

Renoprotective evaluations of different angiotensin inhibitors on Diabetic Nephropathy in Rats

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ABSTRACT

Background: This study was designed to compare the effectiveness of different angiotensin inhibitors; direct renin inhibitor (Aliskiren), angiotensin-converting enzyme inhibitors (Ramipril) and angiotensin II receptor blocker (Irbesartan) in prevention and treatment of nephropathy in a group of rat diabetic nephropathy in rats.

Methods: Thirty rats were divided into two groups. The first group consisted of 6 rats which were considered as the normal control group. The second group included 24 induced diabetic rats. The diabetic model rats were subdivided into four subgroups of six rats each. The first subgroup served as a positive control. The second, third and fourth subgroup received Ramipril, Irbesartan and Aliskiren respectively.

Results: Diabetic nephropathic rats showed a significant increase in blood glucose level, blood pressure, heart rate, serum urea, serum creatinine, in addition to deteriorating renal functions including (urine flow, glomerular filtration rate, Na⁺ and K⁺ excretion rate, albumin and creatinine in the urine). The administration of (Ramipril, Irbesartan, and Aliskiren) caused a significant reduction in blood pressure, blood glucose, serum urea, Na⁺ and K⁺ excretion rate, with a significant improvement in urine flow and glomerular filtration rate. All three drugs induced a significant elevation in serum K⁺ concentration.

Conclusion: Administration of different angiotensin inhibitors (ramipril, irbesartan, & aliskiren) could slow the progression of nephropathy in alloxan induced diabetic rats. Both ramipril and irbesartan have the same renoprotective effects for most parameters.

Key words: Diabetic nephropathy, Aliskiren, Irbesartan, Ramipril

Introduction

Diabetic nephropathy is a major microvascular complication of diabetes, representing the leading cause of end stage renal disease in the world. Diabetic nephropathy is characterized by a progressive increase in urinary albumin excretion (microalbuminuria) and a decline in glomerular filtration rate (GFR), which occurs in association with an increase in blood pressure, ultimately leading to end stage renal disease (1, 2).

Basic and clinical research supports the use of renin angiotensin aldosterone system (RAAS) inhibitors in diabetic nephropathy (3, 4, 5).

Several basic and clinical studies, mainly in diabetic patients, have provided evidence that some antihypertensive agents that inhibit the renin angiotensin aldosterone system (RAAS), like angiotensin II type 1 receptor blockers (ARB) and angiotensin-converting enzyme inhibitors (ACEI), are renoprotective (6, 7). The reno-protection provided by these drugs seems at least partly independent of BP lowering and related perhaps to the inhibition of the RAS (8, 9, 10).

Several mechanisms participate in the renal protection afforded by angiotensin inhibitors. ACEIs increase the permeability selectivity of the filtering membrane, thereby diminishing exposure of the mesangium to proteinaceous factors that may stimulate mesangial cell proliferation and matrix production, two processes that contribute to expansion of the mesangium in diabetic nephropathy. Since angiotensin II is a growth factor, reductions in the intrarenal levels of angiotensin II may further attenuate mesangial cell growth and matrix production (11, 12, 13).

Thus, there do not appear to be significant differences between ACEI and ARBs in type 2 diabetic patients with nephropathy based on a small number of comparison studies.

Other studies in hypertensive type 2 diabetics with early nephropathy comparing ACEIs and ARBs have also failed to show significant differences in the effects of these two drug classes on BP and urinary albumin excretion (14, 15). Only a few studies have addressed the question of whether ACE inhibitors are better than ARBs or vice versa.

This study is designed to compare the effectiveness of different angiotensin inhibitors, direct renin (DR) inhibitor (Aliskiren), ACEI (Ramipril) ARBs (Irbesartan) in prevention and treatment of nephropathy in rat induced diabetes.

Materials and Methods

Animals

Healthy adult albino rats of both sexes were used in the present study. Their weight ranged from 250-300 grams. Rats were grouped and kept in separate animal cages at the animal house of the College of Medicine under prevailing atmospheric conditions (room temperature of about 25°C).

