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EFFECTS OF CHRONIC RAMIPRIL TREATMENT IN STREPTOZOTOCIN–INDUCED DIABETIC RATS

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Abstract : The present investigation was undertaken to study the effects of chronic oral ramipril (1 mg/kg) treatment in streptozotocin (STZ) induced diabetic rats. Single tail vein injection of STZ (45 mg/kg, i.v.) produced a diabetic state exhibiting all the cardinal symptoms such as loss of body weight, polydipsia, polyuria, glucosuria, polyphagia, hypoinsulinaemia and hyperglycaemia. The diabetic state was also found to be associated with bradycardia, hypothyroidism, cardiac depression and cardiomyopathy. Ramipril treatment prevented STZ-induced hypertension, bradycardia, hypothyroidism, hyperchosesterolaemia and partially the cardiomayopathy. Ramipril treatment could not, however prevent STZ-induced loss of body weight, polyuria, polydipsia, polyphagia, hyperglycaemia, hypoinsulinaemia, hypertriglyceridaemia and cardiac depression. Our data suggests that ramipril has a few beneficial effects in the STZ-treated diabetic rats.

Key words : diabetes mellitus ramipril streptozotocin

INTRODUCTION

Hypertension is reported to be more prevalent among diabetics than non-diabetic subjects, and it is reported to aggravate the cardiovascular complications of diabetes (1, 2). Both hypertension and diabetes mellitus are multifaceted and dynamic expressions of pathological disequilibria which are closely related and even intermingled by common factors such as obesity, hyperinsulinaemia, micro-and macrovasulcar disease and cardiac risk factors (3).

Our laboratory is engaged in the study of various antihypertensives on STZinduced diabetic rats. It has been reported

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that cardioselective beta-blocker atenolol, does not improve cardiac dysunfction, cardiomyopathy and other complications (4), whereas, prazosin improves cardiac dysfunction but not cardiomyopathy and hyperlipidaemia (4). Chronic nifedipine treatment prevents STZ-induced cardiomyopathy, hypertension, hyperlipidaemia and hypothyroidism (5). Further, Lakkad et al (6) reported that angiotensin converting enzyme inhibitor (ACEI) enalapril prevents STZ-induced cardiomyopathy, cardiac dysfunction and hypercholesterolaemia in rats. Studies with ramipril suggest that it produces a reversal of left ventricular hypertrophy in hypertensive patients without any

deterioration of pump function (7). Animal studies have shown that ramipril improves myocardial metabolism, reduces myocardial necrosis and reperfusion-induced arryhthmias in experimental myocardialischaemia reperfusion studies (8). ACE inhibitors are being used more and more for the treatment of hypertension as they do not interfere with glucose tolerance in diabetic state (9) and blood lipid profile in hypertensives with or without diabetes (10, 11). They are also reported to reduce cardiac involvement in hypertension to induce remodelling of hypertrophied heart (12) and to improve work capacity and life expectancy in patients with severe heart failure (8). This could be particularly useful for long term hypertensive diabetics who have a higher incidence and greater morbidity and mortality from cardiac diseases. Since previous studies with ACEI enalapril were encouraging, the present investigation was undertaken to study the effects of chronic treatment with ramipril in STZ-induced diabetes in rats.

METHODS

Induction of diabetes and drug treatment: Female albino rats of Wistar strain, weighing 200-230 g, were made diabetic by STZ (45 mg/kg, i.v.) dissolved in citrate buffer (pH 4.5). Control rats were injected with citrate buffer. Urine glucose levels were measured using Glucostix 48 hours after the injection and animals showing glucosuria (>2%) were considered as diabetic. Both control and diabetic animals were randomly divided into subgroups--untreated and treated. Ramipril (1 mg/kg) was given daily by oral route for six weeks. During the six weeks treatment period, the animals were maintained on standard rat chow and water *ad libitum* and they were observed carefully for changes in water intake, food intake, body weight, general behavior and mortality.

