World Journal of Pharmaceutical research

Volume 2, Issue 6, 1999-2011. Research Article ISSN 2277 – 7105

ALISKIREN RETARDS THE PROGRESSION OF RENAL DISEASE IN DIABETES MELLITUS: AN EXPERIMENTAL STUDY IN RATS

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Article Received on
18 August 2013,

Revised on 25 Sept. 2013,
Accepted on 30 October 2013

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ABSTRACT

Objective: This study was undertaken to evaluate the protective effect of aliskiren on renal disease progression in diabetes mellitus.

Materials and methods: Twenty-one adult albino rats were randomly divided into 3 groups: the normal control group (NC) injected with citrate buffer,the diabetic control group (DC)injected with streptozotocin (STZ) (60mg/kg I.P), andthe aliskiren treated group (AT),injected with STZ (60mg/kg I.P) and received aliskiren (10 mg/kg orally daily for 8 weeks).At the end of 8th weeks, blood& urine samples were collected for measurements of random blood sugar (RBS), HbA1c, renal function parameters,malondialdehyde (MDA) and reduced glutathione (GSH). Monocyte chemoattractant protein-1 (MCP-1) and histopathological assessment of degree of renal damage also performed.

Results: Compared to DC group, there is a significant decrease in the levels of blood urea (40.1±2.31), uric acid (4.13±0.191), serum creatinine (0.89±0.074), albuminuria (27.4±2.03), andMDA(1.21±0.013) in the ATgroup,($p \leq 0.05$). Expression of renal MCP-1 significantly decreased associated with a significant reduction in glomerular lesion in AT group compared to NC group ($p<0.001$).

Conclusion: We concluded that aliskiren is helpful in reducing lipid peroxidation,

improvingrenal function and reducingrenal expression of inflammatory markers and consequently reduces the progression of diabetic nephropathy.

INTRODUCTION

Previous studies showed that diabetes mellitus is the main cause for end stage renal disease. Therefore, early intervention and active management of diabetes is important to prevent progressive renal complications [1]. Diabetic nephropathy (DN) is characterized by a progressive increase of protein excretion in urine (proteinuria), particularly albumin, associated with early and continuing rise in systemic blood pressure, and a late decline in glomerular filtration rate (GFR), leading eventually to end stage renal failure[2]. Advanced diabetic nephropathy is also the leading cause of glomerulosclerosis and end-stage renal disease worldwide[3]. Functional changes occur in the nephron at the level of the glomerulus, including glomerular hyperfiltration and hyperperfusion, before the onset of any measurable clinical changes. Subsequently, thickening of the glomerular basement membrane, glomerular hypertrophy, and mesangial expansion take place [4]. Microalbuminuria, however, has a variable course; its progression is unpredictable and does not always lead to development of nephropathy [4]. Multiple mechanisms contribute to the development and outcomes of diabetic nephropathy. Most importantly, the interaction between hyperglycemia induced metabolic changes, hemodynamic changes and genetic predisposition, which sets the stage for kidney injury[5]. The hemodynamic factors include the activation of various vasoactive systems such as the renin-angiotensin-aldosterone system (RAAS) and endothelin system. Therefore, it is important to evaluate the effects of a different pharmacologicalstrategy that blocks the RAAS system.Aliskiren is an orally administered direct renin inhibitor(DRI), and previous studiesrecommended that it is safe andeffective in treatment of hypertension [6].

Aliskiren would also potentiate the antihypertensive and antiproteinuric effect of losartan if both are given to type 2 diabeticsubjects with macroalbuminuriaas a way to minimize progression of renal disease [7].

The involvement of metabolic pathway, among other features leads to nonenzymatic glycosylation, increased protein kinase C (PKC) activity, and abnormal polyol metabolism. Findings from various studies support an association between increased secretion of inflammatory molecules, such as cytokines, growth factors and development of diabetic nephropathy [8]. Inflammation plays a pivotal role in development of diabetic renal disease [9]. Chemokine ligand 2 (CCL2) is a small cytokine belonging to the chemotactic chemokine

family that is also known as monocytechemoattractant protein-1 (MCP-1) [10]. MCP-1 plays a role in the progression of renal disease in diabetes mellitus. Studies showed that harvestedmesangial cells, podocytes, andrenal tubular epithelial cells produce MCP-1 in the presence of high glucose [11].In addition, MCP-1 expression increase in streptozotocin-induced diabetes rats [12].

Oxidative stress also seems to play a central part[13] as studies indicated that there is a close link between production of reactiveoxygen species (ROS) and processes of vasoconstriction, vascular smooth musclecell growth and migration, endothelial dysfunction, modificationof extracellular matrix (ECM) proteins, and increased renalsodium reabsorption [14,15]. Therefore, inhibitionof oxidative stress ameliorates the manifestations associatedwith streptozotocin-induced diabetic nephropathy [14-16].