The animals were maintained on a balanced diet (bread, barley, carrots, lettuce, milk) and fresh-water supply.

Induction of experimental diabetes

Diabetes was induced by a single intraperitoneal injection of 120mg/kg body weight of alloxan dissolved in distilled water immediately before injection 16. Alloxan treated animals were allowed to drink 5% of glucose overnight to prevent the potentially fatal hypoglycemia occurring as a result of massive insulin release following alloxan injection (17).

Rats showing blood glucose levels above 180 mg/dl were considered to be diabetic (18) and used for drug treatment.

Experimental design

Thirty rats were divided into five groups each consisting of 6 rats in order to study the effect of different angiotensin inhibitors (Ramipril, Irbesartan, Aliskiren) during the 21 days study period:

Group I: Normal control rats given D.W

Group II: Control diabetic rats given D.W

Group III: Diabetic rats given Ramipril 10mg/kg.

Group IV: Diabetic rats given Irbesartan 10mg/kg.

Group V: Diabetic rats given Aliskiren 10 mg/kg.

The solution of drugs was freshly prepared in normal saline before administration by an oral gavage every morning.

Collection of samples

1-Urine

After 3 days, and at the end of drug treatment, all of the animals were kept in metabolic cages. Animals were fasted but allowed free access to water. Urine sample were collected after 24 hours in urine collecting bottles from which the urine collected was tested for: Albumin, Creatinine, Na⁺ excretion rate and K⁺ excretion rate, glomerular filtration rate and others.

2-Blood

At the end of drug treatment, all of the animals were fasted overnight but allowed free access to water. The next morning, blood samples were taken by cardiac puncture into a plastic syringe under a combination of ketamine in a dose of 75 mg/kg with xylazine in a dose of 10 mg/kg. At 10th day and at the end of experiment (after 21 days), a 24 hours urine collection was carried out by using the metabolic cage. The urine was checked for the albumin and total protein by using Cybow diagnostic kits (DFI co. Ltd, Gimhae- City, Gyung- Nam, Korea).

Statistical Analysis

All data are expressed as the mean \pm standard error means (M \pm SEM). The results were evaluated by using the Statistical Package for the Social Sciences (SPSS Version 21) computer program and the differences in all parameters between diabetic and non-diabetic rats were analyzed by a

one-way analysis of variables (ANOVA). The comparison between groups was done using Duncan test. A change was considered statistically significant when $P < 0.05$.

The experiments were carried out with the approval of the ethic committee of Hawler Medical University/college of Medicine.

Results

Effect of Ramipril, Irbesartan, and Aliskiren on the blood pressure and heart rate of diabetic rats.

In alloxan-induced diabetic rats, a significant high elevation in blood pressure was seen when compared to the normal control group, Table 1. The heart rate of diabetic rats was moderately higher than that of the normal control. A significant reduction in blood pressure was observed following oral 10 mg/kg administration of all the angiotensin inhibitors (Ramipril, Irbesartan, and Aliskiren) when compared to the diabetic group. Table (1).

Ramipril and aliskiren treated group had a significant reduction in their heart rate, while irbesartan caused non-significant changes when compared to the diabetic group.

Effect of angiotensin inhibitors on the renal function of diabetic rats

The urine flow of the diabetic rats was significantly higher than the normal control group. Aliskiren caused a significant reduction in the urine flow when compared to the diabetic rats, while ramipril, and irbesartan produced a non-significant reduction in the urine flow when compared with both groups. While the glomerular filtration rate (GFR) of diabetic rats was found to be significantly lower than normal rats. Angiotensin inhibitors (Ramipril, Irbesartan, & Aliskiren) induced a significant improvement in the GFR when compared to the diabetic group Table (2) - next page.

In alloxan-induced diabetic rats there was a marked elevation in albuminuria when compared to the control animals. Daily oral administration of angiotensin inhibitors for 21 days caused a significant reduction in albumin excreted through urine when compared to the diabetic group.

