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Original Article

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Palosuran in Gentamicin-Induced Acute Kidney Injury in an Experimental Rat Model

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ABSTRACT

Objective: Urotensin-II is a potent vasoconstrictor peptide and has fibrotic effects in the heart and kidneys and palosuran is a selective urotensin-II receptor antagonist. This study aimed to investigate the effects of palosuran, which has antifibrotic effects, in an acute kidney injury model formed with gentamicin and to reveal new treatment options for acute kidney injury cases.

Methods: A total of 24 Wistar albino rats were randomly divided into 3 groups of 8 animals as the control group, the gentamicin group, and the gentamicin+palosuran group. The rats in the control group received a 1 × 1 mL intramuscular injection of saline for 8 days. To create acute kidney injury in the rats in the gentamicin group, an intramuscular injection of 100 mg/kg/day gentamycine was applied for 8 days. In the gentamicin + palosuran group, an intramuscular injection of 100 mg/kg/day gentamycine was applied for 8 days, together with 300 mg/kg palosuran dissolved in distilled water and administered by oral gavage twice a day for the same period. Serum urea, creatinine, calcium, albumin, total protein levels, and urine gamma-glutamyl transferase and protein levels were evaluated for all the groups. In addition, tubular degeneration, tubular necrosis, tubular regeneration, tubulointerstitial nephritis, microcalcification, and total histological scores of these groups were also examined histopathologically.

Results: The urine gamma-glutamyl transferase levels of the gentamicin and gentamicin + palosuran groups were significantly higher than those of the control group (311 ± 60.5 and 140 ± 39.5 vs. 0.33 ± 0.22, $P < .05$). On the eighth day, the gamma-glutamyl transferase levels of the gentamicin + palosuran group were significantly lower than the gentamicin group (311 ± 60.5 vs. 140 ± 39.5, $P < .05$). A significant decrease was obtained in tubular necrosis in the gentamicin + palosuran group compared to the gentamicin group (2.5 ± 0.3 vs. 1.3 ± 0.4, $P < .05$). A statistically significant decrease was determined in the total histological score in the gentamicin + palosuran group compared to the gentamicin group (6.4 ± 0.7 vs. 4.7 ± 0.7, $P < .05$). Our results showed that the urotensin-II receptor antagonist, palosuran, had positive effects on tubular necrosis, tubular degeneration, tubular regeneration, and the total histological score in an experimental acute kidney injury model formed with gentamicin.

Conclusion: The study results suggest that urotensin-II may play a role in the physiopathology of acute renal failure associated with gentamicin. These results are promising for the use of palosuran in the treatment of acute kidney injury.

Keywords: Acute kidney injury, urotensin-II, palosuran, gentamicin

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INTRODUCTION

Acute kidney injury (AKI) is a clinical syndrome characterized by a sudden and generally reversible reduction in glomerular filtration rate (GFR). Acute kidney injury is defined as an increase in serum creatinine level of >0.3 mg/dL within 48 hours, or a 1.5-fold increase in serum creatinine compared to the known or basal value within 7 days, or urine output of <0.5 mL/kg/h in a 6-hour period.[1] Recent studies from developed countries have reported AKI in 3.2%-9.6% of admissions.[2]

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The most common causes of AKI in hospitalized patients are hypovolemia, hypotension, the use of drugs such as aminoglycosides, the use of radiocontrast agents, and major surgical operations.[2]

Aminoglycosides are widely used in the treatment of severe gram-negative bacterial infections.[3-5] The range in which aminoglycosides can be safely used is very narrow and the most important limiting feature is toxicity. The most common side effects are nephrotoxicity, ototoxicity, and neurotoxicities occurring with neuromuscular blockage.[6] Acute kidney injury is a common complication of aminoglycosides seen in 10%-20% of patients.[3-5]

Among the aminoglycosides, after amikacin, the drug with the broadest spectrum and the strongest antibacterial effect is gentamicin (GM), which has a bactericidal effect.[7] Several hypotheses have been suggested that can clarify the mechanism underlying GM nephrotoxicity. One of these is that the accumulation of GM first in the proximal folded tubules may cause tubulointerstitial inflammation.[8,9] Pathological mechanisms related to nephrotoxicity of GM include elevated transforming growth factor-beta (TGF-β), and endothelin -1, a significant increase in monocyte/macrophage infiltration within the renal cortex and medulla, induction of oxidative stress, apoptosis, and necrosis.[10-12]