Blood sample collection and analysis: At the end of six weeks blood samples were collected from the retro-orbital plexus of rats. The serum was separated by centrifugation of the whole blood at 3000 rpm for 15 min. Serum glucose, cholesterol, triglyceride, HDL cholesterol levels were measured spectrophotometrically by enzymatic method using their respective kits. Serum immunoreactive insulin was assaved by the radioimmunoassay method using the kit from Monobind Costa Mesa, USA. Serum T3 and T4 were estimated by Solid Phase Competitive Binding Enzyme Immunoassay method using the kit from Miles India Ltd.

Measurement of blood pressure, heart rate, and cardiac functions: Blood pressure and heart rate of all the experimental animals were recorded before and at the end of the six week treatment using the Harvard BP Monitor. The LVDP was measured using Neely's working heart mode. The animal was stunned and the heart was dissected out and mounted. The heart was perfused with Chenoweth-Koelle solution (pH 7.4) and aerated with carbogen at temperature 37°C. The isolated heart was allowed to stabilize for 10 minutes at the perfusion pressure of 10 cm H_oO. The LVDP was then recorded at different atrial filling pressures (5 cm H₂O to 25 cm H₂O). The atrial pressure was changed 2.5 cm H₂O each time in a stepwise manner by changing the height of the constant level reservoir.

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Histological study of organ tissue: At the end of the treatment period the heart of each animal was dissected out and fixed in Bouin's fixative. After dehydration of the tissue, paraffin blocks were made and microtomy carried out. Perefectly stained sections were observed under a light microscope.

Statistical analysis: Statistical analysis was performed using Analysis of Variance follwed by Tukey's Test (13). Alpha level of 5% was taken as the level of statistical significance.

RESULTS

General features: Injection of STZ to rats resulted in a diabetic state with an elevated Ramipril in Diabetes-Mellitus 355

urine glucose level (>2%) throughout the study period. No detectable glucose was present in the urine of control animals. Diabetic rats showed a significant reduction in body weight, polydipsia, polyphagia (Table I), hypertension and bradycardia (Table II). Ramipril did not alter any of these parameters except the blood pressure, which was lowered significantly in diabetic treated rats. Diabetic rats exhibited reduced heart weight. However, the ratio of heart weight to body weight was not significantly different in all the groups (Table II).

Cardiac functions: The blood pressure of diabetic rats (148.33 ± 3.46) was significantly higher than that of the control animals (120.5 ± 5.85) . Ramipril lowered the blood pressure (115 ± 2.35) in the diabetic

TABLE I : Changes in the general conditions and mortality with ramipril treatment.

	Control (n=7)	Control treated with Ramipril (n=7)	Diabetic (n=5)	Diabetic treated with Ramipril (n=6)
Body weight (g)	220 ± 3.06	218.75 ± 3.75	$156.66 \pm 4.51^*$	160 ± 3.33*
Water intake (ml/day/rat)	35 ± 2	43 ± 2	$57 \pm 4^{*}$	$68 \pm 2^*$
Food intake (g/day/rat)	20 ± 2	20 ± 1	$35 \pm 9^*$	35 ± 1*
Mortality	0.00%	0.00%	13%	13%

*Significantly different from control (P<0.05)

TABLE II : Influence of ramipril treatment on blood pressure and heart rate.

	Control (n=7)	Control treated with Ramipril (n=7)	Diabetic (n=5)	Diabetic treated with Ramipril (n=6)
Blood pressure (mm Hg)	120.5 ± 5.85	118.12 ± 2.78	$148.33 \pm 3.46*$	115 ± 2.35**
Heart rate (beats/min)	334.5 ± 5.16	348 ± 3.8	311.66 ± 3.66*	$340 \pm 6.1^{**}$
Wet heart Wt./body wt. (mg/g)	4.08 ± 0.1	4.67 ± 0.46	$3.65 \pm 0.5^*$	3.8 ± 0.2*

*Significantly different from control (P<0.05)

**Significantly different from diabetic control (P<0.05)

group (Table II). The heart rate of the diabetic rats (311.66 ± 3.6) was significantly lower when compared to that of the control rats (334.5 ± 5.16) . Ramipril prevented STZ-induced bradycardia (Table II).



