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RESEARCH ARTICLE

INVESTIGATION OF AMINOGUANIDINE THERAPY EFFICIENCY IN THE TREATMENT OF RENAL INJURY DUE TO ACUTE UNILATERAL URETERAL OBSTRUCTION: AN EXPERIMENTAL STUDY IN RAT MODEL "AMINOGUANIDINE TREATMENT IN OBSTRUCTIVE UROPATHY"

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ABSTRACT

Objective: To effect of aminoguanidine (AG) on rats with renal injury as a result of unilateral ureteral obstruction was aimed.
Methods: 58 Wistar albino rats were randomly divided into 5 groups; there was no obstruction while applying laparotomy on the sham group (G1). After laparotomy without applying obstruction, sham + AG group was treated with 100mg/kg aminoguanidine intraperitoneal for 14 days (G2). After performing laparotomy on obstruction group, left ureter was defined and incised with a suture (G3). After performing laparotomy on AG group, left ureter was sutured, after that it was intraperitoneal treated with 100mg/kg aminoguanidine for 14 days (G4). For the last group, after ureteral obstruction, physıological serum was given as dissolver (G5). 14 days later, after bilateral nephrectomy, the tissue and blood samples were collected.
Results: After forming unilateral ureteral obstruction, tubular changes, pelvicalyceal dilation and inflammation in the AG group decreased when compared to the other groups. In obstructive left kidney tissue MDA and OSI values was significantly lower in the group treated with AG (G4) compared to the group without (G3), while the GSH and TAS values were determined higher. In obstruction made groups (G3, G4 and G5), a significant difference was not detected between SOD, CAT, GPx and the TOS levels. TAS levels in G4 and G5 was higher than G3, but OSI was found lower.
Conclusion: According to these results, it was showed that AG as histopathological formed anti-inflammatory and antioxidant effect in the unilateral ureteral obstruction rat kidney tissue

INTRODUCTION

The formation of pathology which prevents any flow in anywhere of the urinary system and the changes associated with this condition was defined as obstructive uropathy, and followed by the parenchymal damage occurring in the kidney described as obstructive nephropathy[(1)]. Increased intro-luminal pressure as a result of obstruction of urinary tract leads to damage to the structure of renal tubular and glomerular. Tubular atrophy and interstitial fibrosis are inflammation, and apoptosis arises as a result of this damage.

In case of unilateral ureteral obstruction associated with low renal blood flow and GFR rate arises hydronephrosis situation. Low blood flow leads to damage in the kidney tissue. Hypoxic ROS products increase and following by monocyte/macrophage infiltration and apoptosis, cell dysfunction occurs [(2, 3, 4)]. Mitochondrial energy production in cells is decreased by ischemic event progresses. After occurrence of cellular imbalance, increased protease and phospholipase activity escalate cellular permeability. Thus, cell destruction occurs by progress of ischemia time[(5)].

In this study, aminoguanidine treatment was applied in order to decrease the oxidative stress and the level of damage formed along renal parenchyma and resulting of reducing oxidative stress byanti-oxidative treatment intended the protection of the renal parenchyma structure.

MATERIAL AND METHODS

In this study, 58 "Wistar Albino" breed adult male and female rats were raised in experimental animal research center, fed with standard feed and tap water, held in the same room 12 hours of night and 12 hours of day environments, weights ranging from 250-300 g ~ 4 months, were utilized.

Experimental Groups; Group 1: Sham group, Group 2: Sham + Aminoguanidine group, Group 3: Unilateral ureteral obstruction group, Group 4: Aminoguanidine administered group after unilateral ureteral obstruction and Group 5: with unilateral ureteral obstruction, physiological serum given as dissolver was defined as the last group.

Kidney samples were fixed in 10% formaldehyde. After the routine tissue follow-up, 5µm thick sections of paraffin embedded tissues were taken. Hematoxylin- eosin (H-E) stained sections were examined by light microscopy. All cut surfaces were examined, pelvicalyceal dilatation, inflammation (chronic, acute or mix inflamation) tubulo epithelial cell changes (in the brush border loss, swelling of the cell, vacuolization) and necrosis were assessed. Assessment is made by semi-quantitative way and was scored from 0 to 3. According to this; Normal renal histology score of 0, renal histological changes is limited to 1/3 of a kidney score of 1, renal histological changes more than 1/3 but less than 2/3 is the score of 2, the renal histological changes over 2/3, necrosis, abscess, and the presence of inflammation in the ureter was assessed as the score of 3.

