Research Article

Comparative Study of The Effects of Telmisartan and Losartan in Experimental Diabetic Nephropathy

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ABSTRACT

The study was designed to evaluate and compare the effects of telmisartan with losartan in experimental diabetic nephropathy. The diabetic rats were divided into four groups of six each. They were administered test drugs such as telmisartan (10mg/kg), losartan (5mg/kg) and vehicle for 16 weeks. Blood samples were collected for the estimation of blood glucose, blood urea nitrogen, serum creatinine. Urine was collected for the measurement of protein and determination of creatinine clearance. STZ diabetic rats exhibited marked hyperglycemia, proteinuria and reduction in creatinine clearance. Telmisartan pretreatment reduced blood glucose level after 8 weeks which was not observed with losartan. Both telmisartan and losartan significantly improved the remaining parameters towards normal. However the beneficial effect produced by telmisartan was significantly greater compared to losartan. Telmisartan, a PPAR- γ modulator has a better renoprotective action compared to losartan. Therefore telmisartan should be preferred over losartan in the treatment of hypertensive diabetic patients with nephropathy.

Keywords: Renoprotection, telmisartan, losartan, PPARγ, diabetes.

INTRODUCTION

The incidence of diabetes mellitus is rising rapidly and is a major cause of end stage renal disease[1]. Hypertension is a common coexisting condition among patients with CKD as either the primary etiology or as a secondary event.[2] The Renin angiotensin system is a well known regulator of blood pressure and determinant of target organ damage. Angiotensin II (Ang II) is the major effector peptide of the renin-angiotensin system and it is implicated in the pathogenesis of essential hypertension, reno-vascular hypertension, congestive heart failure, and renal diseases associated with albuminuria.[3, 4, 5]

Hyperglycemia is an important causal factor in mediating the development and progression of diabetic kidney disease.[6]Angiotensin II formation is stimulated by hyperglycemia and glycation end products[4] and so the renin angiotensin system has a crucial role in diabetic nephropathy. Therefore, the present treatment protocol primarily aims for an efficient glucose and blood pressure control to arrest the initiation and progression of diabetic nephropathy. Highly selective angiotensin II (Ang II) type 1 (AT_1) receptor blockers (ARBs) are now available which block the diverse effects of Ang II. These agents not only lower BP but also reduce proteinuria and are recommended as first-line therapy for most diabetic patients with chronic kidney disease and hypertension. Several ARBs are available for clinical use. Despite belonging to the same drug class, these ARBs vary in some aspects of their chemical structure. This leads to important differences in pharmacokinetic and pharmacodynamic characteristics. Telmisartan has a pharmacology that distinguishes it from other ARBs. Telmisartan has a unique profile among ARBs, with a high affinity for the angiotensin II type I receptor, a long duration of receptor binding, high lipophilicity and a long plasma half life.Telmisartan in addition to blocking the RAS, acts as a selective modulator of peroxisome proliferator-activated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism.[7]This unique property of telmisartan is absent in other conventional ARBs like losartan. Telmisartan blocks the action of angiotensin II and also modulates PPAR-γ receptor. Therefore, it is believed that telmisartan's dual mode of action may provide greater protective benefits compared to losartan against renal damage caused by diabetes and hypertension.

The present work has been undertaken to evaluate and compare the effects of telmisartan and losartan in STZ induced diabetic nephropathy.

MATERIALS AND METHODS

Chemicals

Telmisartan and losartan were purchased from Glenmark Pharmaceuticals Ltd. Mumbai and STZ was purchased from Sigma Chemical Co St Louis, USA.

Animals

Adult albino rats, of either sex, weighing between 250 – 300g were maintained under standard conditions with food and water *ad libitum*. The study was approved by the Institutional Animal Ethical Committee.

Grouping & treatment

Animals were divided into four groups of 6 animals each and treated for 8 wks as follows:

- Group – I : Normal control
- Group – II : Diabetic rats treated with distilled water
- Group – III : Diabetic rats treated with telmisartan (10 mg/kg)
- Group – IV : Diabetic rats treated with losartan (5 mg/kg)

Induction of diabetes in experimental animals

Diabetes was induced in rats by single intra peritoneal injection of streptozotocin (STZ) at a dose of 60 mg/kg body weight dissolved in 0.01 M (ph 4.5) citrate buffer. STZ induces diabetes within 3 days by destroying the beta cells. [8] Three days after STZ injection, rats with blood glucose levels of more than 250 mg/dl were included in the study. Human antihypertensive doses of telmisartan (80 mg) and losartan (20 mg) were converted into animal doses as per the guidelines set by USFDA. In STZ-diabetic rats, nephropathy develops after approximately 8 weeks. Treatment with test drugs (telmisartan and losartan) were started 5 days prior to STZ injection and continued for a period of 16 weeks.