Fig. 1: Effects of streptozotocin diabetes and ramipril treatment on Left Ventricular Developed Pressure on rat heart. (O) indicates control; (□)depicts control ramipril treated; (●) represents diabetic control; (■) indicates diabetic treated with ramipril. Each point and the bar represents mean ± SEM of 5-7 experiments. Indian J Physiol Pharmacol 1997; 41(4)

Increase in left atrial filling pressure produced a gradual increase in LVDP and this was significantly lower in STZ-treated rats (Fig.1). Ramipril treatment of the diabetic rats did not alter the LVDP at any of the filling pressures (Fig. 1).

Biochemical parameters: Hyperglycemia and hypoinsulinaemia were observed in diabetic rats (Table III). These remained unaltered after ramipril treatment. Serum cholesterol and triglyceride levels were found to be significantly high in diabetic rats (Table III). In addition, a significant decrease in T_3 levels was also seen in diabetic rats. The six-weeks ramipril treatment did not alter STZ-induced hypertriglyceridaemia. However, it produced a significant decrease in the serum cholesterol, LDL cholesterol and levels and also increased the serum T_3 levels thereby improving hypothyroidism.

Effects on myocardium: The single tail vein injection of STZ caused distortion of myocaridial fibres. The nuclei were clustered, intercalations disrupted and

TABLE III : Effect of ramipril treatment on various biochemical parameters.

	Control (n=7)	Control treated with Ramipril (n=7)	Diabetic (n=5)	Diabetic treated with Ramipril (n=6)
Glucose (mg/dl)	84.12 ± 2.70	84.79 ± 7.67	520.88 ± 3.92*	528.20 ± 8.56*
Insulin (uU/ml)	41.2 ± 6.9	$22.12 \pm 0.26^*$	$15.38 \pm 1.77^*$	$15 \pm 4.36^{*}$
T ₃ (ng/ml)	1.58 ± 0.19	$1.92 \pm 0.38^*$	$0.64 \pm 0.07^*$	$1.2 \pm 0.01^{**}$
Cholesterol (mg/dl)	83.67 ± 5.01	$49.05 \pm 4.54^*$	$129.86 \pm 5.71^*$	$64.40 \pm 2.2^{**}$
Triglycerides (mg/dl)	78.72 ± 1.31	79.55 ± 1.72	$153.21 \pm 3^*$	155.19 ± 2.68*
LDL (mg/dl)	37.88 ± 1.30	3.96 ± 1.21	70.39 ± 2.95*	48.8 ± 1.35**

*Significantly different from control (P<0.05)

**Significantly different from diabetic control (P<0.05)

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vacuoles were formed (Fig. 2B). The six week ramipril treatment partially prevented these disruptions (Fig. 2C).



Fig. 2: Representative histological view of rat myocardium from control (A), diabetic (B) and diabetic treated with ramipril (C).

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DISCUSSION

Junod et al (14) reported that diabetogenic effects of STZ are dose dependent, ranging from a mild diabetes following a dose of 35 mg/kg to a severe ketotic state, leading to death within 2-3 days after a dose of 100 mg/kg. When treated with intermediate doses of STZ (55 and 65 mg/kg), rats fail to gain weight and develop blood glucose levels 3-4 times higher than normal, survive without insulin supplementation do not develop ketosis. In the present investigation a single tail vein injection of 45 mg/kg STZ was used to induce diabetes. At this dose, rats showed characteristic symptoms of diabetes mellitus like polydipsia, polyuria, polyphagia, weight loss, and glucosuria. Hofteizer and Carpenter (15) suggested that the loss of body weight could be due to dehydration and the catabolism of fats and proteins seen during diabetes mellitus. Treatment with ramipril could not prevent the weight loss in diabetic animals.

Diabetic rats exhibited glucosuria (>2%) 48 hr after the injection of STZ (45 mg/kg) and this persisted throughout the treatment. At the end of six weeks serum glucose levels of the diabetic treated rats were significantly higher than those of the control animals (Table III). A corresponding decrease was observed in the serum insulin levels of the diabetic animals as compared to the control untreated animals. It was interesting to note a decrease in the serum insulin levels in the control treated animals (Table III), the mechanism for which remains obscure. Lakkad et al (6) also reported similar finding with enalapril treatment. Although the serum insulin

levels (22.12 ± 0.26) in the control treated group were lowered, the serum glucose levels (84.79 ± 7.67) were not significantly higher than those of the control untreated group (84.12 ± 2.70) . This suggests an increase in insulin sensitivity in the control treated group.