An increase in reactive oxygen species can cause progressive kidney damage through the expression and activation of proinflammatory mediators such as nuclear factor- κB, leukocyte adhesion molecules, mitogen-activating protein kinases (MAPKs), and TGF-β1.[13] Thus, a pathological process characterized by the infiltration of inflammatory cells such as monocytes, macrophages, and myofibroblasts are involved in progressive kidney damage due to GM.[14,15] The damage formed in the kidney is closely associated with the degree of tubulointerstitial fibrosis.[16]

Urotensin-II (U-II) is known to be the strongest vasoconstrictor peptide, with a more powerful effect than endothelin-1, which is also known to have vasoconstrictor properties.[17] Urotensin-II is a peptide formed from 11 amino acids.[18] The mRNA of this molecule is isolated from the vascular endothelium, heart, brain, spinal cord, kidney, liver, adrenal glands, hypophysis, spleen, leukocytes, small intestine, colon, placenta, and other tissues. Urotensin-II receptors are found in the vascular endothelium, myocardium, brain, smooth and skeletal muscles, adrenal glands, thyroid gland, and renal cortex. Previous studies have shown elevated plasma U-II levels in renal failure, congestive heart failure, diabetes mellitus, essential hypertension, and portal hypertension.[19,20]

As U-II also has fibrotic effects in the heart and kidneys, it has been shown to have a role as an autocrine and paracrine growth factor for renal epithelial cells.[21-23] Palosuran is a non-peptide, orally active, selective, and competitive UT-II receptor antagonist. In previous studies, it has been shown that palosuran decreased mean pulmonary arterial pressure and thickness of the pulmonary artery and arteriolar wall of the experimental pulmonary hypertension rat model. In addition, it has also been shown that it caused a significant reduction in portal pressure of the experimental liver cirrhosis rat model and decreased glucose, triglyceride, cholesterol, HbA1c, and 24-hour urine albumin levels of the experimental rat diabetes model.[24-26]

This study was conducted to reveal potential new treatment options for AKI cases. The aim of the study was to investigate the laboratory and histological effects of the U-II receptor antagonist, palosuran, in an AKI model created with GM.

MAIN POINTS

- In an experimental model of acute kidney injury, palosuran, a urotensin-II receptor antagonist, was found to provide a significant reduction in tubular necrosis and total histological score.
- It was determined that palosurane decreased urinary gamma-glutamyl transferase levels.
- It was determined that palosuran did not provide a significant decrease in serum urea creatinine levels.

METHODS

The study was approved by the Animal Experiments Local Ethics Committee of Dokuz Eylül University School of Medicine on 20.06.2012 with protocol number 50/2012. A total of 24 non-uremic, Wistar albino rats, each weighing 180-220 g, were used in the study. For familiarization with the environment, the rats were placed in metabolic cages 1 week before the study period, in which temperature was maintained at 24° ± 2°C and a 12-hour light–dark cycle was applied. All the animals were fed standard rat feed of 40 g/day and had free access to tap water. Palosuran was obtained from Switzerland with the written protocol applied with the Actelion company.

The rats were randomly divided into 3 groups of 8 animals as the control group, the GM group, and the gentamicin + palosuran (GM + PS) group. Before starting the experiment protocol, the animals were weighed and urine output was measured. The rats in the control group received with a 1 × 1 mL intramuscular injection of saline for 8 days. To create AKI in the rats in the GM group, an intramuscular injection of 100 mg/kg/day was applied for 8 days.[27] In the GM + PS group, an intramuscular injection of 100 mg/kg/day was applied for 8 days, together with 300 mg/kg palosuran dissolved in distilled water and administered by oral gavage twice a day for the same period.[28]

Serum urea, creatinine, calcium, albumin, total protein levels, urine gamma-glutamyl transferase (GGT), and protein levels of control, GM, and GM + PS groups were analyzed. In addition,

tubular degeneration (TD), tubular necrosis (TN), tubular regeneration (TR), tubulointerstitial nephritis (TIN), microcalcification (MC), and total histological scores (THS) of these groups were also investigated histopathologically.

