International Journal of Preventive Medicine

Brief Communication Open Access

Effect of L-arginine and L-NAME on Kidney Tissue Damage in Rats after 24 h of Bilateral Ureteral Obstruction

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How to cite this article: Tirani SA, Pezeshki Z, Nematbakhsh M, Nasri H, Talebi A. Effect of L-arginine and L-NAME on kidney tissue damage in rats after 24 h of bilateral ureteral obstruction. Int J Prev Med 2015;6:60.

ABSTRACT

Background: Bilateral ureteral obstruction (BUO) affects renal function adversely. Previous investigations have implied that nitric oxide (NO) improves renal function in obstructive nephropathy. The aim of the current study was to investigate the role of NO precursor, L-arginine, and NO blocker agent, L-NAME on kidney tissue damage in rats after 24 h of BUO.

Methods: Forty Wistar rats (18 male, 22 female) were divided into four groups as follows; group 1: Sham or negative control group that received saline 3 days prior to the sham operation, group 2: Vehicle or positive control group that received saline 3 days prior to BUO, and groups 3 and 4: L-arginine and L-NAME groups that were treated same as group 2 except L-arginine (300 mg/kg) and L-NAME (4 mg/kg) instead of saline, respectively. Twenty-four hours after obstruction, the serum levels of blood urea nitrogen (BUN), creatinine (Cr), nitrite, and malondialdehyde (MDA) as well as kidney tissue levels of nitrite and MDA were measured and histopathological studies were done on left kidney.

Results: The serum levels of BUN and Cr and kidney and body weights increased and the tissue levels of MDA and nitrite decreased significantly in all BUO groups ($P < 0.05$). However, the tissue damage score was significantly lower in the L-arginine treated group in comparison to the vehicle and L-NAME groups ($P < 0.05$). As expected, the serum level of nitrite significantly increased in the L-arginine group ($P < 0.05$).

Conclusions: Endogenous NO donor; L-arginine, may protect the kidney tissue against BUO. However, this renoprotective role of L-arginine did not attenuate the increased kidney function markers (BUN and Cr) induced by obstruction.

Keywords: Bilateral ureteral obstruction, kidney tissue damage, L-arginine, nitric oxide

Access this article online	
Quick Response Code:	**Website:** www.ijpvmjournal.net/www.ijpm.ir
	DOI: 10.4103/2008-7802.160339

INTRODUCTION

Bilateral ureteral obstruction (BUO) is one of the main causes of acute kidney injury.[1,2] BUO result in tubular defects, interstitial fibrosis, leukocytes infiltration, and decrease in glomerular filtration rate (GFR) and renal blood flow (RBF).[3,4]

Nitric oxide (NO) is synthesized from its precursor L-arginine by NO synthase (NOS). The enzyme NOS is found in three isoforms namely endothelial NOS, neuronal NOS, and inducible NOS (iNOS).[5,6] NO supplementation restores RBF in chronic ureteral obstruction (UO) through its vasodilatory effect as shown by Hegarty *et al*.[7] In addition, interstitial fibrosis, which is a histological hallmark of UO is intensified in mice with targeted deletion of iNOS. This implies the protective and antifibrotic role of iNOS in UO.[8] Furthermore, co-administration of angiotensin converting enzyme inhibitor and L-arginine in rats undergone unilateral ureteral obstruction (UUO) showed that the increase in NO production ameliorates interstitial fibrosis in UUO.[9] NO is also known to influence the maintenance of tubular structure by preventing apoptosis in mice with UUO.[10] Some studies have also shown that administration of L-arginine can affect renal function through NO synthesis in obstructive nephropathy.[11,12] In UUO, the regular kidney function may have the least disturbance while it may be reversed in BUO. However, a question related to the effect of endogenous NO donor or NO blocker on kidney tissue still remained unanswered. In the current study, we investigated the influence of administration of L-arginine and its antagonist, L-NAME, on kidney tissue damage in rats 24 h post-BUO.

METHODS

Experimental animals

The study was performed on 22 female (185 ± 2.3 kg) and 18 male (260 ± 5.8 kg) Wistar rats based on the guidelines of Isfahan University of Medical Sciences Ethics Committee for Animal care and handling. The rats were kept at the temperature of 23–25°C and had free access to rodent chow and tap water.