There are several reports on genesis of hypertension in STZ-induced diabetes mellitus rat model. Kawashima et al (16) were the first to report that rats treated with STZ developed hypertension. Since then many other workers have observed raised blood pressure in STZ treated rats (17,18). In the present study we observed similar changes in blood pressure. Ramipril treatment prevented the rise in blood pressure in the STZ-treated animals (Table II).

Jackson and Carrier (19) reported the occurrence of bradycardia when diabetes is induced by STZ in normotensive rats. Savarese and Berkowitz (20) suggested that the decrease in heart rate could be due to a decrease in beta receptor binding sites in the diabetic heart. According to Garber et al (21) another possible mechanism for bradycardia could be the hypothyroid state of the animal. Ciaraldi and Marinetti (22) reported that hypothyroidism is known to cause a reduction in the density of both alpha and beta receptors in the cardiac tissue. Additional evidence for the role of hypothyroidism in producing bradycardia was provided by the observation that T₃ treatment of STZ-induced diabetic rats prevented bradycardia (23). In the present study the heart rate of diabetic rats were significantly less than that of the control rats. Ramipril treatment could prevent STZinduced bradycardia (Table II). Ramipril treatment increased the T_a levels (Table III)

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as well in the diabetic treated group, thus hypothesis that supporting the hypothyroidism could be one of the causes of diabetes induced bradycardia in STZ treated rats. Although ramipril treatment prevented STZ-induced hypothyroidism, the cardiac function was found to be depressed at higher filling pressures (Fig. 1). Histological study of the myocardium revealed that STZ-induced diabetes mellitus causes degeneration of fibres, loss of fibre intactness and vacuolization. Although ramipril treatment prevented this derangement the cardiac depression at higher filling pressures remained unaltered. This suggests involvement of other factors in the pathogenesis of cardiomyopathy in the present study.

Cardiac depression in the diabetic state could be a result of microangiopathic changes, altered cardiac autonomic functions (24), increased stiffness of ventricular wall (25) and changes in subcellular organelles such as sarcoplasmic reticulum (26), membrane pumps and enzymes in the sarcolemma (27, 28). Hyperlipidaemia may also lead to cardiac depression. It has been reported that diabetic rats are Wistar-Kyoto hyperglycaemic and hypoinsulinaemic but they do not develop hyperlipidaemia or cardiac depression (29). In the present investigation STZ-treatment produced an increase in serum triglyceride level (Table III). Ramipril treatment did not alter the increase in these levels. This suggests that the cardiac depression observed in the present investigation may be a result of the high serum triglyceride levels.

In the present investigation we found an increase in the serum cholesterol levels Indian J Physiol Pharmacol 1997; 41(4)

in the STZ-treated group. This increase in the cholesterol levels was prevented by ramipril (Table III). Increase in serum lipids indicates either the defective removal or over-production of one or more lipoproteins. Insulin plays a role in both production and removal of triglyceride rich proteins which could be the cause of lipid disorders of diabetes. Probably there is defective removal of triglyceride rich lipoproteins in the insulin deficient state (30-33). Insulin administration restores normal conditions. In the present study we observed an increase in the serum cholesterol levels with simultaneous decrease in insulin levels in the STZ-treated group. Ramipril treatment reduced the cholesterol level with further decrease in insulin levels in the control and diabetic animals. This again indicates an increase in the insulin receptor sensitivity.

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In conclusion, chronic ramipril treatment prevented STZ-induced rise in blood pressure, bradycardia, hypercholesterolaemia, and hypothyroidism. However, loss of body weight, rise in serum triglyceride, and glucose levels remained unaffected with ramipril treatment. Our data suggests that ramipril has a few beneficial effects in STZ-induced diabetes and that it may be a preferred antihypertensive in diabetic hypertensive patients.

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