Metabolism cage methods were used for 24-hour urine collection. Before drug administration and on day 8, animals were placed in metabolic cages and 24-hour urine samples were collected. Anesthesia was provided with 40 mg/kg ketamine, then the thorax was opened, and 5 mL intracardiac blood samples were taken. After obtaining aseptic conditions in the abdominal region, an abdominal midline incision was made, and the kidneys were removed for histopathological evaluation. All the rats were then sacrificed by exsanguination. On the same day, the blood samples were centrifuged for 5 minutes at 5000 rpm/min for separation of the serum. Urea, creatinine, calcium, albumin, and total protein levels were evaluated in the obtained serum samples by the spectrophotometric method (Biolabo Reagents, Maizy, France). GGT levels in the urine were evaluated with the same spectrophotometric method (Biolabo Reagents), and urine protein levels were evaluated by the Lowry method. For histopathological evaluation, the membrane was stripped from the kidneys, which then they were divided into 2 with a longitudinal cut. The kidneys were left for approximately 30 minutes in a formalin solution with mercury, then embedded in paraffin blocks, and stored in the pathology laboratory until assay. Sections of 3-5 mm in thickness were cut from the blocks, prepared routinely, stained with hematoxylin and eosin, and then evaluated under a light microscope by a single pathologist blinded to the groups.

Tubular degeneration (TD) was evaluated as vacuolization of small bodies of varying size and their content stained with acidophil in the cytoplasm of epithelial cells of the proximal tubule. Tubular degeneration scoring was done as follows: no TD, 0 points; mild TD: several small foci (0%-10%) of TD immediately below the capsule, 1 point; moderate TD: several foci and TD along the tubulus segment (10%-25%), 2 points; severe TD: extensive and evident TD along the tubulus segment (25%-50%), 3 points; and very severe TD: >50% TD, 4 points.

Tubular necrosis (TN) was described as the observation of nucleus loss in epithelial cells, dark acidophilic cytoplasm, shedding of tubulus epithelium cells to the lumen, and acellular tubulus sections. Tubular necrosis scoring was done as follows: no TN, 0 points; mild TN: several small foci (0%-10%) of TN immediately below the capsule, 1 point; moderate TN: several foci and TN along the tubulus segment (10%-25%), 2 points; severe TN: extensive and evident TN along the tubulus segment (25%-50%), 3 points; and very severe TN: >50% TN, 4 points.

Tubular regeneration (TR) was determined according to the criteria of basophilic-stained cytoplasm in epithelial cells, nuclear pleomorphism, irregular chromatin distribution, and mitosis. Tubular regeneration scoring was done as follows: no TR, 0 points; mild TR, several small foci (0%-10%) of TR immediately below the capsule, 1 point; moderate TR: several foci and TR along the tubulus segment (10%-25%), 2 points; severe TR: extensive and evident TR along the tubulus segment (25%-50%), 3 points; and very severe TR: >50% TR, 4 points.

Tubulointerstitial nephritis (TIN) was described as inflammatory cell infiltration in the perivascular and interstitial areas. Tubulointerstitial nephritis scoring was done as follows: no TIN, 0 points; mild TIN: several small areas (0%-5%) of infiltration concentrated in the perivascular area, 1 point; moderate TIN: infiltration in several foci (5%-10%) generally in the cortical interstitial area, 2 points; severe TIN: extensive and evident areas of infiltration (15%-25%), 3 points; very severe TIN: many infiltration areas of >25%, 4 points.

Microcalcification (MC) was assessed as medullar papillary calcifications. Microcalcification scoring was done as follows: no MC, 0 points; mild MC: a single focus of calcification in the cortex, 1 point; moderate MC: several different foci of calcification in the cortex (1%-10%), 2 points; severe MC: extensive and evident calcification in the cortex (11%-25%), 3 points; and very severe MC: an area of >25% calcification, 4 points.

The total histological score (THS) was calculated as TD/2 + TN + TR + TIN/2 + MC. The total histological score scoring was applied as normal: 0-2 points; mild THS: 3-5 points; moderate THS: 6-8 points; and severe THS: >8 points.

Statistical Analysis

Statistical analysis was performed using the SPSS 22.0 program (IBM Corp., Armonk, NY, USA). Continuous variables were given as mean ± standard deviation if the distribution was normal and as median (minimum–maximum) if the distribution was not normal. In the comparison of independent group differences, the significance test of the difference between the 2 means (independent samples *t* test) was used when the parametric test assumptions are provided. The Mann–Whitney *U* test was used to compare the independent group differences when the parametric test assumptions were not provided. In addition, Pearson's and Spearman's correlation analyses were used for correlation analysis between numerical variables showing normal distribution.

