VALSARTAN TO ATTENUATE RENAL DAMAGE IN UNILATERAL URETERAL OBSTRUCTION

[1]Samycha Jusuf, [1]Alvarino.

[1]Department of Urology, Faculty of Medicine/Indonesia University, Cipto Mangunkusumo Hospital, Jakarta.

ABSTRACT

Objective: *This experimental study aims to observe the effect of valsartan to attenuate renal damage in unilateral ureteral obstruction.* ***Material & method:*** *Experimental study was performed using 30 wistar rats with unilateral ureteral obstruction achieved by ligation of the left ureter. Rats were divided into two groups, no treatment group and valsartan group. At the 14th day, evaluation was performed to compare interstitial fibrosis, hydropic degeneration, and tubular atrophy between the two groups using haematoxylin-eosin staining. Only rats surviving until at least the 7th day are included in the study.* ***Results:*** *From thirteen wistar rats in no treatment group, there were two with moderate interstitial fibrosis and eleven with mild interstitial fibrosis while all rats in valsartan group had mild interstitial fibrosis ($p > 0.05$). There is no significant difference on hydropic degeneration between no treatment and valsartan group (31.46 vs 33.67; $p > 0.05$). There is also no significant difference in tubular atrophy between the two groups (61.78 vs 62.07; $p > 0.05$).* ***Conclusion:*** *Valsartan therapy in antihypertensive dosage has no significant effect in to attenuate interstitial fibrosis, hydropic degeneration, and tubular atrophy in unilateral ureteral obstruction in wistar rats.*

Keywords: *Unilateral ureteral obstruction, valsartan, interstitial fibrosis.*

ABSTRAK

Tujuan: *Penelitian ini bertujuan untuk mengamati pengaruh valsartan untuk memperbaiki kerusakan ginjal pada obstruksi ureter unilateral.* ***Bahan & Cara:*** *Dilakukan penelitian eksperimental menggunakan 30 ekor tikus wistar dengan obstruksi ureter unilateral melalui pengikatan ureter kiri yang dibagi menjadi dua kelompok, tanpa pemberian valsartan dan dengan pemberian valsartan. Pada hari ke-14 dinilai dan dibandingkan fibrosis interstisial, degenerasi hidrofik, dan atrofi tubulus pada kedua kelompok dengan pulasan hematoksilin-eosin. Hanya tikus yang tetap hidup hingga melewati hari ketujuh yang dimasukkan dalam penelitian.* ***Hasil:*** *Dari tiga belas tikus wistar pada kelompok obstruksi ureter unilateral tanpa pemberian valsartan, didapatkan 11 tikus mengalami fibrosis interstisial pada ringan dan 2 tikus mengalami fibrosis interstisial sedang, sementara seluruh tikus wistar pada kelompok dengan valsartan mengalami fibrosis interstisial ringan ($p > 0.05$). Tidak terdapat perbedaan bermakna untuk degenerasi hidrofilik epitel tubulus antara kelompok tanpa valsartan dan dengan valsartan (31.46 vs 33.67; $p > 0.05$). Tidak terdapat pula perbedaan bermakna untuk atrofi tubulus antara kedua kelompok (61.78 vs 62.07; $p > 0.05$).* ***Simpulan:*** *Pemberian valsartan dengan dosis antihipertensi tidak mengurangi tingkat fibrosis interstisial, degenerasi hidrofilik, maupun atrofi tubulus pada obstruksi ureter unilateral pada tikus wistar.*

Kata kunci: *Obstruksi ureter unilateral, valsartan, fibrosis interstisial.*

Correspondence: Samycha Jusuf, c/o: Department of Urology, Faculty of Medicine/Indonesia University, Cipto Mangunkusumo Hospital. Jl. Diponegoro 71, Jakarta 10430. Office phone: 021-3152892. Mobile phone: 081311268684.