The effect of different angiotensin inhibitors on Na^+ concentration in the urine, were non-significantly reduced in comparison to the diabetic animals, although the urinary Na^+ concentration did not return to the normal value. Table (3). The Irbesartan treated rats did not show a significant improvement in Na^+ excretion rate, while Ramipril and Aliskiren treated rats induced a significant reduction in Na^+ excretion rate. As shown in Table (3) there was a significant reduction in Na^+ serum concentration level of angiotensin inhibitors treated rats when compared to the diabetic rats. In comparison to the diabetic rats, the percentage of Na^+ reabsorption in the angiotensin inhibitors treatment groups were non-significantly reduced. Table (3) - next page.

Effect of angiotensin inhibitors on the renal excretion of K^+ of the diabetic rats

Following the induction of diabetes by alloxan, there was a reduction in K^+ urine concentration accompanied by an increase in the urinary potassium excretion rate. There was a significant elevation in K^+ urine concentration in the groups which received different angiotensin inhibitors in comparison with diabetic group.

The angiotensin inhibitor treated rats showed a significant decrease in the K^+ excretion rate in comparison to the diabetic rats, albeit not reaching the normal range. The serum concentration of K^+ was increased significantly in diabetic and treated rats with angiotensin inhibitors when compared to the control group. Table (4) - page 7.

Table 1: The effects of 10 mg/kg of angiotensin inhibitors on the blood pressure & heart rate of the diabetic rats

Parameters	Control (n=6)	Diabetes (n=6)	Ramipril (n=6)	Irbesartan (n=6)	Aliskiren (n=6)
Blood pressure (mm.Hg)	102.1 ± 5.48 a	135.76 ± 6.43 b	106.56 ± 3.44 a	106.2 ± 3.88 a	112.34 ± 3.004 a
Heart rate (Beats/min)	297.33 ± 4.73 a	352.11 ± 10.97 b	312.2 ± 16.4 a	323.4 ± 13.16 ab	310.8 ± 12.7 a

* Similar letters indicate no significant differences.

* Different letters indicate significant differences at $P < 0.05$.

Table 2: The effects of 10 mg/kg of angiotensin inhibitors on the urine flow, GFR, and albuminuria of the diabetic rats

Parameters	Control (n=6)	Diabetes (n=6)	Ramipril (n=6)	Irbesartan (n=6)	Aliskiren (n=6)
Urine flow (ml/min/kg)	0.014 ±0.00216 a	0.034 ± 0.0054 b	0.023 ± 0.006 ab	0.022 ± 0.0033 ab	0.018 ± 0.0038 a
GFR (ml/min/kg)	0.065 ± 0.0123 a	0.025 ± 0.0065 b	0.037 ±0.0089 a	0.038 ±0.0085 a	0.033 ± 0.0047 a
Albumin in urine (mg/dl)	110 ± 40.49 a	500 ± 91.24 b	153.3 ± 14.75 a	155 ± 47.17 a	166.66 ± 42.6 a

* Similar letters indicate no significant differences.

* Different letters indicate significant differences at P < 0.05.

Table 3: Effects of angiotensin inhibitors on the renal excretion of Na⁺ of the diabetic rats

Parameters	Control (n=6)	Diabetes (n=6)	Ramipril (n=6)	Irbesartan (n=6)	Aliskiren (n=6)
Na ⁺ in urine (mEq/dl)	82.3 ± 7.5 a	165 ± 8.96 b	134.166 ±23.88 b	131.66 ±18.28 b	124.5 ± 16.7 b
Na ⁺ excretion rate (mEq/min/kg)	2.14 ± 0.528 a	5.077 ± 0.78 b	2.367 ± 0.23 a	3.88 ± 0.67 b	2.15 ± 0.33 a
S. Na ⁺ Concentration (mEq/L)	138.16 ± 18.9 a	215.16 ± 32.65 b	149.5 ± 21.2 a	153.7 ±33.3 a	146.8 ± 17.73 a
% Na ⁺ reabsorption of filtered load	70.88 ± 4.85 a	95.67 ± 14.57 b	79.55 ± 5.3 ab	83.77 ± 4.71 ab	84.8 ± 4.18 ab

* Similar letters indicate no significant differences.

