

Renoprotective effects of combining ACE inhibitors and statins in experimental diabetic rats

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ABSTRACT

Background and the purpose of the study: The combination of angiotensin II receptor antagonists and HMG CoA reductase inhibitors have shown to confer renoprotection. The purpose of this study was to find out the renoprotective effects of telmisartan and atorvastatin in combination and in monotherapy of Streptozotocin (STZ) induced diabetic nephropathy in rats.

Methods: Diabetes was induced by i.p injection of STZ to rats, after 18 hrs of fasting. Diabetic rats were randomly grouped and treated with telmisartan and atorvastatin in combination as well as monotherapy for 30 days. The serum and urine glucose, creatinine and serum triglyceride, cholesterol, albumin and micro-albumin and blood urea nitrogen, total protein and histological analyses of the left kidney were performed at the end of the study.

Results: By the end of the study, the combination showed significant ($P < 0.05$) improvement in urine glucose, serum cholesterol, serum and urine creatinine, blood urea nitrogen, total protein, serum albumin, micro-albuminuria levels in comparison to monotherapy. However, this combination didn't show significant changes on serum glucose and triglyceride levels. Kidney pathological injury was attenuated by the combination as compared to the diabetic group.

Conclusion: The present study document that, telmisartan and atorvastatin combination have better renoprotective effects but not with individual drug when compared to the diabetic group. The combination also attenuated the progression of diabetic nephropathy by slowing the proteinuria and microalbuminuria and these effects were confirmed by histopathological analysis.

Keywords: Atorvastatin, Telmisartan, Diabetic nephropathy.

INTRODUCTION

Diabetic nephropathy is a major long-term complication of diabetes mellitus. Clinically there is development of microalbuminuria with progression to overt proteinuria, increased in blood pressure and reduced renal function (1). Excessive deposition of extracellular matrix protein in the glomeruli and subsequent mesangial expansion are the main structural alterations in diabetic nephropathy (2).

Accumulating evidences suggest that in patients with diabetes mellitus there is increased rates of lipoprotein oxidation. Hyperlipidemia may be involved in the pathogenesis of renal injury and is also considered a risk factor for diabetic nephropathy (3). Inhibition of HMG CoA reductase by statins not only reduces cholesterol synthesis, but also decrease levels of geranylgeranyl phosphate and farnesyl pyrophosphate, which have important roles in the post translation modification of proteins (4).

Several large clinical trials have recently demonstrated that control of hypertension by angiotensin converting enzyme inhibitors and

angiotensin II receptor antagonists significantly delayed the progression of diabetic nephropathy due to the reduction of blood pressure (5). Angiotensin II is known as a vasoactive substance which has also growth factor properties, being able to induce hypertrophy, proliferation and production of proteins of extracellular matrix in kidney cells and mimic the effect of high glucose concentration in diabetes (6). Treatment with angiotensin II receptor antagonist has shown to normalize urinary protein excretion and renal structural changes (7).

The aim of the present study was to assess the renoprotective effects of a combination of an angiotensin II receptor antagonist with an HMG CoA reductase inhibitor in experimental diabetes.

MATERIAL AND METHODS

Chemicals

Atorvastatin was obtained as a gift sample from Shantam Pharma Pvt Ltd, Gandhinagar and Telmisartan was obtained as a gift sample from

Alembic Pharma, Baroda, Streptozotocin was purchased from Prolabs Marketing Pvt. Ltd., Delhi. Analytical grades, citric acid, tri sodium citrate, sodium dihydrogen orthophosphate, disodium hydrogen phosphate and formaldehyde procured from Merck laboratories, and Nice chemicals.

Animals

All the experiments were carried out with male albino wistar rats, 150-250g (Indian Institute of Sciences, Bangalore, Karnataka). Rats were housed in polyacrylic cages (38×23×10 cm) at maximum four animals per cage. They were housed in an air conditioned room and were kept in standard laboratory conditions under natural light dark cycle (approximately 14 h light/ 10 h dark) maintained humidity 60±5% and an ambient temperature of 25±2°C. All animals had free access to standard diet (Amrut rat feed, Bangalore) and tap water *ad libitum* and allowed to acclimatize for one week before the experiments. Commercial pellet diet contained 22 % protein, 4% fat, 4% fiber, 36% carbohydrates and 10% ash (w/w). The experiment was carried out according to the guidelines of the Committee for the Purpose of Control Supervision of Experiments on Animals (CPCSEA), New Delhi,

India. approved by the institutional animal ethical committee of Acharya and B. M. Reddy College of Pharmacy, Bangalore (Approval No. IAEC / Ph.cology/06/2009-10).

Experimentation

After 18 hrs fasting male wistar albino rats (150-250 gm) diabetes were induced by i.p injection of a single dose of STZ (45 mg/kg). STZ was dissolved in cold citrate buffer (pH 4.5) immediately before use and solution were made fresh daily.

Following the induction of diabetes animals were randomly allocated into 5 groups with 6 rats per group; (1) control rats, (2) diabetic rats with no treatment, (3) diabetic rats treated with atorvastatin (10 mg/kg, p.o.), (4) diabetic rats treated with telmisartan, (10 mg/kg, p.o.), (5) diabetic rats treated with the combination of both atorvastatin, (10 mg/kg, p.o.) and telmisartan (10 mg/kg, p.o.) and were treated for 30 days.

The blood was drawn from the retro orbital plexus of the diabetic wistar albino rats; under light ether anesthesia on the respective days and the serum glucose, creatinine the blood urea nitrogen, cholesterol, triglycerides and albumin were measured. Animals were accommodated in metabolic cages for urine collection for 2 days in order to become familiar

Table 1. Serum glucose, cholesterol, triglyceride, albumin, creatinine and blood urea nitrogen levels in rats following treatment with atorvastatin, telmisartan and the combinations.