INTRODUCTION

Ureteric obstruction is a condition that may occur at any age with varying levels and effects. The cause may be congenital, acquired, or benign or malignant. The severity of the effects is influenced by various aspects, such as the degree of obstruction, chronicity, initial kidney condition, potential for kidney repair, and other factors such as the presence of infection, with the worst effects can be permanent

kidney failure.[1]

The prevalence of obstruction obtained from an autopsy series involving 59.064 subjects of different ages was 3.1%. Up to the age of 20, the prevalence among men and women are equal, then it will occur more frequently in women aged 20-60 years due to pregnancy and gynecologic malignancies, and more often in men over the age of 60 years due to prostate malignancy.[1]

It should be pointed out that there is a difference between hydronephrosis with obstructive nephropathy. Hydronephrosis is a condition in which there is dilatation of the renal pelvis or calyx which can occur with or without obstruction, whereas, obstructive nephropathy and anatomical or functional damage of the kidney are due to obstruction.[1] In adults, obstructive nephropathy is often caused by stones,[2] while in children the cause is generally congenital with the most common cause is ureteropelvic junction stenosis.[3]

Obstruction can lead to renal structural changes, including interstitial fibrosis, tubular atrophy and apoptosis, and inflammation.[1] A variety of factors are expected to play a role in the process, such as oxidative stress and inflammation.[4,5] In turn other factors play a role, such as transforming growth factor β (TGF-β), angiotensin II, nuclear factor κB (NFκB), and tumor necrosis factor-α (TNF-α) produced by renal tubular and interstitial cells itself or from macrophages.[1]

In the state of obstruction, infiltration of macrophages stimulates the synthesis of TGF-ß produced by renal tubular epithelial and interstitial fibroblasts.[1,3] Through both of its receptor, the TGF-ß1 and TGF-ß2, whose functions are mutually reinforcing, these conditions results in the accumulation of collagen with the end result interstitial fibrosis, epithelial cell apoptosis, and tubular atrophy. In the state of unilateral ureteral obstruction, TGF-ß1 receptor will increase in number so that their effect will be doubled.[3]

The renin-angiotensin-aldosterone system also plays a role in this situation. The renin-angiotensin-aldosterone increases the expression of TNF-α and NFκB, while angiotensin II serves to increase the expression of TGF-ß1 in the condition of unilateral ureteric obstruction.[1] The effect of angiotensin II works through both its receptor, AT1 and AT2. Although in adult mammals, AT1 has a stronger role. The increasing expression of NFκB's leads to fibrosis process, while NFκB itself has a positive feedback effect on the production of angiotensin II, so that the produced effect was stronger. TNF-α is a cytokine that can directly cause apoptosis in renal inflammation.[1,3,5]

The group of drugs that affect the renin-angiotensin-aldosterone axis are those from the class of angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin II receptor blocker, both of which are used as the treatment for hypertension. The administration of ACE-I or angiotensin II receptor blockers may reduce the expression of TGF-ß, lowering extracellular matrix production, activation of NFκB, and proliferation of fibroblasts that results in decreased fibrosis. However, ACE-I can only reduce the production of angiotensin II, which is influenced by angiotensin-converting enzyme, while the angiotensin II receptor blockers have an effect on all levels that produce angiotensin II.[6] In addition, angiotensin II receptor blockers do not have the disadvantages of ACE-I, such as cough and angioedema.[7]

Valsartan is a potent nonpeptida tetrazole derivative that can lower blood pressure, so it is used as an antihypertensive therapy. Valsartan works by selective inhibition of the angiotensin II type I receptor (AT1).[8] It's affinity to AT1 is 20.000 times higher than to AT2. Valsartan is available in doses of 10, 20, 40, 80, 160, and 320 mg. All doses are considered safe and well tolerated.[8] Valsartan antihypertensive effect starts to appear at a dose of 80 mg in humans. The dose threshold that can lower blood pressure is 1 mg/kg BW.[8-10] Compared with other groups of angiotensin receptor blocker, valsartan is considered to have faster, higher, and better antihypertensive effect with fewer side effects.[9]

OBJECTIVE

The aim of this study was to observe the effects of valsartan on the improvement of kidney damage in unilateral ureteral obstruction.

MATERIAL & METHOD

This study was an experimental study to determine the effects of valsartan against kidney damage in unilateral ureteral obstruction using experimental animals the wistar rats.