Blood urea nitrogen (BUN), creatinine and albumin analyzes were performed in serums from blood samples.

In tissue samples, MDA, SOD, CAT, GPx, GSH, TOS, TAS and OSI levels are analyzed.

Statistical analysis was performed with SPSS. Quantitative data were summarized with a median, minimum and maximum values. In group comparisons, two sample paired Wilcoxon test and Kruskal-Wallis variance analysis was used. After Kruskal – Wallis variance analysis test, Conover test was applied to paired comparisons. In all tests, the level of significance was accepted as 0.05.

RESULTS

In terms of rats' weights, while there is no statistical difference between the kidney mass in Group 1 and Group 2, there was statistically significant increases in the left kidney weight compared to the right kidney in Group 3, Group 4 and Group 5.

Histopathological examination ; in the Group 1 and Group 2 sham groups, normal renal histology is present with no significant pathologic findings (Figure 1 and 2). The group made, the unilateral left obstruction, produces severe kidney damage and the score was at level 3 (Figure 3). After forming unilateral ureteral obstruction, tubular changes in the AG group, pelvicalyceal dilation and inflammation decreased when compared to the other groups, the score was at level 2 (Figure 4 and 5). Necrosis was not detected in this group. After the unilateral ureter obstruction, the histological picture worsened in the group treated only with the solvent, acute inflammation was observed in the abscess and the ureter (Figure 6 and 7). (Table 1)

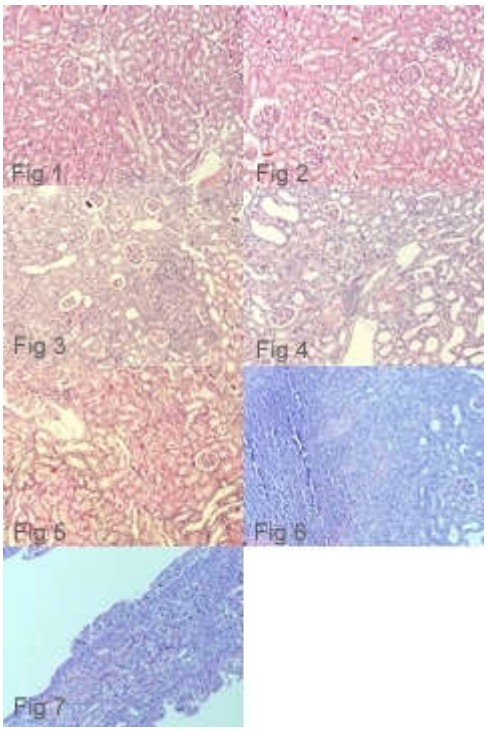

Figure 1 Normal renal histology insham group. Hematoxylin Eosinx 100
Figure 2 Normal renal histology in sham group. Hematoxylin Eosin x 100
Figure 3 Intense inflammation and damage in tubules in obstruction group. Hematoxylin Eosin x 100
Figure 4 Decreased inflammation in aminoguanidine group.Hematoxylin Eosin x 100
Figure 5 Vacuolar degeneration in the proximal tubule in aminoguanidine group. Hematoxylin Eosin x 200
Figure 6 Abscess area in parenchyma in solvent group. Hematoxylin Eosin x 100
Figure 7 Acute inflammation in urothelial epithelium in solvent group. Hematoxylin Eosin x 100

In terms of pelvicalyceal dilatation, the degree of pelvicalyceal dilatation of G1 and G2 was found less compared to G3, G4 and G5. In terms of tubular changes, the degree of tubular changes in G1 and G2 was found less than G3, G4 and G5. The change at G3 and G5 are in a similar rate that has been found to be more than G4. In terms of the degree of inflammatory changes, the degree of inflammatory changes in G1 and G2 has been found less according to G3, G4 and G5. The change at G3 and G5 are in a similar rate that has been found to be more than G4. In terms of parenchymal abscess focus degree, parenchymal abscess focus degree in G5 was also found to be more than G1, G2, G3, and G4.