* Different letters indicate significant differences at P < 0.05.

Table 4: Effects of different angiotensin inhibitors on the renal excretion of K⁺ of the diabetic rats

Parameters	Control (n=6)	Diabetes (n=6)	Ramipril (n=6)	Irbesartan (n=6)	Aliskiren (n=6)
K ⁺ in urine (mEq/dl)	87.5 ± 15.47 a	66.5 ± 3.6 b	78.3 ± 10.14 a	81.46 ± 9.54 a	83.66 ± 8.79 a
K ⁺ excretion rate (mEq/ min/kg)	1.194 ± 0.23 a	2.53 ± 0.41 b	1.31 ± 0.24 a	1.225 ± 0.3 a	1.35 ± 0.29 a
S. K ⁺ conc. (mEq/L)	4.16 ± 0.24 a	4.8 ± 0.61 b	5.7 ± 0.33 b	5.9 ± 0.35 b	5.15 ± 0.42 b

* Similar letters indicate no significant differences.

* Different letters indicate significant differences at P < 0.05.

Table 5: Effect of angiotensin inhibitors on the biochemical parameters of the diabetic rats

Parameters	Control n=6	Diabetes n=6	Ramipril n=6	Irbesartan n=6	Aliskiren n=6
Blood glucose (mg/dl)	106.2 ± 10.99 a	237.2 ± 34.63 b	130.16 ± 6.37 a	126.43 ± 5.23 a	114 ± 7.98 a
S.Urea (mg/dl)	30.42 ± 3.55 a	44.19 ± 2.21 b	35.2 ± 2.36 a	33 ± 3.38 a	35.7 ± 1.74 a
S.Creatinine (mg/dl)	0.76 ± 0.044 a	1.296 ± 0.17 b	1.12 ± 0.04 b	1.14 ± 0.044 b	1.13 ± 0.044 b

* Different letters indicate significant differences at P < 0.05

Effect of angiotensin inhibitors on the biochemical parameters (blood glucose, serum urea, & serum creatinine) of the diabetic rats

Following the treatment of diabetic animals with angiotensin inhibitors Ramipril, Irbesartan, and Aliskiren at a dose of 10 mg/kg for 21 days, a significant reduction in the blood glucose and serum urea were noticed when compared to the diabetic group. The ramipril, irbesartan, and aliskiren did not significantly change the serum creatinine in comparison to the diabetic rats. Table (5).

Discussion

Several randomized trials have shown that improved glycemic control in both type 1 and 2 diabetic patients decreases the risk of diabetic nephropathy and other complications. Although significant improvement in the treatment of diabetic nephropathy has occurred over the past 25 years, as a result, pharmacological inhibition of the RAS has been proposed as a key strategy

in reducing kidney damage beyond the predicted effects as a result of blood pressure reduction. (19, 20, 21).

In diabetic rats, a definite and elevated blood pressure was seen when compared to the normal control group, while the heart rate of diabetic rats was moderately higher than that of the normal group. The result obtained from experiments on rats through detecting the effect of (Ramipril, Irbesartan, and Aliskiren) on blood pressure and heart rate, showed that there was a statistically significant decrease in blood pressure with a non-significant decrease in heart rate. Ramipril and irbesartan were better than aliskiren in decreasing Blood pressure. The hypotensive effect of different angiotensin inhibitors may be explained by the vasodilating effects of ACEI on the glomerular efferent arterioles, where it prevents the Ang-II formation (22). Inhibition of angiotensin lowers systemic vascular resistance and blood pressure; this is not surprising when the renal vessels are exceptionally sensitive to the vasoconstrictor actions of angiotensin II (23). Angiotensin inhibitor increases renal blood flow without increasing GFR; thus reducing the filtration fraction. Both the afferent and efferent arterioles are dilated as well

as causing systemic arteriolar dilatation. ACEI increases the compliance of large arteries, which contributes to systolic pressure reduction (24).