For differences, $P < .05$ value was considered statistically significant.

RESULTS

According to the serum biochemical results of the groups on the eighth day, the serum urea and creatinine values in the GM group were found to be at a higher level compared to the control group. There was no significant difference in serum creatinine levels between the GM and GM + PS groups (2.5 ± 0.20 vs. 2.6 ± 0.17, $P > .05$). A decrease in serum urea levels was determined in the GM + PS group compared to the GM group but not

Table 1. The Serum Biochemical Results of the Groups on Day 8.

	Control	GM	GM + PS	*P*
Serum urea (mg/dL)	36 ± 2	71.3 ± 5	57 ± 3.3	>.05
Serum creatinine (mg/dL)	0.7 ± 0.04	2.5 ± 0.2	2.6 ± 0.17	>.05
Calcium (mg/dL)	8.3 ± 0.07	8.6 ± 0.14	8.2 ± 0.14	>.05
Total protein (g/dL)	5.8 ± 0.14	5.8 ± 0.06	5.7 ± 0.13	>.05
Albumin (g/dL)	2.4 ± 0.2	3 ± 0.06	3 ± 0.1	>.05

GM, gentamicin; GM + PS, gentamicin + palosuran.

to a statistically significant level (71.3 ± 5 vs. 57 ± 3.3, $P > .05$). No significant difference was observed between the 3 groups in terms of calcium, total protein, and albumin levels. The serum biochemical results of the groups are shown in Table 1.

On the eighth day, the urine GGT levels of the GM and GM + PS groups were significantly higher than the control group (311 ± 60.5 and 140 ± 39.5 vs. 0.33 ± 0.22, $P < .05$). The GGT levels of the GM + PS group on the eighth day were significantly lower than those of the GM group (311 ± 60.5 vs. 140 ± 39.5, $P < .05$). No significant difference was found between the groups in respect of the urine protein levels. The 24-hour urine protein and GGT levels are shown in Table 2.

The histopathological data of the control group are given in Figure 1, the histopathological data of the GM group are given in Figure 2, and the histopathological data of the GM + PS group are given in Figure 3. A decrease in TD was determined in the GM + PS group compared to the GM group but not to a statistically significant level (2 ± 0.14 vs. 1.8 ± 0.3, $P > .05$). An increase in TR was determined in the GM + PS group compared to the GM group but not to a statistically significant level (1.7 ± 0.3 vs. 1.8 ± 0.3, $P > .05$). A significant decrease was determined in TN in the GM + PS group compared to the GM group (2.5 ± 0.3 vs. 1.3 ± 0.4, $P < .05$). A statistically significant decrease was determined in the THS in the GM + PS group compared to the GM group (6.4 ± 0.7 vs. 4.7 ± 0.7, $P < .05$).

DISCUSSION

In our study, it has been shown that the U-II receptor antagonist palosuran has positive effects on TN, TD, TR, and THS in an experimental AKI model created with GM. In addition, palosuran has been shown to have positive effects on urinary GGT levels, which are used to show TN in the early period. However, no effect on renal function tests has been detected.

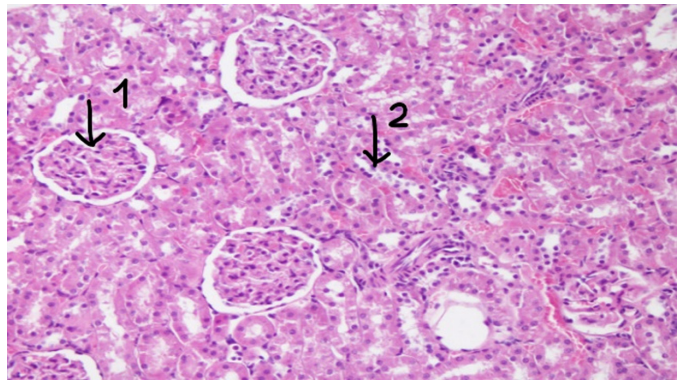

Figure 1. Light microscopic view of the kidney of the control group, where glomeruli (arrow 1) and tubule (arrow 2) structures are seen (H&E, ×200). H&E, hematoxylin and eosin.