Collection of urine:

The rats were individually housed in metabolic cages (1 per one metabolic cage) provided with a wire mesh bottom and funnel with stainless steel sieves to retain feces and allow the urine to pass and 24 hr urine sample was collected. Urine samples were collected at 0 weeks, 4 weeks, 8 weeks, 12 weeks and 16 weeks.

Biochemical Parameters

1. **Measurement of blood glucose concentration:** To measure blood glucose level, the blood samples were collected from the retro orbital sinus. The blood glucose level was estimated 72 hrs after STZ administration and thereafter every 4 weeks for 16 weeks by Automatic Autoanalyzer based on the principle of Glucose Oxidase Method.[9]
2. **Blood urea nitrogen (BUN) :** The blood urea nitrogen levels were evaluated with the diagnostic kit by Automatic Autoanalyzer based on the principle of glutamate dehydrogenase-urease method.[10]
3. **Plasma creatinine:** The plasma creatinine levels were evaluated by diagnostic kit based on principle of Jaffe's Method.[11]
4. **Urine creatinine :** Renal function was estimated by determining urine creatinine concentration in all experimental animals by automatic analyzer using diagnostic kit.[11]
5. **Total urinary protein** : was measured by precipitation with trichloroacetic acid and the precipitate was dissolved in 1N NaOH and quantitated by Biuret method.[12]
6. **Determination of glomerular filtration rate :** Glomerular filtration rate was calculated using the following formula [13]
   GFR (ml/min) = Urinary Creatinine(mg/dl) x Urine volume (ml)x1000g / Plasma creatinine (mg/dl) x Body weight (g) x 1440 (min)

Statistical Analysis

All data are shown as mean ± SEM. Results were analyzed by analysis of variance. Significance between the groups was estimated using student t test. A p value of < 0.05 is considered as statistically significant.

RESULTS

Blood glucose concentration

Table 1: Effect of telmisartan and losartan on blood glucose in rats with STZ induced diabetic nephropathy

Groups	0 wks	4 wks	8 wks	12 wks	16 wks
Normal control	86.7 ± 2.4	88.8 ± 6.12	88.3 ± 2.8	87.8 ± 2.16	87.1 ± 2.74
Diabetic control	274.2 ± 5.27[a]	280.4 ± 3.12[a]	286.3 ± 5.16[a]	292.6 ± 5.58[a]	315.3 ± 5.9[a]
Diabetic + telmisartan	275.1 ± 4.38	277.3 ± 4.95	266.5 ± 5.89[b]	272.6 ± 3.65[b]	299.3 ± 5.53[b]
Diabetic + losartan	272.5 ± 5.25	278.5 ± 3.17	284.9 ± 6.01	288.6 ± 5.16	312.3 ± 6.83

Values are mean ± SEM for 6 animals in each group; $P^a < 0.001$ when compared with normal control; $P^b < 0.01$ when compared with diabetic control

Urinary protein

Table 2: Effect of telmisartan and losartan on urinary protein in rats with STZ induced diabetic nephropathy

Groups	0 wks	4 wks	8 wks	12 wks	16 wks
Normal control	0	0	0	0	0
Diabetic control	0	1.0 ±0.07^{a}	1.6 ± 0.06^{a}	1.96 ± 0.08^{a}	2.0 ± 0.14^{a}
Diabetic + telmisartan	0	0.26 ± 0.05^{bc}	0.4 ± 0.05^{bc}	0.48 ± 0.05^{bc}	0.51 ± 0.05^{bc}
Diabetic losartan	0	0.48± 0.05^{b}	0.81 ± 0.07^{b}	0.93 ± 0.12^{b}	0.81 ± 0.05^{b}

Values are mean ± SEM for 6 animals in each group; P^{a}< 0.001 when compared with normal control; P^{b}< 0.01 when compared with diabetic control ; P^{c}< 0.05 when compared with losartan treated diabetic group.

Blood urea nitrogen

Table 3: Effect of telmisartan and losartan on BUN levels in rats with STZ induced diabetic nephropathy

Groups	0 wks	4 wks	8 wks	12 wks	16 wks
Normal control	18.0 ±0.83	18.16 ± 0.55	19.33 ± 0.66	19.0 ± 0.53	20.16 ± 0.73
Diabetic control	20.1 ± 0.54	58.66 ± 1.02^{a}	60.33 ± 1.16^{a}	61.16 ± 1.68^{a}	63.33 ± 1.72^{a}
Diabetic + telmisartan	16.8 ± 0.79	27.66 ± 1.06^{b}	29.8 ± 1.25^{bc}	30.6 ± 1.75^{bc}	33.83 ± 1.38^{bc}
Diabetic + losartan	15.6 ± 0.79	28.5 ± 0.89^{b}	32.8 ± 1.52^{b}	34.5 ± 1.55^{b}	36.33 ± 1.58^{b}