The urine flow of the diabetic rats was significantly higher than the normal control group, while the glomerular filtration rate (GFR) of diabetic rats was found to be significantly lower than in the normal rats. In the present study, the result obtained from the experiment on rats for detecting the effect of different angiotensin inhibitors (Ramipril, Irbesartan, and Aliskiren) on urine flow and glomerular filtration rate, showed a significant improvement in the urine flow and GFR. Both ramipril and irbesartan had a superior renoprotective effect than that of aliskiren. The renal protection effect of different angiotensin inhibitors may be explained by ACEI probably attenuating the progression of renal insufficiency in patients with a variety of nondiabetic nephropathies, and may arrest the decline in GFR even in patients with severe renal disease (25, 26, 27). Normally, GFR is slightly reduced by angiotensin II; however, during renal artery hypotension, the effects of angiotensin II on the efferent arteriole predominate, leading to increased renal blood flow, hence increasing GFR. Therefore, blockade of the renin-angiotensin system may cause acute renal failure in patients with bilateral renal artery stenosis and in patients with unilateral stenosis who have only a single kidney (7). Angiotensin II variably influences GFR via several mechanisms such as constricting the afferent arterioles, which reduces intra-glomerular pressure and GFR, or by contracting the mesangial cells, which decreases the capillary surface area within the available glomerulus which subsequently leads to decrease GFR, and it could be due to constricting effect on the efferent arterioles, which increases intra-glomerular pressure which increases GFR (28, 29).

The result of this experiment is in agreement with a study by Weidmann et al (1995) who concluded that GFR is better preserved in ACEI treated groups (30). Furthermore, Lebovitz et al (1996) (31) declared that enalapril prevented falling in GFR in hypertensive patients. However, the result of the present study does not agree with Kasiske et al (1993) who found that ACEI is more superior to B blockers in decreasing GFR among diabetic patients (32). Parving and Rossing (1994) concluded that lisinopril has no significant effects in decreasing GFR in diabetic nephropathic patients (33). Moreover, Barnett et al (2005) showed that the rate of GFR decrease was equivalent in both enalapril and telmisartan treated patients (34).

In the present study a marked elevation in albuminuria was seen in diabetic rats. Increased glomerular pressure associated with diabetes can be enhanced by Ang II -mediated constriction of the glomerular arterioles, causing further elevation in microcirculatory pressure within the glomerulus, and leading to excretion of albumin, and thus to the development of microalbuminuria and proteinuria (35). While after the oral administration of (ramipril, irbesartan and aliskiren) a significant reduction in urinary albumin was noticed. In addition, both ramipril and irbesartan better reduced the albumin in the urine. This result is in agreement with studies conducted by Chan et al (2000), and Jerums et al (2001) who reported that treatment with ACEI & aliskiren decrease albumin excretion rate (36, 37).

Studies in streptozotocin diabetic rats have demonstrated that both Ang II and ACEI blocked the development of hypertension and significantly decreased albuminuria (38). Whereas in the DETAIL (Diabetic exposed to telmisartan and enalapril) study there were no significant differences in albumin excretion rate in both enalapril and telmisartan treated patients (34).

Several mechanisms have been suggested for antiproteinuric effects of RAS inhibition. First, it may be related to a reduction in intraglomerular blood pressure independently of systemic blood pressure by vasodilatation preferentially of the postglomerular arterioles (39). Second, RAS inhibition may improve the charge and size selectivity of the glomerular membrane (40), which may be related, in part, to reduced loss of glomerular nephrin, which has been suggested to play a central role in the function of the glomerular filtration barrier (41).

The administration of both ramipril and aliskiren induced a significant change in Na^+ excretion rate, while irbesartan did not show any significant improvement in diabetic rats. On the other hand the effect of ramipril, irbesartan, and aliskiren on the Na^+ concentration in the urine was non-significant.

