ABSTRACT

There are no shortcuts to success!!!

- Beverley Sills

1.0

Hypertension is approximately twice as frequent in patients with diabetes as compared with patients without the disease. In the UK Prospective Diabetes Study (UKPDS) epidemiological study, each 10-mmHg reduction in mean systolic blood pressure was found to be associated with reductions in risk of 12% for any complication related to diabetes, 11% for myocardial infarction and 13% for microvascular complications. The choice of antihypertensive therapy in the diabetic population needs to be considered in the context of not only reducing the blood pressure, microvascular and macrovascular complications but also providing cardioprotection and prevention of interstitial fibrosis.

The renin-angiotensin aldosterone system (RAAS) plays an important role in the development and progression of devastating disorders in patients with hypertension. Mineralocorticoid receptors in cardiac muscle lead to increased cardiac collagen synthesis and the action of aldosterone on these receptors may account for altered cardiac structure and function in hypertension and cardiac failure. Aldosterone is directly involved in cardiac fibrosis. The Randomized Aldactone Evaluation Study (RALES), involving 1663 patients with moderately severe or severe congestive heart failure and receiving a low dose of spironolactone (25mg/day) in addition to standard therapy, showed that spironolactone substantially reduces the risk of both morbidity and death among patients with severe heart failure.

Apart from aldosterone, Angiotensin II (Ang II) is another important component of the RAAS that is known to stimulate fibrous tissue. The cardiovascular effects of angiotensin are mainly mediated by AT_1 receptor

Further, chronic ACE inhibitor treatment determines an increase in plasma renin activity due to a lack of negative feedback. Telmisartan is a specific AT_1 receptor blocker. Various clinical trials have demonstrated that telmisartan induces left ventricular hypertrophy regression and vascular remodelling and it is a partial agonist of peroxisome proliferator-activated receptor-γ (PPAR-γ).

Pharmacologic suppression of angiotensin II either does not reduce plasma aldosterone levels, or reduce for a very short time period. Hence, simultaneous suppression of Ang II and aldosterone may be more beneficial. Candesartan treatment improves the early stage of LV remodeling in patients with essential hypertension and the addition of spironolactone during candesartan treatment is clinically efficacious to reduce the LV hypertrophy which has already developed.

Despite many reports of beneficial role of spironolactone and telmisartan on cardiovascular diseases, direct studies on their effects on diabetes induced metabolic and cardiovascular complications are scanty. In light of this, the objective of the present investigation was to study the effect of spironolactone, telmisartan and their combination on cardiovascular complications associated with streptozotocin induced type-1 and type-2 diabetes mellitus in rats and to assess the effect of spironolactone, telmisartan and its combination on isoproterenol induced cardiac hypertrophy in rats.

Female healthy wistar rats weighing 180-220 gm were made type 1 diabetic by i.v. injection of streptozotocine (STZ) ($45mgkg^{-1}$). For induction of type 2 diabetes, two-day-old Sprague dawley neonates of either sex were injected intraperitoneally (i.p.) with 90 mg/kg STZ. For isoproterenol induced cardiac hypertrophy model, isoproterenol was administered intra-peritoneally in the dose of 5mg/kg once daily for 10 days to healthy wistar rats of either sex Spironolactone and telmisartan were dissolved in distilled water and were administered orally (p.o.) at a dose of 20mg/kg/day and 5mg/kg/day respectively for 8 weeks, with food and water *ad libitum*.

Injection of type 1 and type 2 diabetes in rats resulted into cardinal signs of diabetes i.e. loss of body weight, polyphagia and polydipsia. Chronic treatment with spironolactone or telmisartan did not prevent loss of body weight in diabetic rats. However, treatment with the combination of spironolactone with telmisartan significantly prevented the loss of body weight in type 1 and type 2 STZ-diabetic rats.

Type 1 STZ-diabetic rats were found to exhibit significant hyperglycemia and hypoinsulinemia as compared to control rats. Chronic treatment with spironolactone did not produce significant decrease in the elevated serum glucose levels. Chronic treatment with telmisartan or its combination with spironolactone produced a significant decrease in elevated serum glucose levels. Chronic treatment with spironolactone, telmisartan or their combination produced a significant increase in decreased serum insulin levels. Neonatal type 2 STZ-diabetic rats were found to exhibit significant hyperglycemia and hyperinsulinemia as compared to control rats. Chronic treatment with spironolactone and combination of spironolactone with telmisartan did not produce significant decrease in the elevated serum glucose levels and serum insulin levels. However, chronic treatment with telmisartan produced a significant decrease in elevated serum glucose and serum insulin levels. The improvement in glycaemic control in type 1 and type 2 diabetes by telmisartan indicate that it may prevent various micro and macrovascular complications of hyperglycemia.