Experimental protocol

The rats were divided into four groups as follows: Group 1 (n = 10) as the sham operated control group that received vehicle (saline) 3 days prior to the sham operation, group 2 (n = 14) received vehicle (saline) for 3 days, and then was subjected to BUO, groups 3 (n = 10, L-arginine group) and 4 (n = 6, L-NAME group) were treated the same as group 2 except that L-arginine (300 mg/kg, ip) and L-NAME (4 mg/kg, ip)[13] were administered instead of vehicle, respectively. L-arginine from Fluka-Garantie (Switzerland) and L-NAME from Sigma-Aldrich (USA) were purchased. Twenty-four hours after the obstruction, blood samples were collected via heart puncture under chloral hydrate (450 mg/kg, ip) (Merck, Germany) anesthesia, the animals were sacrificed humanely and both kidneys were removed. Blood samples were centrifuged at 6000 rpm for 20 min and the serum collected was stored at −20°C for biochemical measurements. Left kidney was fixed in 10% formalin for histopathological investigations and right kidney was homogenized in 2 mL saline, centrifuged at 15,000 rpm for 5 min and the supernatant was stored at −20°C for measurements of tissue nitrite and malondialdehyde (MDA).

Induction of bilateral ureteral obstruction

Bilateral ureteral obstruction was performed under general anesthesia with chloral hydrate. Both kidneys were exposed by flank incision and ureters were ligated using 4–0 silk. The whole procedure was done for the sham operated group except for ureter obstruction.

Measurements

The levels of serum creatinine (Cr) and blood urea nitrogen (BUN) were measured using a quantitative diagnostic kit (Pars Azmoon, Tehran, Iran). The serum and kidney levels of nitrite were measured by an ELISA assay kit (Promega Corporation, USA). MDA assessment in serum and kidney were done manually. For measuring MDA, a mixture of trichloroacetic acid (15%) and thiobarbituric acid (0.375%) were prepared. Then, 1 mL of the sample was added to 2 mL of the prepared mixture and incubated in boiling water for 45–60 min. When the samples cooled to the room temperature, they were centrifuged at 1000 rpm for 10 min, and the absorbance was read at 536 nm.[14]

Histopathological analysis

Left kidney was fixed in 10% formalin and embedded in paraffin for histopathological assessments. Then, sections with 4 μm thickness were stained with hematoxylin and eosin to evaluate kidney tissue damage using light microscopy. Kidney tissue damage score (KTDS) was evaluated by a pathologist who was blind to the study protocol from 0 to 5 based on six pathological parameters including hyaline cast, vacuolization, flattening, and degeneration of epithelium and tubular cells, and dilatation and debris of tubules.

Statistical analysis

The data were expressed as mean ± standard error. Serum levels of Cr, BUN, nitrite, and MDA; and kidney nitrite and MDA were compared using the one-way analysis of variance followed by the least significant difference test. The Kruskal–Wallis and Mann–Whitney tests were used to compare the groups with regard to the pathological damage scores. The $P < 0.05$ was considered statistically significant.

RESULTS

Serum levels of blood urea nitrogen, creatinine, nitrite, malondialdehyde

The serum levels of BUN and Cr were increased significantly in all BUO groups (P<0.05), and the serum level of MDA was decreased significantly in vehicle group (P<0.05). The result also indicated that the serum level of nitrite was significantly higher in L-arginine group as expected when compared to sham, positive control, and L-NAME groups ($P < 0.05$) [Figure 1].

Kidney tissue level of nitrite and malondialdehyde

The tissue level of nitrite and MDA significantly decreased in all BUO groups when compared with the sham group ($P < 0.05$). In addition, the tissue MDA level in the L-NAME group was significantly different from the L-arginine group at the significant level of 0.1 [Figure 1].

Histopathological findings, kidney, and body weight

When compared with the sham group, the KTDS was significantly higher in the positive control, L-arginine, and L-NAME groups, which underwent BUO ($P < 0.05$) [Figure 1]. The data also indicated that the KTDS in L-arginine group were significantly lower than the values in the saline and L-NAME