Groups	Serum glucose (mg/dl)	Serum cholesterol (gm/dl)	Serum triglyceride (mg/dl)	Blood urea nitrogen (mg/dl)	Serum albumin (mg/dl)	Serum creatinine (mg/dl)
Control	89.28±4.611	51.57±6.928	117.30±10.11	19.38±1.471	3.281±0.156	0.612±0.0612
Diabetic	423.41±21.05	106.48±5.812	181.35±8.046	53.2±3.392	2.353±0.158	1.078±0.0593
^b D + Atorvastatin	415.19±16.08 ^{ns}	79.02±4.840 ^{ns}	157.49±12.00 ^{ns}	33.64±1.840 ^a	2.807±0.238 ^{ns}	0.98±0.0296 ^{ns}
D + Telmisartan	409.42±22.75 ^{ns}	100.02±1.871 ^{ns}	162.14±5.386 ^{ns}	37.17±2.003 ^a	2.976±0.086 ^{ns}	0.957±0.0796 ^{ns}
D + Atorvastatin + Telmisartan	413.99±34.93 ^{ns}	77.89±8.746 ^a	147.27±10.52 ^{ns}	31.92±1.784 ^a	3.012±0.248 ^a	0.933±0.0849 ^a

.P^a< 0.05 (s) compared to diabetic group

B,D: Diabetic

Table 2. Urine glucose, microalbuminuria, creatinine and total protein in rats after treatment with atorvastatin, telmisartan and their combinations

Groups	Urine glucose (mg/dl)	Microalbuminuria (mg/dl)	Urine creatinine (mg/dl)	Total protein (mg/dl)	Histopathology (Score)
Control	13.09±3.701	6.75±0.8447	58.76±1.096	54.68±5.337	0.65
Diabetic	988.09±13.37	13.94±0.3922	15.94±0.5970	138.01±7.970	3
D + Atorvastatin	375±18.71 ^a	9.38±0.5730 ^a	23.51±0.8325 ^a	107.25±11.20 ^{ns}	2.33 ^a
D + Telmisartan	371.79±12.73 ^a	9.33±0.2842 ^a	28.97±1.233 ^a	117.20±4.060 ^{ns}	2.5 ^a
D + Atorvastatin + Telmisartan	368.12±20.69 ^a	8.73±0.1763 ^a	34.19±1.168 ^a	95.62±9.407 ^a	1.75 ^a

.P^a< 0.05 (s) compared to diabetic group

B,D: Diabetic

with the environment of the cage. On the, second day, 24 hrs urine samples were collected from all groups to determine urine glucose, creatinine, albumin and total protein. Urine was centrifuged and kidneys were removed and fixed in formalin solution for histological studies.

Statistical Analyses

All measurements which were performed were blinded by the same operator. Data presented mean \pm SEM. The treated groups were compared with the respective diabetic groups using ANOVA followed by Dunnett multi comparison test using Graphad Instat. $P < 0.05$ considered as significant.

RESULTS

The results of the combination treatment showed significant ($P < 0.05$) changes in serum cholesterol, creatinine, albumin and blood urea nitrogen levels in comparison with diabetic group, but not with individual drug. However, this combination did not show any effect on serum glucose and triglyceride levels when compared with diabetic group.

The combination also showed significant ($P < 0.05$) changes in urine glucose, creatinine, microalbuminuria and total protein levels in comparison with diabetic group.

There was a minimal improvement in atorvastatin and telmisartan groups when compared to diabetic group. There was a slight improvement in atorvastatin group in comparison to telmisartan group.

DISCUSSION

The present study demonstrated that combination of an angiotensin II receptor antagonist and an HMG-CoA reductase inhibitor attenuated the increase in albuminuria in the kidney of experimental diabetes. The combination of atorvastatin and telmisartan showed significant ($P < 0.05$) reduction in albuminuria. Angiotensin converting enzyme inhibitors or angiotensin II receptor antagonist have potent antiproteinuric and

renoprotective actions. In rat models, angiotensin converting enzymes or angiotensin II antagonist have prevented proteinuria and several structural injury including interstitial lesions (8). The results of this study demonstrated that the combination has improved excretion of protein in urine significantly ($P < 0.05$). Results of this study also demonstrated that the combination of atorvastatin and telmisartan have significant ($P < 0.05$) effects on reduction of albuminuria and improved ($P < 0.05$) plasma concentration of creatinine and urea ($p < 0.05$). These findings are consistent with earlier reports that a combination of enalapril and lovastatin attenuated the increase in albuminuria and prevented the diabetes associated with increase in plasma concentration of creatinine and urea, a marker of renal function (9). The present study also demonstrated that there was significant ($P < 0.05$) reduction in polyuria as compared to diabetic group. These observations are supported by earlier reports that polyuria was more severe in the early weeks of diabetes and gradually decline after treatment although it was significantly higher than control group (10).

Lipid lowering agents have variable degrees of renoprotection in a number of experimental models (11). Results of the present study demonstrated that the combination has significant ($P < 0.05$) effect in reduction of serum cholesterol when compared to diabetic group. However, this combination did not show significant changes in serum triglyceride levels in comparison to the diabetic group.

CONCLUSION

In conclusion, the result of the present study showed that combination of atorvastatin and telmisartan compared to mono therapy have better renoprotective effects in diabetic group. The combination also attenuated the progression of diabetic nephropathy by slowing the proteinuria and microalbuminuria and these effects were confirmed by histopathological analyses.

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