0.033*	*0.012*	*0.012*

Table 2: Statistical analysis (p values) of biochemical parameters and microscopic scores between the groups. Microscopic score, MPO, MDA and nitrite level were statistically different between lycopene and control groups. MPO: myeloperoxidase, MDA: malondialdehyde.

In biochemical analysis, the mean value of MPO level was 0.05 ± 0.03 in the lycopene group, 0.10 ± 0.03 in the control group and 0.04 ± 0.01 in the sham group. The MPO level was lower in the lycopene group than in the control group (p=0.033) and higher in the control group than in the sham group (p=0.012). The mean value of the MDA level was 4.64 ± 1.49 in the lycopene group, 8.37 ± 3.31 in the control group and 18.76 ± 9.30 in the sham group. The MDA level was lower in the lycopene group than in the control group (p=0.007) and the sham group (p=0.013) and lower in the control group than in the sham group (p=0.012). The mean value of the nitrite level was 0.06 ± 0.01 in the lycopene group, 0.14± 0.09 in the control group and 0.05 ± 0.01 in the sham group. The nitrite level was lower in the lycopene group than in the control group ($p < 0.001$) and higher than in the sham group (p=0.013) and higher in the control group than in the sham group (p=0.012) (Table 2 and 3).

	MPO level	MDA level	Nitrit level
Lycopene Group	0.05 ± 0.03	4.64 ± 1.49	0.06 ± 0.01
Control Group	0.10 ± 0.03	8.37 ± 3.31	0.14 ± 0.09
Sham Group	0.04 ± 0.01	18.76 ± 9.31	0.05 ± 0.01

Table 3: Mean ± Standard deviation of biochemical parameters of the groups. MPO, MDA and nitrite level were lower in lycopene group than control group due to the protective effect of lycopene. MPO: myeloperoxidase, MDA: malondialdehyde.

In the scintigraphic analysis, the preoperative mean uptake values were 49.94 ± 1.15 in the lycopene group, 50.39 ± 1.38 in the control group and 50.27 ± 1.34 in the sham group. Preoperative Tc-99m DMSA uptake values were not statistically significant between the groups (p=0.821). The postoperative mean uptake values were 6.26 ± 5.17 in the lycopene group, 2.09 ± 1.03 in the control group and 49.17 ± 1.37 in the sham group. The postoperative uptakes of the lycopene and control groups were very low when compared with the sham group. There was no statistically significant difference between the lycopene and control groups (p=0.063). The uptake values of the lycopene and control groups were lower than those of the sham group as expected (p=0.013 and p=0.012, respectively).

Discussion

Lycopene, which is a major carotenoid found in tomatoes, has been demonstrated to show antioxidant activity both in vitro and in vivo[11]. Lycopene may play a role in diseases related to oxidative stress due to its antioxidant activity. Besides antioxidant activity, lycopene has many other roles in the immune system, metabolic pathways and cell-cell communication. Lycopene normalizes the change of intrathymic T-cell differentiation caused by tumorigenesis[12].

It has an inhibitory effect on basal endometrial cancer cell proliferation and suppresses insulin-like growth factor-1 which is a major regulator of mammary and endometrial cancer cell growth[13]. It inhibits proliferation and regulates cell-cell communication by enhancing gap-junction communication[14]. Lycopene consumption decreases the risk of degenerative diseases, certain kinds of cancer and cardiovascular diseases. A higher lycopene intake is associated with a reduced risk of lethal prostate and a reduction of prostate specific antigen levels[15]. Lycopene also has a potential role in long-term diabetic complications, preeclampsia, leukoplakia, oral submucous fibrosis, gingivitis and periodontitis[16].

In a study by Aydin et al, lycopene was used after bile duct ligation in obstructive jaundice. Lycopene significantly recovered the liver functions, reduced MDA, nitric oxide levels and enhanced all antioxidant enzyme activity in all tissues. It was reported that lycopene markedly recovered the liver and kidney tissue injuries seen in obstructive jaundice[17]. Lycopene has also been found to have a radioprotective effect against ionizing radiation in tissues such as liver[18], lymphocytes[19] and lacrimal glands[5].

Ureteropelvic stenosis, ureterovesical stenosis and kidney or ureteral stones may cause obstruction at different levels of the urinary system. Functional and biochemical changes in the kidney secondary to

obstruction are known as obstructive nephropathy. Various intraluminal and extraluminal reasons including retroperitoneal fibrosis, blood clots, trauma, tumors, foreign materials and intra-abdominal surgical operations such as caesarean section, hysterectomy and colonic surgery may result in ureteral obstruction[20]. Kidney functions may not be disrupted for a long time in partial chronic obstruction, whereas acute obstruction demonstrates symptoms in a short time and causes a rapid deterioration of kidney functions. Acute ureteral obstruction may lead to functional and biochemical changes in the kidney by decreasing the renal blood flow, glomerular filtration rate and even irreversible renal damage in cases of inadequate diagnosis and management[21]. Obstructive nephropathies lead to increased backpressure on the kidney and hydronephrosis that cause tubular dilatation, tubular atrophy, and apoptosis of renal tubular and interstitial cells[22]. Hydronephrosis causes elevated intrapelvic pressure and renal hemodynamic changes, which result in ischemic oxidative stress[23].

On day one after unilateral ureteral obstruction, tubular cell death is observed and this increases with time. Tubular dilatation and apoptosis peak in the distal tubules and collecting duct after 2 weeks[24]. In the 3rd week, several of the glomeruli are destroyed, several tubules display atrophy and significant inflammatory cell infiltration is present in the interstitial space[25].

In the current study, in the macroscopic evaluation there was marked hydronephrosis and marked dilatation in pelvises and ureters as expected in the lycopene and control groups that had undergone ureteral ligation. In the microscopic evaluation, the microscopic score was statistically lower in the lycopene group than in the control group, which demonstrated the structural protective effect of lycopene in ureteral obstruction.

Reactive oxygen species (superoxide, hydrogen peroxide and hydroxyl radical) are intermediary metabolites, which can destroy proteins, lipids, nucleic acids, carbohydrates and cause inflammation, apoptosis and fibrosis[26]. The degree of oxidative stress depends on an imbalance between reactive oxygen species and antioxidant defense within the kidney[27]. Excess reactive oxygen species result from some intracellular processes, but mainly in the mitochondria through the electron transport chain and β-oxidation of fatty acids[28]. MDA is a reactive carbonyl compound which is generated by membrane lipid peroxidation[26].

MPO activity can be used as a marker for neutrophil and macrophage infiltration into tissues (29). Total nitrite concentration shows lipid membrane damage[30]. In the current study, the biochemical parameters (MPO, MDA and nitrite levels) in the lycopene group were statistically lower than in the control group, which showed that lycopene decreases oxidative stress and protects the kidney. The Tc-99m DMSA uptakes of the kidneys were higher in the lycopene group than in the control group and very low in both groups when compared with the sham group. This was due to the long period of ureter ligation causing irreversible tubular damage.

In conclusion, the results of this study have shown that lycopene is an effective agent for protection of the kidney after ureteral obstruction as demonstrated by the microscopic parameters and decreased reactive oxygen species. Further studies are necessary for routine clinical usage.

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Corresponding author
MURAT SADIC, M.D.
Clinic of Nuclear Medicine, Ankara Training and Research Hospital
Ankara
(Turkey)