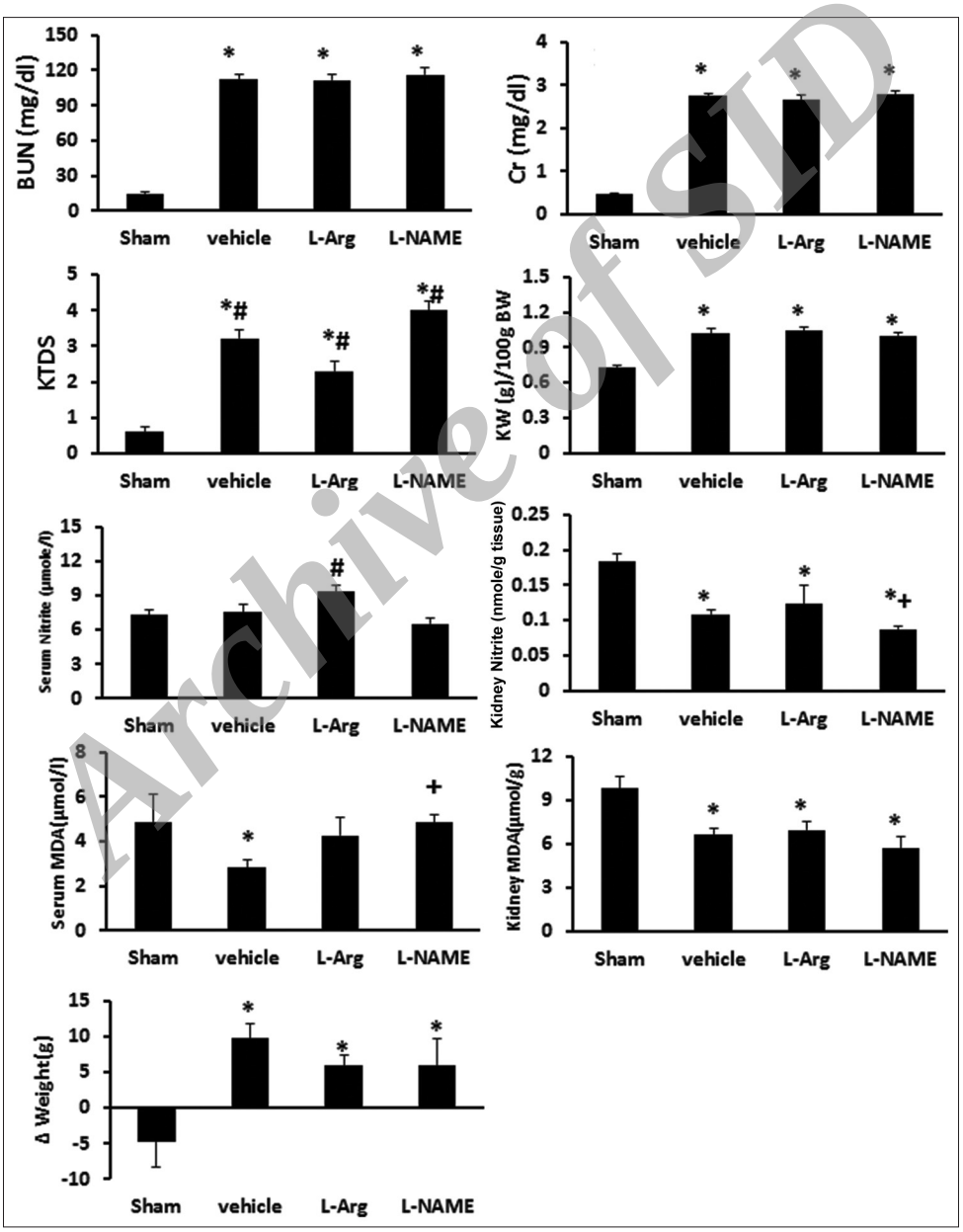

Figure 1: The serum levels of blood urea nitrogen creatinine, nitrite, malondialdehyde (MDA), the kidney tissue levels of nitrite and MDA, kidney tissue damage score, kidney weight per 100 g body weight, and weight change (Δ weight) in sham operated group and 3 uterus bilateral ligation groups treated with vehicle (saline), L-arginine, and L-NAME. Star (*) and #indicated significant difference from sham and other groups ($P < 0.05$) respectively. Plus sign indicated significant difference at significant level of 0.1

groups ($P < 0.05$). Body and kidney weight increased significantly in all the BUO groups when compared with the sham group ($P < 0.05$) [Figures 1 and 2].

