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EFFECT OF CHRONIC TREATMENT WITH ATENOLOL AND PRAZOSIN IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract : Diabetes-mellitus was induced in rats by single intravenous injection of (45 mg/kg) streptozotocin (STZ). STZ diabetic rats showed hypertension, decreased cardiac functions, cardiomyopathy and hypercholesterolemia observed at the end of six weeks. Chronic treatment with atenolol (10 mg/kg) for six weeks in the diabetic rats reduced the elevated blood pressure, but failed to prevent STZ induced other complications. Chronic treatment with prazosin (1 mg/kg, po) in the diabetic rats, reduced the elevated blood pressure and also partially prevented hypercholesterolemia, cardiac dysfunctions and in particular the cardiomyopathy. The results suggest that prazosin may be a better option as compared to atenolol in hypertension when it is associated with diabetes mellitus.

Key words : atenolol cardiomyopathy diabetes mellitus prazosin streptozotocin

INTRODUCTION

Diabetes and hypertension when co-exist are major risk factors in the development of retinopathy, nephropathy and specific cardiomyopathy leading to myocardial infraction (1). Diabetes may be associated with hyperlipidaemia which has a deteriorating influence of high free fatty acid concentration on myocardial membrane and function (2). Further, controlling blood pressure in diabetics is positively beneficial as far as the progression of nephropathy is concerned.

Different beta-blockers may show different metabolic effects in diabetic hypertensive subjects. Non-selective beta-blockers have been shown to affect serum lipid levels adversely (3). It is postulated that selective beta-blockers such as atenolol are likely to affect adversely the serum lipid levels to a lesser extent then non-selective one such as propranolol.

Hydralazine does not affect the glucose tolerance in man and it has been shown to prevent diabetes induced cardiac dysfunction and hyperlipidaemia in rats (4). Prazosin with alpha-adrenoceptor blocking activity has been shown to increase plasma glucose levels in patients with chemical diabetes (5). In contrast chronic therapy of prazosin (3 mg/day) for one week significantly improved the glucose tolerance with slight increase in insulin levels in another group of diabetic patients (6). Effect of prazosin on lipid levels is also controversial. In the present investigation we have studied the effects of chronic treatment with atenolol and prazosin on streptozotocin induced diabetic rats.

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METHODS

I. Induction of diabetes and drug treatment: Healthy female albino rats of Wistar strain, weighing 160-200 g, were rendered diabetic by i.v. injection of streptozotocin (STZ) (45 mg/kg) dissolved in citrate buffer (pH = 4.5). Control rats were injected with citrate buffer alone. Urine glucose levels were measured 48 hours after the injection, using enzymatic test strips (Miles India Ltd.) and animals showing glucosuria (>2%) were selected as diabetic rats. Both control and diabetic animals were randomly divided into two subgroups:- Untreated or treated. Atenolol (10 mg/kg) or prazosin (1 mg/kg) was given daily by oral route for six weeks. The animals were maintained during this period with food and water given *ad libitum*. Parameters such as water intake, food intake, changes in body weight and mortality were observed.

II. Blood sample collection and analysis : At the end six weeks the rats were fasted for 12 hr. Blood samples were collected from the retro-orbital plexus and serum was separated. Serum glucose levels were determined by glucose oxidase peroxidase method using the GOD/POD kit from Span Diagnostics, India. Serum immunoreactive insulin was assayed by the radio immunoassay method using the kit from Monobind Costa Mesa, U.S.A. Serum T_3 and T_4 were estimated by Solid Phase Competitive Binding Enzyme immunoassay method using the kit from Miles India Ltd. Serum cholesterol, total lipids and triglycerides were measured by enzymatic method using their respecitve kits.

III. Measurement of blood pressure, heart rate and cardiac functions : After the completion of six weeks treatment, animals were anesthetized with pentobarbitone sodium and carotid artery was cannulated to record blood pressure and heart rate using pressure transducer connected to polygraph. Later, the animals were sacrificed, hearts were excised quickly and mounted as per the modified Neely's Working heart technique. Hearts were perfused with Chenoweth Koelle solution (pH 7.4) which was constantly bubbled with carbogen and maintained at 37°C. The millimolar concentrations of the solutes in the buffer were as follows : NaCl, 120.0; KCl, 5.6; $CaCl_2$, 2.18; $MgCl_2$, 2.1; $NaHCO_3$, 19.2; and glucose, 10.0. The aortic outflow was connected to a compliance chamber containing 2 to 3 ml of air, which in turn was connected to a 70 cm polythene tubing to have an after load to the heart. Hearts were allowed to stabilize for 10 min at the perfusion pressure of 10 cmH_2O. The left ventricular developed pressure (LVDP), rate of contraction (+ve dp/dt) and rate of relaxation (-ve dp/dt) were then recorded at different atrial filling pressure (10 cm H_2O to 25 cm H_2O). The atrial pressure was changed by 2.5 cm H_2O each time in a stepwise manner by changing the height of constant level reservoirs.