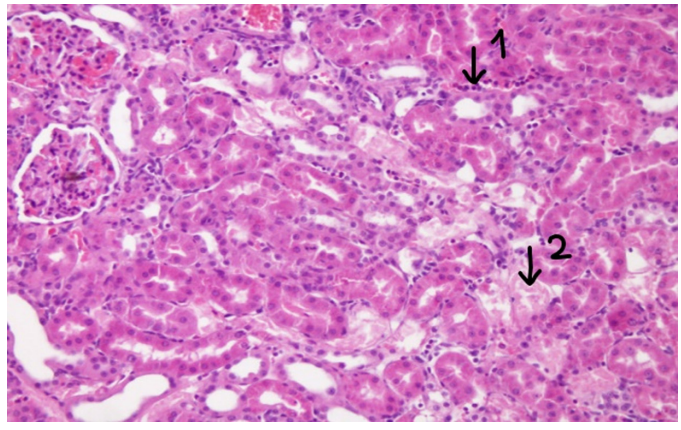

Figure 2. Light microscopic view of kidney of the GM group, mild tubulointerstitial inflammation (arrow 1) and tubular necrosis (arrow 2) are seen (H&E, ×200). H&E, hematoxylin and eosin; GM, gentamicin.

In a rat model of kidney damage created with GM, Park et al[27] investigated the effect of paricalcitol. The rats were administered with GM at a dose of 100 mg/kg/day for 14 days, and at the end of the study, a significant increase was seen in serum creatinine levels. The rat kidneys were examined histologically, and dilatation in

Table 2. The Urine Protein (mg/dL) and GGT (IU/L) Results of the Groups on Day 0 and Day 8

	Control		GM		GM + PS	
	Day 0	Day 8	Day 0	Day 8	Day 0	Day 8
Urine protein (mg/dL)	4 ± 0.4	3.2 ± 0.38	3.1 ± 0.21	5.2 ± 0.38	3.4 ± 0.12	4.7 ± 0.27
GGT (IU/L)	1.32 ± 0.45	0.33 ± 0.22	1.4 ± 0.7	311 ± 60.5	0.1 ± 0.1	140 ± 39.5

GM + PS, gentamicin + palosuran; GM, gentamicin.

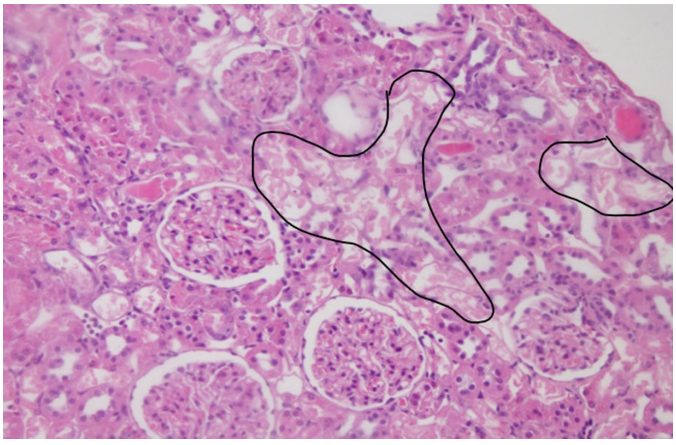

Figure 3. Light microscopic view of kidney of the GM+PS group, where areas of severe tubular necrosis (within the marked area) are seen (H&E, ×200). H&E, hematoxylin and eosin; GM+PS, gentamicin+palosuran.

tubular epithelial cells, tubular vacuolar changes, and necrotic changes were observed.[27] In the current study, intramuscular injection of GM at a dose of 100 mg/kg/day was administered to the rats for a period of 8 days, and an AKI model was created, consistent with previous studies in the literature.

Although urinary GGT levels are not a surrogate of renal functions, it has been shown in some studies to increase as a result of tubular damage. In a study by Whiting et al[29] which investigated the relationship between GM nephrotoxicity and enzymuria, the urine GGT levels of rats with tubular damage created with GM were seen to have increased in parallel with a decrease in renal activity. Naghibi et al[30] evaluated the relationship between urine GGT levels and tubular damage in experimental vancomycin nephrotoxicity in rats, and no significant relationship was determined between urine GGT levels and tubular damage. However, with the administration of GM in the current study, TN, TD, and the accumulation of inflammatory cells in interstitial areas were determined in kidneys, and the urine GGT levels were determined to be statistically significantly increased in the rats observed with histological tubular damage. These results suggest that an increase in urine GGT levels shows tubular damage.