The aim of this study is to evaluate the protective effect of aliskiren on renal disease progression in patients with type 2 diabetes mellitus.

MATERIALS and METHODS

Animals

A total of 21 male albino rats weighing (140-350) g, were used in this study. All experiments were conducted in the Department of Pharmacology, College of Medicine, Al Qadaysia University, according to the guidelines for the Care and Use of Laboratory Animals in scientific research. The animals were placed in an animal house, in a group caging system, at controlled temperature (25±2◦C) and ambient humidity. Lights were maintained on a 12-h light/dark cycle. The animals had free access to water ad libitum.

Drugs

Aliskiren was used in a dose of 10 mg/ kg orally[17]. A 300 mg tablets (Novartis, Batch No. S0102) was dissolved in distilled water, and given to the rat according to the body weight once daily by stomach tube [18].

Animal model of diabetic mellitus

Diabetes mellitus was induced in the overnight fasted rats by a single i.p. injection of streptozotocin (STZ, Sigma) at a dose of 60 mg/ kg body weight[19]. STZ was dissolved in 1M citrate buffer (pH 4.5) (10) and freshly prepared before injection. Hyperglycemia in rats followed up for 72 hours, by using glucometers. Male rats with random blood glucose concentration more than 250 mg/dl(13.9 mmol/L) were considered diabetic and used for study [20].

Experimental Protocol

After 2 weeks of acclimatization period, the animals randomized into 3 groups (of 7 rats each): Rats in first group were injected with citrate buffer only and used as normal control group (NC). While the rats in other two groups were injected with streptozotocin (STZ) at a dose of 60mg/kg i.p. and treated as following: Diabetic control group (DC) rats received no treatment. Aliskiren treated group (AT) rats received aliskiren 10 mg per kg orally once daily. At the end of the study (after 8 weeks),after the urine was collected by metabolic cage, the animals were sacrificed with sodium pentobarbital at 50 mg/kg BW I.P.[21], About 2.5ml of blood was obtained from each rat by cardiac puncture using disposable syringe. Then the abdomen was opened through a midline incision and the kidneys were quickly removed. The kidneys were washed with ice cold normal saline to remove any RBCs or clots. De-capsulation of kidneys was performed. Then the right kidney was transported to the deep freezer and stored at -80C^{o} until analysis of oxidative stress measurement. The left kidney was fixed in 10% formalin for histopathological evaluation.

Following investigations were performed:

1. Random blood sugarRBS andHbA1c
2. Renal function parameters include blood urea, creatinine, uric acid and albuminuria
3. Oxidativestress parameters including malondialdehyde (MDA) and reduced glutathione (GSH).
4. Immunohistochemistry for assessment of MCP1.
5. Histopathological examination of the kidney for assessment of glomerular lesion.

Biochemical Procedures

Glucose concentration was determined by GPO/PAP manual method, HbA1c was determined by using fluorescence immunoassay technology.Blood urea, serum creatinine,uric acid was determined by using colorimetric method and albumin in urine was determined by using direct strip method. After preparation of kidney homogenate was done by weighing 50mg of renal cortex (that had been previously stored in the deep freezer) and homogenizing it with a tenfold volume (1:10 w/v) [22] of the appropriate buffer, homogenizing buffer, such as phosphate-buffered saline (PBS), 0.1M; pH=7.4 [23]. Content of GSH in renal tissue was determined using methods of Beutler[24]. Content of MDA in renal tissue was determined by using competitive inhibition enzyme immunoassay technique (cusabio; Catalog No. CSB-E13712Rb).

Immunohistochemistry

Immunohistochemistry was performed with a polyclonal goat antibodies, raised against mouse MCP-1 and staining procedure was carried out according to the manufacturer's instructions (Santa Cruz Biotechnology, Inc).The stain intensity was scored to 0: Indicated no staining, 1: Weak, 2: Moderate, 3: Strong, 4: Very strong stain intensity [25].

Histological examination of the aorta

For histological evaluation of glomerular sclerosis, the specimens were processed in usual manner, and embedded in paraffin and cut into 5 µm thick sections. The tissue sections were stained with hematoxylin and eosin. The assessment of glomerular damage was performed according to the severity glomerular changes: 0; normal, 1; (mild) glomerular damage, 2; (moderate) glomerular damage, 3; (moderate to severe) glomerular damage and 4; (sever) glomerular damage [26].

Statistical analysis

Statistical analyses were performed using SPSS 13.0 version. Data were expressed as mean ± SEM. Paired t-test was used to compare the mean values within each group at different time. Analysis of Variance (ANOVA) was used for the multiple comparison among all groups. The histopathological grading was assessed by Mann-Whitney test. In all tests, $P< 0.05$ was considered to be statistically significant.