IV. Histological study of myocardium: Left ventricle from heart were isolated, fixed in Bouin's fixative, sections were cut and stained with Haemotoxylin and Eosin. The slides were examined by light microscope.

V. Statistical analysis: All values are expressed as mean ± SEM. Statistical analysis was performed using one way Analysis of Variance followed by Tuckey's multivarient test.

RESULTS

Injection of streptozotocin in rats resulted in a diabetic state with presence of glucose in urine. Diabetic rats showed a significant reduction in body weight, heart rate, polydipsia, and increase in blood pressure (Table I). Atenolol treatment did not alter the responses to any of these parameters except blood pressure which showed a significant reduction in blood pressure as compared to diabetic untreated (control) rats. Hyperglycemia and hypoinsulinaemia produced by STZ injection in rats were unaltered after atenolol treatment. Serum total lipids, total cholesterol, and triglyceride levels were found to be significantly increased in diabetic rats. In addition, a significant decrease in T_3 and T_4 levels was also seen in STZ-diabetic rats. Atenolol treatment for six weeks did not alter

TABLE I : General characteristics of STZ-Diabetic rats and effect of Atenolol.

Parameter	*Control*	*Control treated with Atenolol*	*Diabetic control*	*Diabetic treated with Atenolol*
Body weight (g) (after 6 weeks)	210.00 ± 3.54	190.54 ± 5.59*	127.50 ± 4.33*	102.00 ± 9.69*
Wet heart weight/body weight (mg/gm)	4.2 ± 0.3	3.94 ± 0.19	7.9 ± 0.8*	5.96 ± 0.50*
Water intake (ml/day)	33.0 ± 2.10	30.00 ± 5.32	104.60 ± 4.08*	105.00 ± 2.43*
Mean blood pressure (mmHg)	125.00 ± 1.86	119.16 ± 3.27	165.20 ± 4.36*	107.50 ± 4.78*
Heart rate (beats/min)	353.00 ± 7.14	346.67 ± 6.67	243.00 ± 14.75*	212.00 ± 11.43*
Serum glucose (mg/dl)	82.20 ± 5.04	93.10 ± 2.67	359.40 ± 20.92*	364.75 ± 20.56*
Serum insulin (μU/ml)	13.4 ± 1.8	15.0 ± 3.77	6.88 ± 0.43*	7.62 ± 0.24*
Serum total lipids (mg/dl)	350.08 ± 8.60	371.00 ± 19.91	483.75 ± 27.07*	491.20 ± 18.08
Serum cholesterol (mg/dl)	65.74 ± 1.60	67.9 ± 4.10	114.25 ± 3.62*	118.03 ± 9.20*
Serum triglyce rides (mg/dl)	332.33 ± 20.63	309.66 ± 16.34	488.80 ± 31.20*	409.00 ± 20.23*
T_3 (ng/ml)	2.1 ± 0.03	1.93 ± 0.08	1.87 ± 0.06	2.0 ± 0.07*
T_4 (μg%)	4.33 ± 0.15	3.60 ± 10.05	4.38 ± 0.53	3.08 ± 0.53

*Significantly different from control (P<0.05); (n = 5-8).

the diabetic induced hyperlipidaemia and hypothyroidism.

Like atenolol, prazosin treatment did not prevent streptozotocin induced loss of body-weight, polydipsia or bradycardia (Table II). However, blood-pressure was found to be comparable with non-diabetics. Diabetes induced hyperglycemia, hyperlipidaemia and hypoinsulinaemia were also not altered significantly (Table II). There was some increase in triglyceride levels but it was not significant.

Increase in left atrial filling pressure caused graded increase in LVDP (Fig.1), +ve dp/dt

TABLE II : Effect of Prazosin on control and STZ-diabetic rats.

Parameter	*Control*	*Diabetics*	
		Untreated	*Prazosin treated*
Body weight (after 6 weeks)	205.83 ± 3.75	126.00 ± 9.41*	133.00 ± 9.95*
Wet heart weight/body weight (mg/g)	4.31 ± 0.33	7.68 ± 0.79*	3.57 ± 0.08**
Water intake (ml/day)	31.00 ± 2.00	105.00 ± 5.00*	111.00 ± 4.00*
Mean blood pressure (mm Hg)	124.00 ± 3.60	165.20 ± 4.36*	115.00 ± 7.36**
Heart rate (beats/min)	356.00 ± 3.67	243.00 ± 14.76*	260.00 ± 24.49*
Serum glucose (mg/dL)	79.50 ± 4.92	359.40 ± 20.92*	375.20 ± 15.30*
Serum insulin (μU/ml)	13.35 ± 3.16	6.88 ± 0.43*	10.50 ± 3.17
Serum total lipids (mg/dL)	318.40 ± 12.42	485.89 ± 40.26*	516.50 ± 26.39*
Serum triglycerides (mg/dL)	320.00 ± 21.36	458.80 ± 31.21*	533.60 ± 64.69**
Serum cholesterol (mg/dL)	76.61 ± 5.75	115.42 ± 2.76*	137.40 ± 10.93*
T_4 (μg/dL)	4.26 ± 0.23	3.55 ± 0.37	2.14 ± 0.54*