In the study by Park et al[27] investigating the effect of paricalcitol on kidney damage created with GM in rats, there was determined to be a decrease in serum creatinine and urea levels, and renal histological improvement was observed. It was suggested that paricalcitol improved fibrosis and kidney damage formed with GM in association with an improvement in the inflammatory process. The potential mechanism of these anti-inflammatory and antifibrotic effects of paricalcitol was thought to be the cutting of the extracellular signal-regulated kinase pathway.[27]

Previous studies have shown that U-II is effective in increasing the inflammatory response and that U-II upregulation mediated by TGF-β plays an important role in renal fibrosis and dysfunction.[22,31] Tian et al[22] reported that U-II levels increased in AKI induced by GM and that these increased U-II levels could be effective in this process. Therefore, the effect of palosuran in AKI can be related to the anti-inflammatory effects associated with reduced inflammation and TGF-β levels mediated by U-II.

In a study by Ali,[11] OKY-046, which is a thromboxane A2 synthesis inhibitor, was shown to regress kidney damage associated with GM. However, Yamada et al[32] evaluated the effect of the renin–angiotensin suppressor and deoxycorticosterone acetate and although significant results were obtained in GFR in kidney damage created with GM, they did not observe any effect on tubular damage. Urotensin-II is a peptide with a vasoconstrictor property and has been shown to play a role in GFR regulation through tubuloglomerular re-absorption and GFR reflex control.[33] Palosuran is a U-II receptor antagonist, and apart from the effect in the anti-inflammatory process, it may regress nephrotoxicity associated with GM by providing an increase in renal blood flow.

Onat et al[28] investigated the effect of palosuran in an experimental rat model of lung fibrosis. It was observed that less edema and less accumulation of inflammatory cells were determined in rats applied with palosuran. As a result of this study, they suggested that palosuran may be effective in lung fibrosis due to its antifibrotic properties. In another study, the administration of palosuran in an experimental rat model of pulmonary hypertension was observed to reduce mean pulmonary arterial pressure and the thickness of pulmonary artery and arteriole walls.[24] Furthermore, Trebicka et al[25] observed a significant reduction in portal pressure and increased renal blood flow with the administration of palosuran to rats with experimental liver cirrhosis. From the results of that study, it was suggested that palosuran could be a new treatment option in portal hypertension.[25]

Following the demonstration of an increase in U-II and receptors in diabetes in experimental models in the literature, the effect of the U-II receptor antagonist, palosuran, has been investigated in diabetic rats. In a study where palosuran was administered to rats with experimentally induced diabetes, a reduction was observed in serum glucose, triglycerides, cholesterol, HbA1c levels, and in the amount of 24-hour urine albumin. Histologically, less tubular damage in the kidneys and in the beta islet cells in pancreatic tissue was detected. From that study, it was suggested that U-II peptide could be effective in the pathogenesis of diabetes.[26]

Sidharta et al[34] administered 125 mg palosuran twice a day for mean 13 days to diabetic patients with macroalbuminuria, and the study results showed a reduction in albumin expression in 24-hour urine in 26.2% of the patients with moderate renal dysfunction and in 22.3% of the patients with severe renal

dysfunction. It was concluded that palosuran could be beneficial in diabetic patients with renal dysfunction.[34] In our current study, it was determined that administration of palosuran to rats with GM-induced AKI showed improvement in TN and degeneration. Based on the data obtained in this study, palosuran can be considered to be effective in the treatment of AKI associated with GM.

In a study by Clozel et al,[35] palosuran was administered to rats in an experimental post-ischemic AKI model, and a reduction in serum creatinine levels was determined. In the histological evaluation of the kidneys, significant regression in tubulointerstitial lesions was also observed. With the inhibition of MAPK phosphorylation, it was also shown that there could be anti-inflammatory effects of palosuran.[35]

In our study, no significant difference was found in urea and creatinine levels in the G + P group compared to the GM group. This may be due to the short duration of palosuran treatment and the small study population.

Our study has some limitations. The inability to measure plasma U-II level and U-II protein expression in the kidney for technical reasons are as follows: the small study population, the short duration of the study, and the low number of serum and urine markers for AKI.

CONCLUSION

The data obtained in the current study showed that the U-II receptor antagonist, palosuran, had positive effects on TN, TD, TR, and the THS in an experimental AKI model created with GM. In addition, palosuran was shown to have positive effects on urine GGT levels, which are used to show TN in the early period. However, no effect was observed in the kidney function tests. It can be considered that palosuran could have anti-proliferative and anti-inflammatory effects and increase renal blood flow, thereby providing histopathological improvement in the kidney.