RESULTS

Effect of diabetes mellitus

Compared to NC group, RBS, HbA1c%, blood urea, serum creatinine, uric acid, albuminuria, content of MDA weresignificantly increased while content of GSH decreased in animals in the DC group ($p\leq 0.05$), (Table 1 and Table 2).

Table (1): Showing the levels of random blood sugar, glycated hemoglobin and parameters of renal function. Results are expressed in mean ± standard deviation in the normal control (NC) group, diabetic control (DC) group and aliskiren treated (AT) group groups

Parameters	*Groups*		
	NC	**DC**	**AT**
RBS (mg/dl)	133±3.9	495.9±20.44*	489.6±20.04
HbA1c%	4.39±0.13	6.76±0.149*	6.57±0.139
Blood urea (mg/dl)	26.1±1.24	51.1±0.91*	40.1±2.31**
Creatinine (mg/dl)	0.51±0.051	1.16±0.065*	0.89±0.074**
Uric acid (mg/dl)	3.23±0.241	5.41±0.124*	4.13±0.191**

Albuminuria (mg/dl)	9.1±0.32	50.1±1.52*	27.4±2.03**

*p ≤ 0.05, as compare to NC group,

**p ≤ 0.05, as compare to DC group.

RBS: random blood sugar; HbA1c: glycated hemoglobin

Immunohistochemical analysis showed that expression of renal MCP-1 significantly increased in DC group compared to NC group (p≤ 0.05). Histopathological findings showed that theDC group rats hada significant glomerular lesion compared to NC group (p≤ 0.05), (Table 3).

Effects of aliskiren treatment

Compared to DC group aliskirentreatment didn't produce a significant effect on level of RBS andHbA1c. However, it managed to reduce increase the levels of blood urea (40.1±2.31), serum creatinine (0.89±0.074), uric acid (4.13±0.191) and Albuminuria (27.4±2.03) in the AT group at (p ≤ 0.05), (Table 1).

There was also a significant reduction in content of MDA in the AT group (1.21±0.013) compared to the DC group at (p≤ 0.05) but it failed to improve the level of GSH, (Table 2).

Table (2): Renal oxidative stress parameters. Results are expressed in mean ± standard deviation in the normal control (NC) group, diabetic control (DC) group and aliskiren treated (AT) group groups

	Groups		
parameters	**NC**	**DC**	**AT**
MDA (µmol/L)	0.989±0.0557	1.825±0.0514*	1.21±0.013**
GSH (mmol/L)	1.135±0.0415	0.733±0.0235*	0.797±0.0199

*p ≤ 0.05, as compare to NC group,

**p ≤ 0.05, as compare to DC group.

MDA: malondialdehyde; GSH: reduced glutathione

Immunohistochemistry

The result of immunohistochemical analysis for rat's kidney tissue of MCP-1 was significantly different between all the 3 study groups (Table 3, Figure1). The median

intensity of this marker was highest in DC group (very strong) and lowest in NC group (negative). Aliskiren treated group was associated with a median stain intensity of (moderate) for MCP-1 that is significantly lower than the DN group.

Table (3): Change in median kidney tissue for both (MCP1) immunostain intensity and histopathology changes of glomerular lesion in normal control (NC), diabetes control (DC) and aliskiren treated groups (AT).

	Groups		
Marker and lesion	**NC**	**DC**	**AT**
MCP-1	Negative	Very strong*	Moderate**
Glomerular lesion	Normal	Moderate*	Mild**

*p < 0.05, as compare to NC group ,
**p < 0.05, as compare to DC group

MCO-1: monocyte chemoattractant protein-1 (MCP-1)

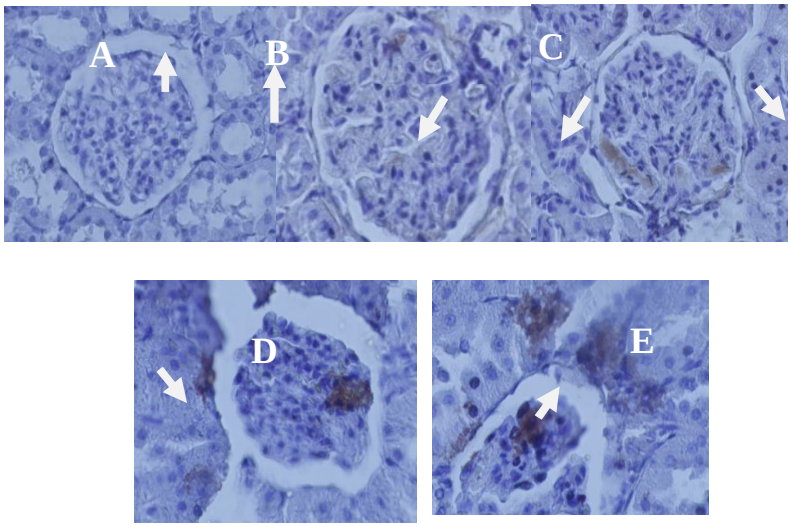

Figure (1):Immunohistochemical staining for MCP-1 expression in renal tissue of rats (x40), A (negative stain), B (weak stain intensity), C (moderate stain intensity), D (strong stain intensity), E (very strong stain intensity).
Aliskiren treated group was associated with a moderate stain intensity for MCP-1 (C)