There was a non-significant reduction in the level of serum Na^+ concentration in all treated rats. The percentage of Na^+ reabsorption in the angiotensin inhibitor treatment group was non-significantly reduced but still lower than the control group.

However, it has been suggested that angiotensin II can act presynaptically to potentiate the release of norepinephrine from sympathetic nerve terminals and thus enhancing the renin release from the renal tubule (42, 43). The rise in sodium level in diabetic rats could be related to the fact that angiotensin II stimulates the zona glomerulosa of the adrenal cortex to increase the synthesis and secretion of aldosterone which acts on the distal and collecting tubules to cause retention of Na^+ and excretion of K^+ and H^+ . The stimulant effect of angiotensin II on aldosterone synthesis and release is enhanced under conditions of hyponatremia or hyperkalemia and reduced when concentrations of Na^+ and K^+ in plasma are altered in the opposite directions (44). Very low concentrations of angiotensin II stimulate Na^+/H^+ exchange in the proximal tubule; an effect that increases Na^+ , Cl^- , and angiotensin II may reduce Na^+ excretion in part by diminishing medullary blood flow.

In the present study, the significant decrease in the K^+ excretion rate accompanied by a non-significant rise in the serum K^+ level were detected following administration of ramipril, irbesartan, and aliskiren. These effects could be explained by suppression of endogenous aldosterone and worsening kidney function (45). Despite some reduction in the concentration of aldosterone, significant K^+ retention is rarely encountered in patients with normal renal function who have not been taking other drugs that cause K^+ retention (46).

In this study, the level of blood glucose in diabetic rats was significantly increased when compared to the control group. This result in accordance with studies of Bilal et al (1998), Azuma

et al (2007) who suggested that elevated blood glucose levels in diabetes are caused by a defect in production and or secretion of the hormone insulin (47, 48). In this study the elevation of blood glucose level indicate that this effect is caused by the direct influence of alloxan on pancreatic beta cells.

Treatment with angiotensin inhibitors Ramipril, Irbesartan, and Aliskiren caused a significant reduction in the blood glucose level of diabetic rats. However, aliskiren was better in reducing blood glucose than the other two drugs.

In this study the hypoglycemic effect of angiotensin inhibitors may be due to increased rate of glucose uptake into the cell, and to improve glucose metabolism (49, 50). Clarification of the mechanism of this effect is in progress. In patients with essential hypertension, plasma insulin and blood glucose levels increase together, indicating reduced insulin sensitivity. Because of this, even without the onset of diabetes, a latent rise in blood glucose level may be seen. Angiotensin receptor blocker and ACEI can improve glucose metabolism via blocking the inhibitory effect of angiotensin II on insulin signal transmission (51, 52). On the other hand, the vasodilatory action of angiotensin inhibitor may increase the access of insulin and glucose to the skeletal muscle tissue, the main site of insulin-mediated removal of glucose 53. The result of hypoglycemic effects of ramipril, irbesartan, and aliskiren in the diabetic rats is in agreement with other studies done by Jacobsen et al (2003), Lau et al (2004), Dizaye and Rashid (2009) (54, 55, 56). Subsequent studies indicated that telmisartan also suppressed the new onset of diabetes (53).

In the present study ramipril, irbesartan, and aliskiren did not significantly decrease serum creatinine, and this effect was compatible with the finding of Lewis et al (2001) who suggested that the level of serum creatinine was not significantly changed by irbesartan in nephropathic patients (21). Along the same lines, are the data from other studies which showed that serum creatinine levels did not significantly change in the ACEI group (57, 58). However, the result of this study was in disagreement with the study of Brenner et al (2001), who found that there was 25% reduction for doubling the serum creatinine level after using ACEi (20).

Conclusion

Administration of different angiotensin inhibitors (ramipril, irbesartan, and aliskiren) could slow the progression of nephropathy in alloxan induced diabetic rats. Both ramipril and irbesartan had the same renoprotective effects for most parameters.

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