Table 1 Histopathological examination of all groups

Groups	Pelvicalyceal Dilatation	Tubular Changes	Inflammation	Parenchymal Abscess Focus	Urethral Inflammation
Group 1	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Group 2	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Group 3	3 (3-3)^{x}	3 (3-3)^{x}	3 (2-3)^{x}	0 (0-0)	0 (0-0)
Group 4	3 (2-3)^{x}	2 (2-2)^{x}	2 (2-3)^{x}	0 (0-0)	0 (0-0)
Group 5	3 (3-3)^{x}	3 (2-3)^{x}	3 (3-3)^{x}	3 (0-3)^{y}	3 (0-3)^{y}

^{x} : Comparison with Group 1 and 2 $p<0.001$
^{y} : Comparison with Group 1, 2, 3 and 4 $p<0.001$

In terms of urethral inflammation, the degree of urethral inflammation in G5 was found to be more compared to G1, G2, G3, and G4. There was no statistically significant difference between groups in terms of necrosis.(Table 1)

There is no difference in BUN, creatinine and albumin levels between G1 and G2.There is no difference between creatinine and albumin levels when compared to G3 and G4. The BUN value of the G3 were also found to be higher than G4. There is no difference between BUN and albumin levels when compared to G3 and G5. The creatinine value of the G3 were also found to be higher than G5. (Table 2)

Table 2 The serum BUN, creatinine and albumin levels

	BUN	Kreatin	Albumin
Groups	**Median (Min-Max)**	**Median (Min-Max)**	**Median (Min-Max)**
Group 1	27 (22-30)	0.42(0.1-0.52)	1 (0.9-1.2)
Group 2	26 (22-28)	0.47 (0.39-0.52)	1.2 (0.9-1.3)
Group 3	31.5 (29-36)[x]	0.59 (0.51-0.63)[x]	0.9 (0.5-1)[x]
Group 4	29 (21-34)[y]	0.58 (0.48-0.77)[x]	0.8 (0.7-1.1)[x]
Group 5	32 (22-35)[x]	0.52 (0.32-0.64)[x,z]	1 (0.5-1.1)[x]

[x] : Comparison with Group 1 and 2 p<0.05
[y] : Comparison with Group 3 p<0.05
[z] : Comparison with Group 4 p<0.05

The MDA value is detected highest at G3. Statistically significant differences between G1 and G2 were not found in MDA analysis. The MDA value of G3 was detected in significantly higher than the value of G1 and G2. The MDA value of G3 was detected in significantly higher than the value of G4 as well. (Table 3)

There was no statistically significant difference among all groups in SOD analysis.(Table 3)

A significant statistical difference was not emerged between G1 and G2 in CAT, GSH-Px, TOS and TAS analysis. A statistically significant difference was not detected between G3, G4 and G5CAT, GSH-Px, TOS and TAS analysis.(Table 3)

CAT values of tissue samples wherein G1 and G2 are determined significantly higher than the G3, G4 and G5. (Table 3)

GSH-Px values of tissue samples in G1 and G2 are determined significantly higher than the G3. Furthermore, a statistically significant difference was not located between the GSH-Px values of G4 and G5.(Table 3)

GSH levels in G1 are also found significantly higher rate than G2 in GSH analysis. A statistically significant difference was not detected between G2, G3 and G5. Also there was no statistically significant difference found between G1 and G4. Nonetheless, GSH values of tissue samples in G1 and G4 are determined significantly higher than the GSH values of G2, G3 and G5.(Table 3)

TOS values of tissue samples in G1 and G2 are determined significantly lower than the TOS values of G3, G4 and G5.(Table 3)

TAS values of tissue sample in G3 is determined significantly lower than G4 and G5. A statistically significant difference was not detected between G4 and G5.(Table 3)

A statistically significant difference was not appeared between G1, G2, G4 and G5 in OSI analysis. The OSI value of G3 is determined significantly higher than the rest of the all groups. (Table 3)

DISCUSSION

Unilateral ureteral obstruction can occur as a result of many clinical pathology. Although obstruction is eliminated in case of obstruction, kidney damagecan still develop. If the mechanisms of deterioration in kidney function that occurs following obstruction can unmask, using pharmacological agents for this situation can be decreased damage to the kidneys.

After Postrenal obstruction the pressure increase occurs and the increase causes dilatation in the collecting system, the increase of fibroblast activity in interstitial space, mononuclear cell growth, leads to an increase in the number of macrophages and the inflammatory process begins in the cells.

Table 3 Chemical results for the left renal.