Values are mean ± SEM for 6 animals in each group; P^{a}< 0.001 when compared with normal control; P^{b}< 0.01 when compared with diabetic control; P^{c}<0.05 when compared with losartan treated diabetic group

Creatinine clearance

Table 4: Effect of telmisartan and losartan on creatinine clearance (ml/min) in rats with STZ induced diabetic nephropathy

Groups	0 wks	4 wks	8 wks	12 wks	16 wks
Normal control	1.1 ± 0.12	1.1 ± 0.11	1.4 ± 0.19	1.3 ± 0.12	1.1 ± 0.08
Diabetic control	1.2 ± 0.10	2.2 ± 0.13^{a}	1.0 ± 0.05	0.5 ± 0.05^{a}	0.3 ± 0.07^{a}
Diabetic + telmisartan	1.1 ± 0.09	1.4 ± 0.16^{b}	1.2 ± 0.08	0.9 ± 0.07^{b}	1.0 ± 0.05^{b}
Diabetic + losartan	1.2 ± 0.11	1.5 ± 0.13^{b}	1.1 ± 0.08	0.9 ± 0.06^{b}	0.9 ± 0.07^{b}

Values are mean ± SEM for 6 animals in each group; P^{a}< 0.01 when compared with normal control; P^{b}< 0.05 when compared with diabetic control

DISCUSSION

This study was conducted to compare the effects of telmisatan and losartan and in order to gain a more detailed insight into possible renoprotective mechanisms of ARBs (telmisartan and losartan) that may occur in addition to BP lowering in overt nephropathy. Results of the present study confirm that STZ, which is commonly used to induce diabetes in experimental animals, causes hyperglycemia and diabetic nephropathy slowly progressing to end stage renal disease. The untreated diabetic rats demonstrated hyperglycaemia, macroproteinuria, and increase in blood urea nitrogen & plasma creatinine as well as decrease in glomerular filtration rate (GFR). Long –standing hyperglycemia is known to be a significant risk factor for the development of diabetic nephropathy. Hyperglycemia may directly result in mesangial expansion and injury by an increase in the mesangial cell glucose concentration. Glucose can also bind reversibly and eventually irreversibly to proteins in the kidneys and circulation to form advanced glycosylation end product (AGEs), which can contribute to renal damage by stimulation of growth and fibrotic factors via receptors fopr AGEs.[14] Our results showed that telmisartan pretreatment significantly ($p<0.05$) reduced blood glucose levels at the end of 8 weeks and this is in agreement with the results of a previous study

in which telmisartan is shown to have glucose lowering property.[15] This may be attributed to the partial agonist action of telmisartan on PPARγ which stimulates insulin sensitivity, thereby decreasing the blood glucose level independent of the action of renin angiotensin system.[7]The blood glucose level lowering effect was not seen in losartan treated diabetic rats possibly due to absence of action on PPARγ. The end stage of diabetic renal disease is usually characterized by changes in both proteinuria and a subsequent decline in GFR. Development of lesions in glomerular capillaries of the kidneys allows proteins to escape because of the changes in the basement membrane.[16] In this study there was no proteinuria in the control groups. The diabetic rats exhibited a sustained increase in urinary protein excretion ($P < 0.001$). It is noteworthy that in both treatment groups there was a significant ($P < 0.01$) reduction in the progression of proteinuria but the improvement in urinary protein excretion rate tended to be significantly ($p < 0.05$) greater in telmisartan pretreatment group compared to losartan group (Table:2). This is in accordance with the results of AMADEO study[17] and may be attributed to the glucose lowering property of telmisartan through the agonistic action on PPAR gamma receptor in addition to RAS blockade and blood pressure control. Also the levels of BUN and creatinine clearance are indicators of renal function. Increased blood urea levels were observed in STZ diabetic rats as compared with controls. The study revealed that both telmisartan and losartan pretreatment significantly ($p < 0.01$) reduced the elevated levels of blood urea nitrogen in diabetic rats (Table: 3) However, telmisartan treated diabetic rats produced a significantly ($p<0.05$) greater degree of reduction in BUN levels compared to losartan treated group after 8 weeks. The protective effect of telmisartan and losartan on kidney function may be attributed to the blockade of angiotensin II action which is known to produce deleterious effects on kidney by affecting blood pressure and renal haemodynamics, production of growth promoting and profibrotic factors, renal tubular and glomerular hypertrophy and oxidative stress in kidney.[18] Further, PPAR- γ receptors have been localized in urinary system including glomerulus, collecting ducts, proximal tubules and renal vasculature. There have been reports suggesting that activation of PPAR- γ triggers protection in different models of renal failure like chronic allograft renal damage [19] and renal ischaemia- reperfusion injury.[20] The studies have suggested that activation of PPAR- γ receptors directly attenuate glomerular diseases possibly by inhibiting mesangial growth, which occurs early in the process of nephropathy. Telmisartan can function as both an ARB and as a PPAR activator. This could be a possible explanation for greater BUN lowering effect of telmisartan compared to losartan in diabetic rats. The creatinine clearance test has been used to estimate the glomerular filtration rate (GFR). In this study, both telmisartan and losartan produced a beneficial effect on creatinine clearance in treated diabetic rats. However, there were no significant differences in the estimated GFR between the groups. This is in agreement with the study conducted by AMADEO trial. This study implies that telmisartan produces greater beneficial effect on renal function compared to losartan with respect to anti proteinuric and BUN lowering action with the only exception being a significant difference in creatinine clearance between the two treatment groups. However, creatinine clearance measurements may be inaccurate. A major limitation of creatinine clearance is that its accuracy worsens in relation to the amount of tubular creatinine secretion. Often as GFR declines, the contribution of urine creatinine from tubular secretion increases, further increasing the discrepancy between true GFR and measured creatinine clearance and indeed when using the generally recommended calculation according to the formula no difference was detected.