Experimental animals in this study were 30 male wistar rats aged three months weighing between 150-200 grams, healthy and had no anatomical defects. Thirty wistar male rats were divided into two groups, each group of 15 animals. The first group (A) was a control group, in which the wistar rats were treated only with left ureteral ligation without any therapy. The second group (B) was the treatment group where the wistar rats with left ureteral ligation were given with valsartan therapy.

Subsequently, laparotomy was done in experimental animals with anesthesia using diethyl ether. Midline abdominal incision was made and the left ureter was identified. The proximal ureter was tied using prolene 4-0. Abdomen was closed with 3.0 silk sutures and topical betadine. The animals did not receive other drugs or substances other than standard feed, drink, and valsartan was given to the treatment group only. Valsartan doses given to experimental animals group B was 1.44 mg/200 mg/day, which was the dose that had been converted using dose conversion table based on the dose of 80 mg/day in humans with 70 kg body weight. At the end of the study, no animal died of surgical wound infection.

On day 14, the experimental animals were killed with diethyl ether and laparotomy. The left kidney was taken and preserved with formal dehyde. The kidney was processed by standard histological methods using haematoxylin-eosin staining and subjected to histological examination. The data were written in forms.

Interstitial fibrosis was calculated per 100 tubuli and grouped into mild (< 25% per 100 tubuli), moderate (26-50% per 100 tubuli), and severe (> 50% per 100 tubuli) based on published guidelines on scoring and reporting for interstitial fibrosis.[11] Data on hydropic degeneration were calculated per 100 epithelial tubuli, while data on atrophic tubuli were calculated per 100 tubuli. Both data were presented in the form of numerical data.

The independent variable in this study was renal fibrosis, hydropic degeneration and the tubular atrophy. The dependent variable was valsartan administration.

Data were presented based on the evaluation of anatomic pathology examinations for each independent variable. For categorical data, comparisons was performed using Fisher's Exact Test, while for numerical data, means were compared using Mann Whitney U test. The entire testing was conducted in one direction (one tailed analysis). Data analysis was performed using SPSS 16.0.

RESULTS

From group A, two mice were not included in final calculation because of death before the seventh day. The cause of death in one of the mice was too deep anesthesia with diethyl ether while in the other mouse was not known. All the rats in group B were included in final calculation because no mice died before the seventh day of the study.

Of thirteen wistar rats in group A, eleven mice had mild interstitial fibrosis with the lowest level interstitial fibrosis of 0 per 100 tubuli and the highest 15% per 100 tubuli. There were two of them with moderate fibrosis rate of 30%. In two rats from group A with fibrosis level 0 per 100 tubuli, there was a profile of hydropic interstitial fibrosis and degeneration. In group B, all rats had interstitial fibrosis with mild degree of fibrosis. In this group, the lowest interstitial fibrosis was 8% per 100 tubuli and the highest was 17% per 100 tubuli.

For hydropic degeneration, all experimental animals in group A had degenerated hydropic epithelial tubuli with the highest 60 per 100 epithelial tubuli and the lowest 20 per 100 epithelial tubuli. In group B, the highest value was 60 per 100 epithelial tubuli and the lowest 30 per 100 epithelial tubuli.

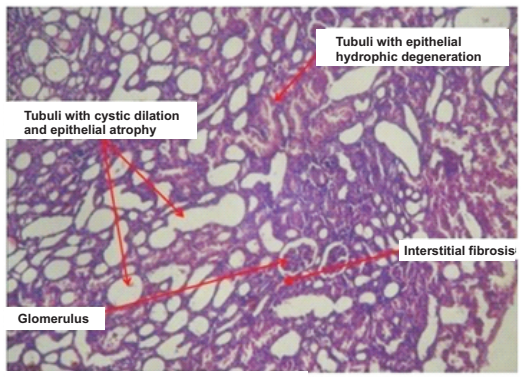
Atrophic tubuli were also assessed in this study. For experimental animals in group A, the highest score was 76 per 100 tubuli while the lowest score was 33 per 100 tubuli. In group B, the highest score was 75 per 100 tubuli and the lowest was 40 per 100 tubuli. The data on tubular atropy is presented in table 1.

After the above data had been processed, there were no significant differences for interstitial fibrosis per 100 tubuli between groups that were not given with valsartan and the group receiving valsartan ($p = 0.206$, Fisher's Exact Test).