Ethics Committee Approval: Ethics committee approval for this study was obtained from the Animal Experiments Local Ethics Committee of Dokuz Eylül University School of Medicine (Approval Date: June 26, 2012; Approval Number: 50/2012).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

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Declaration of Interests: The authors have no conflict of interest to declare.

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REFERENCES

1. KDIGO. Clinical practice guideline for acute kidney injury. *Kidney Int*. 2012;2:1-138.
2. Fang Y, Ding X, Zhong Y, et al. Acute kidney injury in a Chinese hospitalized population. *Blood Purif*. 2010;30(2):120-126. [CrossRef]
3. Humes HD. Aminoglycoside nephrotoxicity. *Kidney Int*. 1988;33(4):900-911. [CrossRef]
4. Moore RD, Smith CR, Lipsky JJ, Mellits ED, Lietman PS. Risk factors for nephrotoxicity in patients treated with aminoglycosides. *Ann Intern Med*. 1984;100(3):352-357. [CrossRef]
5. Meyer RD. Risk factors and comparisons of clinical nephrotoxicity of aminoglycosides. *Am J Med*. 1986;80(6B):119-125. [CrossRef]
6. Edelstein CL, Schrier RW. Chapter 10. Acute renal failure: patogenesis, diagnosis and management. In: Schrier RW, eds. *Renal and Electrolyte Disorders*. 6th ed. PA, USA: Lippincott Williams & Wilkins; 2003:401-455.
7. Mycek MJ, Howland RD. *Lippincott Illustrated Review of Pharmacology*. 3rd ed. Philadelphia: Williams & Wilkins; 2004:130-171.
8. Alexandridis G, Liberopoulos E, Elisaf M. Aminoglycoside-induced reversible tubular dysfunction. *Pharmacology*. 2003;67(3):118-120. [CrossRef]
9. Parlakpinar H, Ozer MK, Sahna E, Vardi N, Cigremis Y, Acet A. Amikacin-induced acute renal injury in rats: protective role of melatonin. *J Pineal Res*. 2003;35(2):85-90. [CrossRef]
10. Geleilete TJ, Melo GC, Costa RS, Volpini RA, Soares TJ, Coimbra TM. Role of myofibroblasts, macrophages, transforming growth factor-beta endothelin, angiotensin-II, and fibronectin in the progression of tubulointerstitial nephritis induced by gentamicin. *J Nephrol*. 2002;15(6):633-642.
11. Ali BH. Gentamicin nephrotoxicity in humans and animals: some recent research. *Gen Pharmacol*. 1995;26(7):1477-1487. [CrossRef]
12. El Mouedden M, Laurent G, Mingeot-Leclercq MP, Taper HS, Cumps J, Tulkens PM. Apoptosis in renal proximal tubules of rats treated with low doses of aminoglycosides. *Antimicrob Agents Chemother*. 2000;44(3):665-675. [CrossRef]
13. Tang WW, Feng L, Mathison JC, Wilson CB. Cytokine expression, upregulation of intercellular adhesion molecule-1, and leukocyte infiltration in experimental tubulointerstitial nephritis. *Lab Invest*. 1994;70(5):631-638.
14. Schlondorff DO. Overview of factors contributing to the pathophysiology of progressive renal disease. *Kidney Int*. 2008;74(7):860-866. [CrossRef]
15. Segerer S, Nelson PJ, Schlöndorff D. Chemokines, chemokine receptors,and renal disease: from basic science to pathophysiologic and therapeutic studies. *J Am Soc Nephrol*. 2000;11(1):152-176. [CrossRef]
16. Mackensen-Haen S, Bader R, Grund KE, Bohle A. Correlations between renal cortical interstitial fibrosis, atrophy of the proximal tubules and impairment of the glomerular filtration rate. *Clin Nephrol*. 1981;15(4):167-171.