Type 1 and type 2 diabetic rats as well as the hypertrophic rats exhibited significantly higher cholesterol, LDL-cholesterol, VLDL–cholesterol and triglycerides levels as compared to those of control rats. Chronic treatment with spironolactone, telmisartan, or their combination significantly reduced the elevated cholesterol, LDL-cholesterol, VLDL –cholesterol and elevated triglycerides levels in diabetic as well as in the hypertrophic rats. There was a significantly lowered HDL-cholesterol levels in diabetic and hypertrophic rats as compared to control rats and chronic treatment with spironolactone, telmisartan

or their combination significantly elevated the lowered HDL-cholesterol levels in diabetic and hypertrophic animals. Lowering of cholesterol and triglyceride levels with increase in the HDL-cholesterol by spironolactone, telmisartan or their combination may produce beneficial effects on STZ-induced cardiovascular complications.

Type 1 and type 2 diabetic rats exhibited significantly higher serum creatinine levels as compared to those of control rats. Chronic treatment with spironolactone, telmisartan or their combination significantly reduced the elevated creatinine levels of diabetic rats.

Increased serum CK and LDH levels in diabetic rats indicate cardiac muscular damage. In the present study, type 1, type 2 diabetic rats and hypertrophic rats exhibited significantly higher serum lactate dehydrogenase (LDH) and creatinine kinase levels (CK) levels as compared to those of control rats. Chronic treatment with spironolactone did not produce a significant reduction of LDH and CK levels in the type 1 diabetic animals. In type 2 diabetic rats as well as in hypertrophic rats, spironolactone produced a significant reduction of CK levels but did not alter significantly the LDH levels. Chronic treatment with telmisartan or its combination with spironolactone significantly reduced the elevated serum LDH and CK levels of type 1 and type 2 diabetic rats as well as that of hypertrophic rats. The decrease in LDH and CK levels further substantiates the beneficial effects of these drugs in reducing the cardiovascular risk in diabetes mellitus.

Serum CRP levels were found to be significantly increased in type 1, type 2 diabetic rats and hypertrophic rats as compared to control rats. Chronic treatment with spironolactone, telmisartan or their combination significantly reduced the elevated C-reactive protein levels of diabetic and hypertrophic rats.

At the end of the studies, the mean blood pressure was significantly increased in type 1, type 2 diabetic and hypertrophic rats as compared to those of control rats. Chronic treatment with spironolactone, telmisartan or their

combination significantly prevented the increase in blood pressure in all these animals.

The heart rate was significantly decreased in STZ-diabetic rats as compared to those of control rats at the end of type 1 diabetes study. Chronic treatment with spironolactone, telmisartan or their combination significantly exhibited increase in the heart rate in diabetic animals. In type 2 diabetic and iso-hypertrophic rats, at the end of the study, the heart rate was significantly increased as compared to those of control rats. Chronic treatment with spironolactone, telmisartan or their combination exhibited a significant decrease in the heart rate in type 2 diabetic and hypertrophic animals. Type 1, type 2 diabetic and hypertrophic rats also exhibited significantly decreased rate of pressure development and decay as compared to those of control rats. Chronic treatment with spironolactone, telmisartan, or their combination significantly elevated decreased rate of pressure development and decay of diabetic and hypertrophic rats.

Left ventricular hypertophy does not usually occur in diabetes unless hypertension coexists and diabetic hypertensive patients have greater interventricular septum and posterior wall thickness than non-diabetic hypertensive patients. In present study, type 1, type 2 diabetic and hypertrophic rats, the cardiac hypertrophy index and left ventricular hypertrophy index were significantly higher. Chronic treatment with spironolactone, telmisartan or their combination significantly reduced the elevated cardiac hypertrophy and left ventricular hypertrophy index of diabetic and hypertrophic rats. Type 1, type 2 diabetic and hypertrophic rats exhibited significantly higher left ventricular collagen levels as compared to those of control rats. Chronic treatment with spironolactone, telmisartan or their combination significantly reduced the elevated left ventricular collagen levels of diabetic and hypertrophic rats. All these results further support the contention that spironolactone, telmisartan or their combination is beneficial in STZ-induced cardiovascular dysfunction.

Oxidative stress is not only associated with complications of diabetes, but has been linked to insulin resistance *in vitro* and *in vivo*. The plasma of Type 2 diabetic patients is more susceptible to lipid peroxidation than in non-diabetic subjects Hence, in present study, anti-oxidant parameters were studied in type 2 diabetes and hypertrophic models. Type 2 diabetic and hypertrophic rats exhibited significantly decreased superoxide dismutase (SOD), catalase and glutathione (GSH) levels and significantly increased malondialdehyde (MDA) levels in left ventricle as compared to those of control rats. Chronic treatment with spironolactone, telmisartan and their combination significantly increased left ventricular SOD, catalase and glutathione levels and decreased MDA of diabetic and hypertrophic rats.

In conclusion, our data suggests that spironolactone, telmisartan and their combination prevents not only the STZ induced metabolic abnormalities but also cardiovascular complications as evident from the reduction in cholesterol, triglyceride, LDH, CK, CRP, collagens levels, decrease in cardiac hypertrophy and left ventricular hypertrophy which are the initial symptoms of congestive heart failure. Although, the combination of spironolactone plus telmisartan did not show beneficial effects as compared the monotherapy, the combination therapy may be beneficial for long term when the angiotensin receptor blocker monotherapy leads to aldosterone breakthrough. Further long term studies are required to confirm compare the beneficial effect of the monotherapy versus combination therapy.