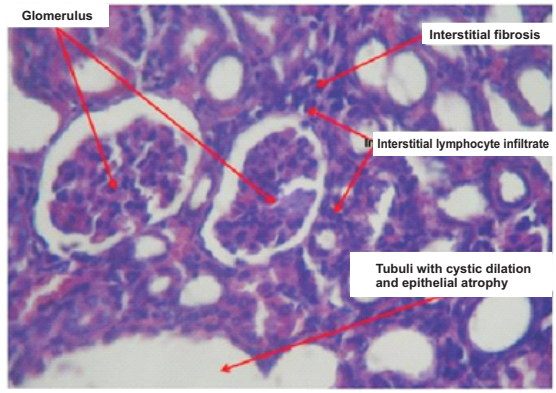
Both groups also showed no significant differences in hydropic degeneration of epithelial tubuli per 100 tubuli. However, the group receiving valsartan showed a higher average than the group without valsartan administration (33.67 vs 31.46; $p = 0.052$). For atrophic tubuli per 100 tubuli, the group without valsartan showed a slightly better mean than those with valsartan administration, but this was not significantly different (61.78 vs 62.07; $p = 0.555$).

Table 1. Distribution of atrophic tubuli.

Wistar Strain	Interstitial Fibrosis per 100 tubuli		Hidropic degeneration of tubular epithelium per 100 tubular epithelium		Tubular atrophy per 100 tubuli	
	A	B	A	B	A	B
1	mild	mild	26	33	70	75
2	moderate	mild	20	30	60	72
3	mild	mild	35	35	70	68
4	mild	mild	30	35	65	65
5	mild	mild	50	35	40	40
6	mild	mild	20	33	75	74
7	moderate	mild	60	33	33	75
8	mild	mild	30	35	64	69
9	mild	mild	35	30	60	72
10	mild	mild	20	35	76	45
11	mild	mild	30	33	60	72
12	mild	mild	25	35	60	40
13	mild	mild	28	35	70	45
14	-	mild	-	33	-	74
15	-	mild	-	35	-	45

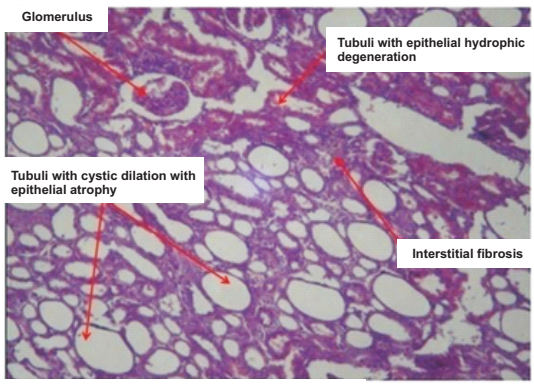


Renal micrograph photo P1 10x objective HE

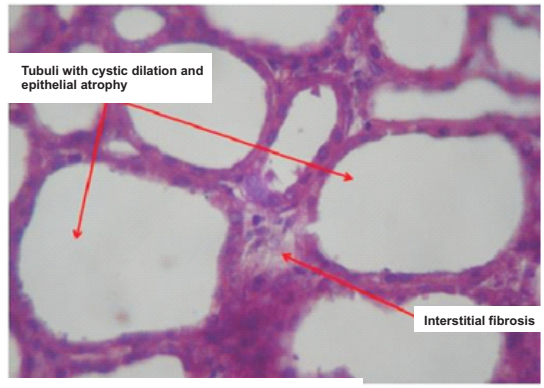


Renal micrograph photo P1 objective HE

Wistar rats with unilateral ureteral obstruction without valsartan administration



Renal micrograph photo with valsartan 10x objective HE



Renal micrograph photo with valsartan 10x objective HE

Wistar rats with unilateral ureteral obstruction with valsartan administration

Figure 1. Micrograph photos of haematoxylin eosin staining results.

DISCUSSION

Valsartan is an angiotensin II receptor blocker. Its use in research is expected to reduce the occurrence of kidney damage due to unilateral ureteral obstruction. These results are expected through valsartan mechanism of action, which will selectively inhibit AT1 that plays a role in increasing TGF-ß1 expression. Thus, renal fibrosis that occurs in experimental animals treated with valsartan was expected to be milder than in those not receiving valsartan.