Groups	Group 1	Group 2	Group 3	Group 4	Group 5
MDA	12.5 (8.9-17.7)	17.3 (14.1-22.1)	22.0 (15.9-24.1)[a,b,d]	14.8 (12.7-17.1)	18.8 (14.1-25.8)[d]
SOD	0.9 (0.7-1.1)	0.9 (0.7-1.0)	0.7 (0.5-0.8)	0.7 (0.7-0.9)	0.6 (0.6-0.9)
CAT	23.1 (18.2-41.4)[c,d,e]	23.3 (20.8-43.9)[c,d,e]	20.7 (11.5-28.5)	23.6 (20.3-31.3)	24.9 (16.1-32.1)
GPx	246.2 (189.8-254.3)[c]	220.8 (164.4-253.0)[c]	178.8 (111.9-194.5)	178.9 (153.6-230.4)	177.4 (150.6-254.1)
GSH	13.6 (10.1-18.3)[b,c,e]	13.1 (8.8-17.9)	13.2 (11-18.5)	19.2 (12.7-42.7)[b,c,e]	19.2 (13-35.5)
TOS	2.5 (1.6-3.5)	2.8 (1.8-3.8)	2.9 (1.9-4.3)[a,b]	2.9 (1.9-3.7)[a,b]	2.4 (1.8-3.6)[a,b]
TAS	0.3 (0.2-0.6)	0.4 (0.1-0.7)	0.3 (0.1-0.4)	0.5 (0.2-0.6)[c]	0.3 (0.2-0.5)[c]
OSİ	7.3 (3.0-13.9)	7.7 (3.2-25.5)	10.0 (7.2-25.2)[a,b,d,e]	6.8 (4.8-16.8)	8.5 (4.6-14.4)

[a] : Comparison with Group 1 p<0.05
[b] : Comparison with Group 2 p<0.05
[c] : Comparison with Group 3 p<0.05
[d] : Comparison with Group 4 p<0.05
[e] : Comparison with Group 5 p<0.05

The formation of free radicals, which began after the release of cytokines and following the chain of events that has not been fully elucidated today, the loss of nephron occurs through in cell apoptosis and interstitial fibrosis. Any given agent or intervention to be made at any of these stages can be provided to prevent progression of any nephron damage. Nevertheless, several studies are available in the literature.

After nephron damage occurs in renal, since there is no mitotic activity on these nephrons, the number of nephrons cannot be increased. For this reason, intervention in the inflammatory process may be possible to prevent an irreversible nephron damage. Due to AG anti-inflammatory and antioxidant effects, by preventing the effect of cytokines in inflammatory processes, fibrosis and apoptosis and by eliminating the effects of oxygen radicals occurrence can reduce renal damage.

In a study by Huang *et al*., after AG is employed for 8-12 weeks on spontaneous hypertensive rat, when it is compared

with the untreated group, it was shown that with the effect on nitric oxide-mediated vascular tone to cause a significant reduction in mean arterial pressure. In addition, AG has been shown to significantly increase the rate of kidney L- arginine and asymmetric dimethyl arginine (ADMA) [6].

In a study by Abou-Salem OM, it was histologically shown that AG treatment administered in pre- treatment periodprotects patients, treated with doxorubicin, from the negative effects of reactive oxygen species, occurring nephrotoxicity was reduced with AG[7]. In our study, based on histopathological evaluation, in the group, which was given AG, tubular changes, pelvicalyceal dilatation and the degree of inflammation was reduced compared to other groups and thus reducing of nephrotoxicity effect was observed.

In a study by Abraham and Rabi, it was demonstrated that renal damage was reduced at rats with renal damage created by a single dose of cyclophosphamide by giving AG before treatment. It has been shown that this effect is related to AG's ability to inhibit nitric oxide-mediated protein degradation and the activity of poly ADP ribose polymerase (PARP) [8]. In our study as well, inflammation was reduced in the group that was given AG and also found that necrosis did not occur.

In a study by Li *et al*. that was carried out on diabetic rats, AG treatment reduced the renal injury at rats with developing nephropathy by inhibiting the release of collagen and by increasing the creatinine clearance was shown to reduce the value of blood creatinine. It is also found to reduce the value of BUN and the rate of albumin excretion [9].In also our study, it was found that BUN values in a AG given group with similar fashion was significantly lower when compared to the group without AG. However, it was not observed any significant change at the creatinine levels in the AG given group.