CONCLUSION

The study demonstrates that telmisartan, based on its unique pharmacokinetic profile and mechanism of action is superior to losartan as a renoprotective agent in diabetic kidney disease. This supports the use of telmisartan over other conventional ARBs like losartan in prevention and treatment of diabetic nephropathy.

REFERENCES

1. INTERNATIONAL DIABETES FEDERATION: *Diabetes Atlas*, 2004 2nd ed.,
2. Arauz-Pacheco C, Parrott MA, Raskin P: The treatment of hypertension in adult patients with diabetes (Technical Review). Diabetes Care 25, 134–147, 2002
3. Burnier M, Brunner HR. Angiotensin II receptor antagonists. Lancet. 355, 2000, 637–645.
4. Bumier M. Angiotensin II type 1 receptor blockers. Circulation. 103, 2001, 904–912.
5. Rodgers JE, Patterson JH. Angiotensin II-receptor blockers: clinical relevance and therapeutic role. Am J Health Syst Pharm. 58, 2001, 671–683.
6. Nandi A, Kitamura Y, Kahn CR, Accili D (2004). Mouse Models of Insulin Resistance. Physiol. Rev., 84, 623-647.
7. Benson SC, Pershadsingh HA, Ho CI, et al. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPAR gamma modulating activity. Hypertension 43, 2004, 993-1002
8. Karunanayake EH, Hearse D J, Mellows G. The metabolic fate and elimination of streptozocin. Biochemical Society Transactions 3, 1975, 410-14.
9. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann ClinBiochem. 6,1969, 24- 27.

10. Marsh WH, Fingerhut B, Miller H. Automated and manual direct methods for the determination of blood urea. Clin Chem. 11, 1965, 624-627.

11. Newman DJ, Price CP. Renal function and nitrogen metabolites. Burtis CA, Ashwood ER Eds. Teitz Textbook of clinical chemistry 3rd edition. Philaldelphia: WB Saunders, 1999, 1204- 1206.

12. Johnson MA, Rohifs EM, Silverman LM. Determination of proteins in urine in Burtis CA, Ashwood ER Eds. Teitz Text book of clinical chemistry 3rd edition. Philaldelphia: WB Saunders, 1999; p 525- 526

13. Post TW, Rose BD: Assessment of renal function: plasma creatinine; BUN; and GFR. In UpTo Date 9.1. Edited by BD Rose. 2001

14. Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 329, 1993, 977–986.

15. Hamed AA, Malek HA. "Effect of telmisartan in experimentally induced diabetes mellitus in rats". Int J Health Sci (Qassim). 1(2), 2007, 249-256.

16. Diabetes Hall P: Prevention of progression in diabetic nephropathy. Diabetes Spectrum 19, 2006, 18–24.

17. Bakris G, BurgessE, Weir M, Davidai G, Koyal S, AMADEO study investigators. Telmisartan is more effective than losartan in reducing proteinuria in patients with diabetic nephropathy. Kidney Int 74, 2008, 364-369.

18. Cooper ME. Interaction of metabolic and haemodynamic factors in mediating experimental diabetic nephropathy. Diabetologia. 44, 2001, 1957-1972.

19. Kiss E, Popavic ZV, Bedke J, Adams J, Bonrouhi M, Babelova A, et al. Peroxisome proliferator-activated receptor (PPAR) gamma can inhibit chronic renal allograft damage. Amj Pathol 176, 2010, 2150-2162.

20. Doi S, Masaki T, Arakawa T, Takahashi S, Kawai T, Nakashima A, et al. Protective effects of peroxisome proliferator-activated receptor gamma ligand on apoptosis and hepatocyte growth factor induction in renal ischemia-reperfusion injury. Transplantation 84, 2007, 207-213.

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