DISCUSSION

The obstructed kidney is characterized by some morphological changes mainly resulted from the reduction of RBF. Early signs of hydronephrosis are tubules dilatation, cell flattening, and atrophy.[13] However, in chronic ureteral obstruction, interstitial fibrosis is considered as a hallmark of renal failure.[15] The findings of our study showed that KTDS is lower in the L-arginine group while administration of L-NAME exacerbated the damage score. It seems that this effect of L-arginine is related to its role in NO synthesis as our results also showed that serum nitrite level increased by L-arginine administration. In general, it has been shown that NO has a protective role in unilateral obstructive nephropathy by ameliorating interstitial fibrosis[16] but we could not find any study to evaluate the influence of NO on kidney tissue damage in acute BUO. Nevertheless, the investigation carried out by Reyes *et al*. indicated that decreased RBF and GFR followed by obstruction are affected by NO reduction.[11] Their study showed that administration of L-arginine for rats immediately after the unilateral release of BUO for 24 h ameliorates GFR and decreases renal vascular resistance.[11] The findings of a study by Hegarty *et al*. 2001. Furthermore, showed that N^G-monomethyl-L-arginine decreased RBF both in the cortex and the medulla 24 h after ureteral obstruction in rats.[7] In general, impairment of NO synthesis pathway reduces RBF in ureteral obstruction. Previous studies have shown that the RBF reduction is between 40% and 50% in animal models of ureteral obstruction.[4,17] We assume that the RBF reduction and consequently kidney tissue damage in L-arginine group are lower than that in the positive control and L-NAME groups. In fact, administration of L-arginine for 3 days prior to ureteral obstruction increased NO content, which prevented severe RBF reduction through its vasodilatory role.

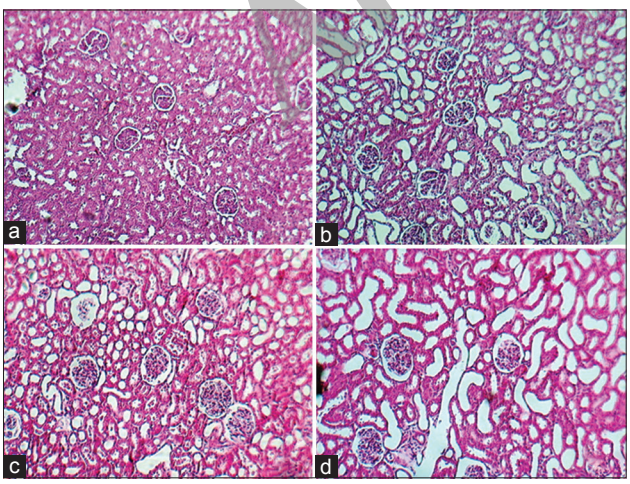

Figure 2: Hematoxylin and eosin stained sections of kidney in studied groups including: (a) Sham operated group, (b) control positive group, (c) L-arginine group, (d) L-NAME group

Based on the finding of previous studies in rats with BUO serum and tissue levels of L-arginine and consequently NO synthesis is low.[18] Accordingly, different investigators have found that L-arginine deficiency adversely affects hemodynamic changes such as RBF reduction and ureteral pressure in animal models with ureteral obstruction.[11,19] These findings imply that L-arginine administration compensate the L-arginine deficiency and increase NO synthesis in obstructed kidney while its antagonist, L-NAME, inhibits NOS and decreases NO level. All the items mentioned above explain the higher levels of nitrite (NO metabolite) in serum and tissue in L-arginine group in comparison with the positive control and L-NAME groups that underwent BUO. Furthermore, it explains the lower level of tissue NO in the L-NAME group compared to other groups in our study. However, NO level was higher in L-arginine group in comparison to positive control and L-NAME groups insignificantly.

Free radicals have an important role in BUO pathophysiology. The pretreatment of rats with probucol and RBF monitoring 4 h and 3 days after the obstruction release showed that probucol improves renal function because of its antioxidant property. This indicates to the formation of free radicals after BUO release.[20] Furthermore, production of renal reactive oxygen species (ROS) increases after obstruction release based on the findings of a previous study.[21] These findings suppose that ROS production is increased after BUO release while their formation following BUO has not investigated yet. Based on our findings, the tissue level of MDA decreased significantly in L-arginine, vehicle, and L-NAME groups. We assume that RBF, which was 40–70% of normal RBF[13] was not sufficient for formation of free radical by 24 h of ureteral obstruction and kidney tissue damage in acute BUO is because of hypoxia, happening as a result of RBF reduction,[22,23] enhancement in the urine volume that flattens the cell shape[24] and reduction in tubular function resulted to their earlier atrophy.[25]

CONCLUSIONS

Endogenous NO donor, L-arginine, may protect the kidney tissue against BUO for a short period of time even when the kidney function is disturbed. Of course, the time of obstruction plays an important role for this effect of L-arginine.

ACKNOWLEDGMENTS

This study was supported by Isfahan University of Medical Sciences.

Received: 02 Dec 14 **Accepted:** 30 Apr 15
Published: 08 Jul 15

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Source of Support: Isfahan University of Medical Sciences.
Conflict of Interest: None declared.