In a study by Orszaghov *et al*. of diabetic rats, AG's structural analogue of pyridoxalaminoguanidine (PAG) was used.PAG used in rats has been shown to reduce DNA damage. It also found that antioxidant capacity was reduced with AG. It has been proposeddiabetes -related complications due to its low toxicity and high efficacy [10]. Our study also shown that AG decreases oxidative stress and increases antioxidant effect.

In a study conducted on rats by Abdel-Zahar *et al*., it has been shown that using AG by inhibition of nitric oxide and reduction of intracellular GSH has protective effectabout acetaminophen-induced hepatic and renal injury prevention [11]. In our study, GSH levels was found to be significantly higher in the group treated AG and thus considered to be effective in reducing renal damage.

In a study conducted on rats by Parker *et al*., protective effect of AG about prevention of kidney damage was investigated through reactive oxygen species (ROS) of gentamicin, an aminoglycoside. In renal tissue, while MDA and NO levels increased with the Gentamicin and decreased with the amino guanidine, GSH-Px, SOD and CAT levels increased with amino guanidine. In addition, blood creatinine and BUN levels are decreased. Morphological changes in the kidney including tubular necrosis, intracellular edema, glomerular and basement membrane alterations were evaluated and it has been shown that AG reduce the kidney damage coming from gentamicin in both biochemical findings and histopathological findings[12]. In our study also, MDA and OSI values increases with oxidative stress at obstructed kidney, decreases in the group treated with AG and GSH and TAS values was determined to increase. In addition, BUN values in the group treated with AG was determined to be significantly lower than in the group not given AG.

In a rat study by Wang *et al*., AG showed the protective effect of the dose and time dependent manner against renal damage due to endotoxin. It has also been found to reduce NO-mediated changes in renal function. Especially the group treated with 100 mg/kg/day has been shown to have the best response [13]. In our study, it was found that renal damage was decreased with AD treatment of 14 days of 100 mg/kg/day.

In a study by Birrell *et al*., type 1 diabetic baboon model studies, AG's effects on diabetic nephropathy has been studied for 4 years. The baboons was treated with AG have been shown to reduce the thickening at renal glomerular basement membrane and It is stated that AG treatment will contribute as primary therapy in the prevention of diabetic nephropathy [14]. In a study by Nakamura *et al*., AG treatment creates antinflammatory effectoncolitis in rats formed by trinitrobenzene sulfonic and it was shown to elicit proliferative effectin cells of the colon mucosa [15]. After histopathological examination result of our study as well, it was determined that the treatment with AG decreased inflammatory effect.

In a study by Ozturk *et al*., AG's benefits are evaluated on the skin flaps in diabetic rats and MDA and NO levels decreased in groups treated with AG, meanwhile SOD and GSH content was found to be increased. The flap has been shown to play an important role in the prevention of necrosis [16].In a study conducted by Yilmaz *et al*., AG's protective effect was evaluated on preventing liver damage due to chronic biliary obstruction and it was indicated that in tissues as a result of giving AG decreases MDA level, increases the level of GSH. These findings has supported AG can be used in the biliary obstruction [17].In a rat model study by Sahna *et al*., a rat group which had undergone renal ischemic reperfusion injury was evaluated and MDA level in the tissues was evaluated 24 hours after renal ischemia. It was shown that MDA levels increased after ischemia and MDA levels in the AG treated group was statistically shown a decrease [18]. In a rat model study to assess amikacin induced nephrotoxicity rate by Parlakpınar *et al*., It was found that MDA value, related to amikacin increased at the cell level, decreased after the administration of AG, the falling GSH levels were found to increase [19].In our study, while a group was given AG decreased MDA values, GSH levels were increased. As a result, although a variety of agents was used and proposed in the prevention of kidney injury after unilateral ureteral obstruction, a standard operation in the treatment has still not been established. The reason for this inability to known to cause of the formation of kidney damage and many of the experimental stages cannot go forward from research. In our study, we found that AG has positive effects on the reduction in obstructed renal function loss as both histopathological and as well as biochemically. Therefore, not to let obstruction lead to further loss of function in clinical situations encountered in urinary obstruction, we think it would be useful to use of AG treatment until obstruction corrected.

CONCLUSION

According to these results, it was showed that AG as histopathological formed anti-inflammatory and antioxidant effect in the unilateral ureteral obstruction rat kidney tissue.

Obstructive uropathy sometimes impair the quality of life by causing irreversible damage and can lead to long-term and high costs treatment. More wide spread and multicenter studies are needed for AG, which can be used to prevent this, to take part in a clinical use.

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