Histopathological findings

The glomerular lesions of kidney tissue were graded as normal, mild, moderate and severe lesions (Figure2). The median histopathological grade of glomerular changes was significantly different between all the 3 study groups. The median was highest in DC group (moderate) and lowest in the NC group (normal). Aliskiren treated group was associated with

a median glomerular change (mild) that is significantly lower than the DC group.

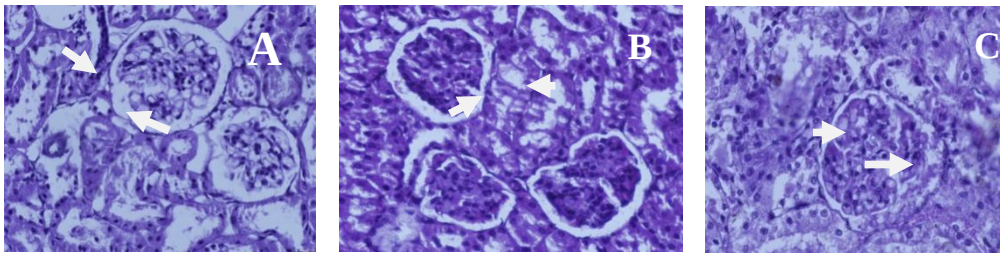

Figure (2):Photomicrograph of kidney section for rats shows the lesion, which A: normal,B: mild lesion characterized by (mild mesengial expantion, few leukocytic infiltration, mild thickness of basement membrane, mild increasing in the cellularity), C: moderate lesion characterized by (moderate mesengial expantion, more leukocytic infiltration, moderate thickness of basement membrane, moderate increasing in the cellularity). Stained With Haematoxylin and Eosin (X40).

DISCUSSION

This study showed that STZ induced diabetic rats showed a significant elevation in the level of RBS and HbA1c in the CD group compared to the NC group evidencing the success of induction of diabetes by streptozotocinin rats[27-29]. Our study showed that although aliskiren had no significant effects on RBSand HbA1c in type1 of diabetes rats in the AT group compared to the DC group, but it had a significant effect in reducing levels of blood urea, serum creatinine, uric acid and albuminuria. This can be explained due to the fact that aliskiren is a direct renin inhibitor that acts mainly in reducing blood pressure without a direct role on glucose metabolism [30-32]. Aliskiren can reduce (pro)renin receptor expression inglomeruli, tubules, and cortical vessels[33].So we didn’t expect significant changes in glucose and HbA1c parameters between the AT and the DC groups, but on the other hand, there was a significant improvement in parameters of renal function (Table 1).

Previous studies showed evidences suggesting that enhanced oxidative stress plays a significant role in the development of cardiovascular complications in cases of diabetes mellitus [34, 35]. Accordingly MDA was measured in this study as a sensitive tool to assess degree of lipid peroxidation [36]. Our data revealed significant increase in the levels of MDA in the DC group compared to the NC group. In addition, the MDA levels in the AT group were significantly lower compared to the DC group (Table 2). On the other hand, there was no such improvement in the levels of GSH in renal tissue using aliskiren treatment.This is in accordance with the results obtained by Guido Lastra et al in 2009 and Yamamoto et al; 2009[37, 38] who demonstrated that aliskiren significantly improved oxidation status in

hyperglycemic rodent model.

Furthermore, our immunohistochemical analysis showed that aliskiren significantly reduced the expression of inflammatory marker MCP-1 in the renal tissue illustrating aliskiren benefit in reducing the degree of inflammation. This was in agreement with the results obtained by Jun et al in 2009[39]. This can be explained by the fact that aliskiren blocks RAAS which in turn can reduce the expression of MCP-1in the body[25, 40]. The combined outcome of this study is that aliskiren can significantly reduce the progression of diabetic nephropathy in rats through inhibition of angiotensin II which in turn minimize blood pressure(a very important factor to prevent progression of DN)[17, 41]; improve renal function (through improving the levels of blood urea, serum creatinine, uric acid and albuminuria) and reducing the degree of oxidative stress and counteract inflammation.

CONCLUSION

We conclude that aliskiren is helpful in reducing lipid peroxidation, improving renal function and reducing renal expression of inflammatory markers and consequently reduces the progression of diabetic nephropathy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. The authors also declare that they have no financial relation with the corporation and company mentioned in this paper.

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