The results of this study indicated that the kidneys of the experimental animals not receiving valsartan therapy had a higher rate of fibrosis compared to those in the group receiving valsartan, but the difference was not significant. This was because renin-angiotensin-aldosterone system was not the only mechanism that plays a role in the occurrence of fibrosis in ureteric obstruction.[1-5] There are many other factors that are described in the following scheme.[1]

From Figure 2, it can be explained that the role of valsartan as ARB is only on one side; to prevent kidney damage in ureteric obstruction. In addition, valsartan as selective inhibitor is just on AT1R alone, while angiotensin II activates NFκB through AT1R and AT2R.[5] Studies in AT1R knock-out mice found infiltration of monocytes in the interstitial tissue and the activation of NFκB, in which both of these processes can be inhibited by inhibiting AT2R.[12] Thus, selective inhibition in AT1R only, as carried out by valsartan, was not significant to improve the kidney in ureteric obstruction. Combined inhibition of both angiotensin II receptors might result in more significant improvement.

Oxidative stress also plays a role in causing kidney damage in ureteric obstruction.[1,2,5] The provision of valsartan does not inhibit fibrosis that

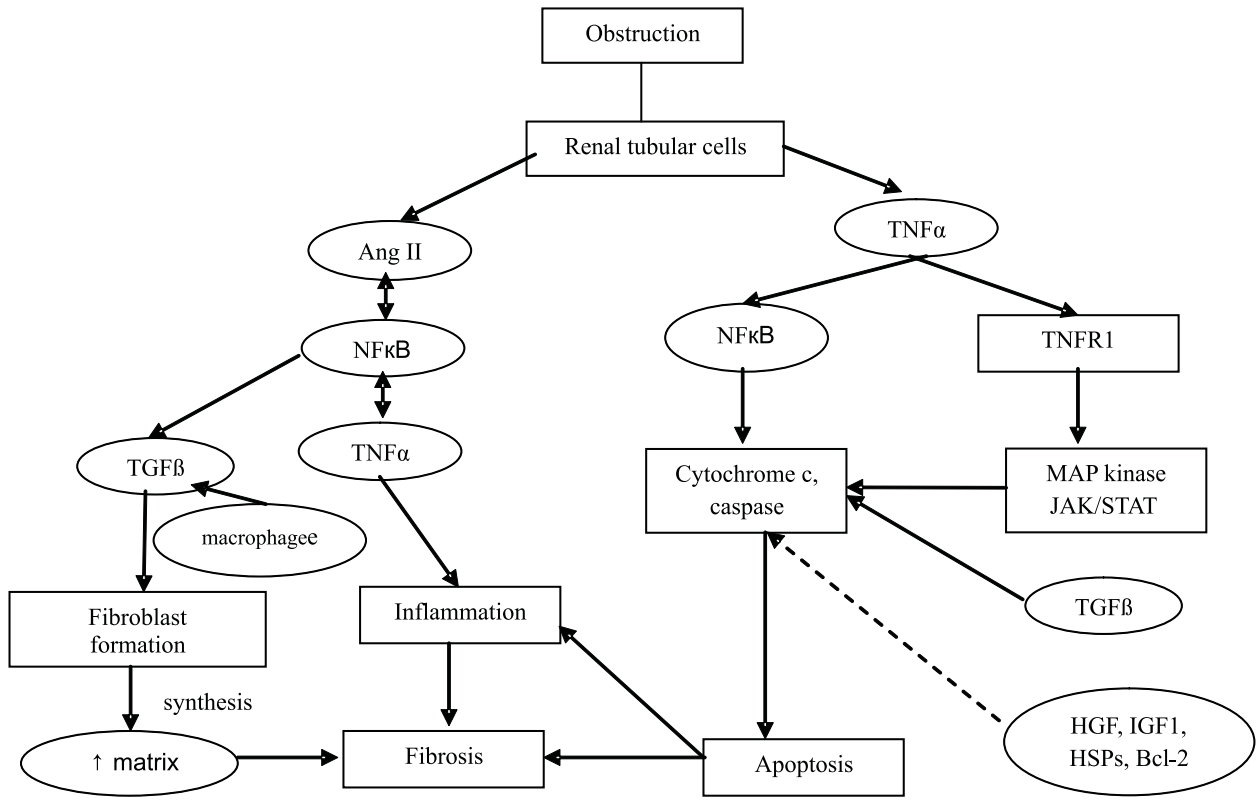


Figure 2. Scheme of interstitial fibrosis and apoptosis in ureter obstruction.