17. Ong KL, Lam KS, Cheung BM. Urotensin II: its function in health and its function in disease. *Cardiovasc Drugs Ther*. 2005;19(1):65-75. [CrossRef]
18. Tölle M, van der Giet M. Cardiorenovascular effects of urotensin II and the relevance of the UT receptor. *Peptides*. 2008;29(5):743-763. [CrossRef]
19. Maguire JJ, Kuc RE, Davenport AP. Orphan-receptor ligand human urotensin II: receptor localization in human tissues and comparison of vasoconstrictor responses with endothelin-1. *Br J Pharmacol*. 2000;131(3):441-446. [CrossRef]
20. Lim M, Honisett S, Sparkes CD, Komesaroff P, Kompa A, Krum H. Differential effect of urotensin II on vascular tone in normal subjects and patients with chronic heart failure. *Circulation*. 2004;109(10):1212-1214. [CrossRef]
21. Langham RG, Kelly DJ, Gow RM, et al. Increased expression of urotensin II and urotensin II receptor in human diabetic nephropathy. *Am J Kidney Dis*. 2004;44(5):826-831. [CrossRef]
22. Tian L, Li C, Qi J, et al. Diabetes-induced upregulation of urotensin II and its receptor plays an important role in TGF-b1-mediated renal fibrosis and dysfunction. *Am J Physiol Endocrinol Metab*. 2008;295(5):E1234-E1242. [CrossRef]
23. Matsushita M, Shichiri M, Fukai N, et al. Urotensin II is an autocrine/paracrine growth factor for the-porcine renal epithelial cell line, LLCPK1. *Endocrinology*. 2003;144(5):1825-1831. [CrossRef]
24. Onat AM, Pehlivan Y, Turkbeyler IH, et al. Urotensin inhibition with palosuran could be a promising alternative in pulmonary arterial hypertension. *Inflammation*. 2013;36(2):405-412. [CrossRef]
25. Trebicka J, Leifeld L, Hennenberg M, et al. Hemodynamic effects of urotensin-II and its specific receptor antagonist palosuran in cirrhotic rats. *Hepatology*. 2008;47(4):1264-1276. [CrossRef]
26. Clozel M, Hess P, Qiu C, Ding SS, Rey M. The urotensin-II receptor antagonist palosuran improves pancreatic and renal function indiabetic rats. *J Pharmacol Exp Ther*. 2006;316(3):1115-1121. [CrossRef]
27. Park JW, Bae EH, Kim IJ, et al. Renoprotective effects of paracalcitol on gentamicin-induced kidney injury in rats. *Am J Physiol Ren Physiol*. 2010;298(2):F301-F313. [CrossRef]
28. Onat AM, Turkbeyler IH, Pehlivan Y, et al. The efficiency ofa urotensin II antagonist in an experimental lung fibrosis model. *Inflammation*. 2012;35(3):1138-1143. [CrossRef]
29. Whiting PH, Brown PA. The relationship between enzymuria and kidney enzyme activities in experimental gentamicin nephrotoxicity. *Ren Fail*. 1996;18(6):899-909. [CrossRef]
30. Naghibi B, Ghafghazi T, Hajhashemi V, Talebi A. Vancomycin-induced nephrotoxicity in rats: is enzyme elevation a consistent finding in tubular injury? *J Nephrol*. 2007;20(4) :482-488.
31. Shiraishi Y, Watanabe T, Suguro T, et al. Chronic urotensin II infusion enhances macrophage foam cell formation and atherosclerosis in apolipoprotein E-knockout mice. *J Hypertens*. 2008;26(10):1955-1965. [CrossRef]
32. Yamada M, Hishida A, Honda N. Effects of desoxycorticosterone acetate (DOCA) plus saline drinking on gentamicin-mediated nephropathy in rats. *Ren Fail*. 1992;14(4):499-505. [CrossRef]
33. Shenouda A, Douglas SA, Ohlstein EH, Giaid A. Localization of urotensin-II immunoreactivity in normal human kidneys and renal carcinoma. *J Histochem Cytochem*. 2002;50(7):885-889. [CrossRef]
34. Sidharta PN, Wagner FD, Bohnemeier H, et al. Pharmacodynamics and pharmacokinetics of the urotensin II receptor antagonist palosuran in macroalbuminuric, diabetic patients. *Clin Pharmacol Ther*. 2006;80(3):246-256. [CrossRef]
35. Clozel M, Binkert C, Birker-Robaczewska M, et al. Pharmacology of the urotensin-II receptor antagonist palosuran (ACT-058362; 1-[2-(4-benzyl-4-hydroxy-piperidin-1-yl)-ethyl]-3-(2-methyl-quinolin-4 -yl)-ureasulfatesalt): first demonstration of a pathophysiological role of the urotensin System. *J Pharmacol Exp Ther*. 2004;311(1):204-212. [CrossRef]