Ang II, angiotensin II; HGF, human growth factor; HSPs, heat shock proteins; IGF, insulin-like growth factor; JAK/STAT, Janus kinase/signal transducers and activators of transcription; MAP, mitogen-activated protein, NF-κB, nuclear factor κB; TGF, transforming growth factor; TNF, tumor necrosis factor; TNFR1, tumor necrosis factor receptor 1. (Figure adapted from Campbell-Walsh Urology 10th edition).

occurs due to oxidative stress. Antioxidant therapy in combination with valsartan can be considered as a preventive therapy to prevent kidney damage. Research conducted by Lubis and Alvarino by combining valsartan and curcumin showed a significant difference in the level of renal tubular fibrosis in ureteral obstruction compared with the group that received valsartan alone.[13] Therefore, hydropic degeneration and tubular atrophy in both experimental groups did not result in significant difference.

The results of this study were in contrast to those in previous studies, which showed the presence of protective effect for obstructive nephropathy provided by the ARB class.[14-18] For example, the administration of Losartan in experimental animals with unilateral ureteral obstruction showed a deterrent effect on the occurrence of unilateral ureteric obstruction and the prevention of obstructive nephropathy in patients with diabetes mellitus type II. Whereas, the provision of irbesartan was considered effective for protecting the occurence of obstructive nephropathy in patients with type II diabetes mellitus and this effect does not depend on the effect of antihypertensive that it produces.

It should be noted that in this study, losartan is used in doses of 10 mg/kg/day, while the antihypertensive therapy, the dose of losartan in adults is 50 mg/day in adults weighing 70 kg.[7,15] There is a difference between the dose given as antihypertensive therapy with a dose that is renoprotective.

Similar study conducted by Wu et al, comparing kidney damage in unilateral ureteral obstruction using valsartan alone and the combination of valsartan with aliskiren.[19] In that study, there were significant differences for the degree of fibrosis between the valsartan-only and combination group. Wu et al also explained that there is a meaningful difference to the level of fibrosis between experimental animals without any treatment given to the experimental animals were given valsartan therapy alone, valsartan dose used was 30 mg/kg/day. As for experimental animals treated with valsartan 15 mg/kg/day showed no significant difference.

It should be noted that the dose of valsartan given to obtain renoprotective effect of the study was 30 mg/kg/day. In our study, we used the valsartan antihypertensive dose of 80 mg/day that we converted to the dose according to the type of experimental animals using a dose conversion table. With the same animal weight, about 200 grams, and based on the study by Wu et al., the renoprotective effect of valsartan can appear by providing as much as 6 mg/day, while we only provided 1,44 mg/day.

Valsartan has a dose dependent effect.[8-10] The threshold dose that can provide antihypertensive effect is 1 mg/kg while to reduce as much as 30 mmHg, it takes the dose of 1.4 mg/kg.[9] Thus, it can be concluded that the administration of valsartan alone in antihypertensive dose not have a significant renoprotective effect on ureteric obstruction. Other factors causing tubular interstitial fibrosis cannot be inhibited by the administration of valsartan. The combination of valsartan with antioxidant administration may provide better renoprotective effect than valsartan alone.

This study was limited only to the histologic parameters with haematoxylin eosin examination. The addition of other parameters, such as the size and weight of the kidney, serum creatinine, and immunohistochemical examination, as well as other markers, are expected to make the study more meaningful.

CONCLUSION

The provision of valsartan in antihypertensive dose does not reduce the level of interstitial fibrosis, hydropic degeneration, and tubular atrophy in unilateral ureteric obstruction in wistar rats. This is because valsartan, as angiotensin II receptor blockers, cannot block all pathways that can lead to fibrosis.

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