*Significantly different from control (P<0.05); (n = 5-7);
**Significantly different from diabetic controls (P<0.05).

(Fig.2) and -ve dp/dt (Fig.3). At higher filling pressures there was a decrease in LVDP, +ve dp/dt and -ve dp/dt in diabetic rats. Atenolol treatment failed to prevent this decrease in diabetic hearts. In fact in hearts obtained from atenolol treated diabetic rats showed a further significant decrease in these parameters. Prazosin treatment however, prevented to some extent depression in cardiac functions in diabetics rat heart. There was a significant increase in LVDP (Fig.1). +ve dp/dt (Fig.2) and -ve dp/dt (Fig.3) in hearts obtained from diabetic rats treated with prazosin as compared to diabetic controls. The results of working heart preparation can well be correlated with histological studies and cardiac hypertrophy index. Histological studies reveal that myocardium of diabetic heart show distorted fibres with clustered nuclei and

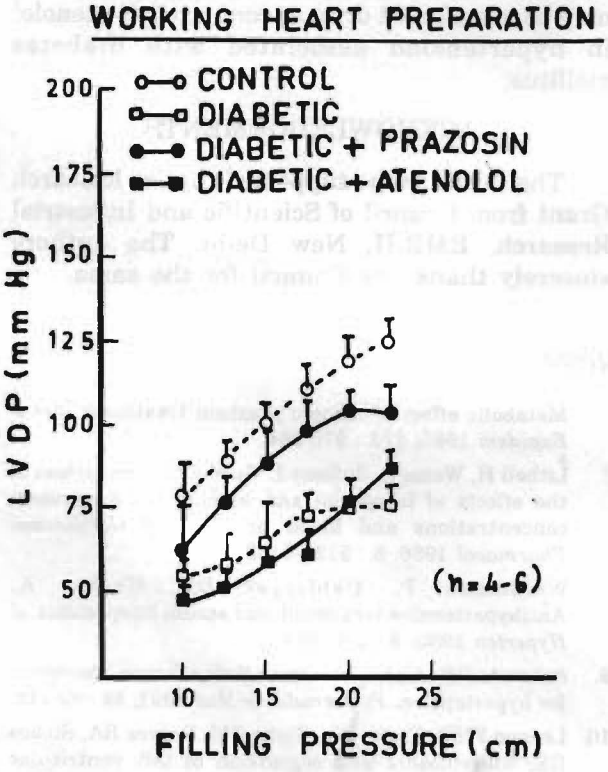

Fig. 1 : Effects of 6 weeks treatment of atenolol and prazosin on Left Ventricular Developed Pressure of control and steptozotocin induced diabetic rat heart.

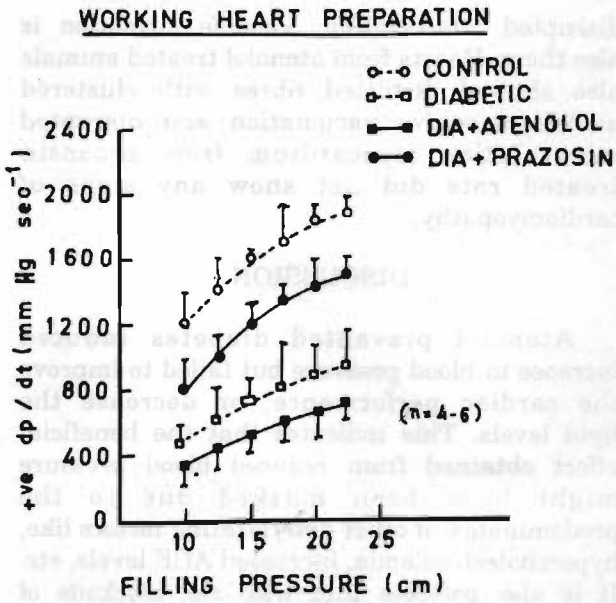

Fig. 2 : Effects of 6 weeks treatment of atenolol and prazosin on rate of rise (+ve dp/dt) of control and steptozotocin induced diabetic rat heart.

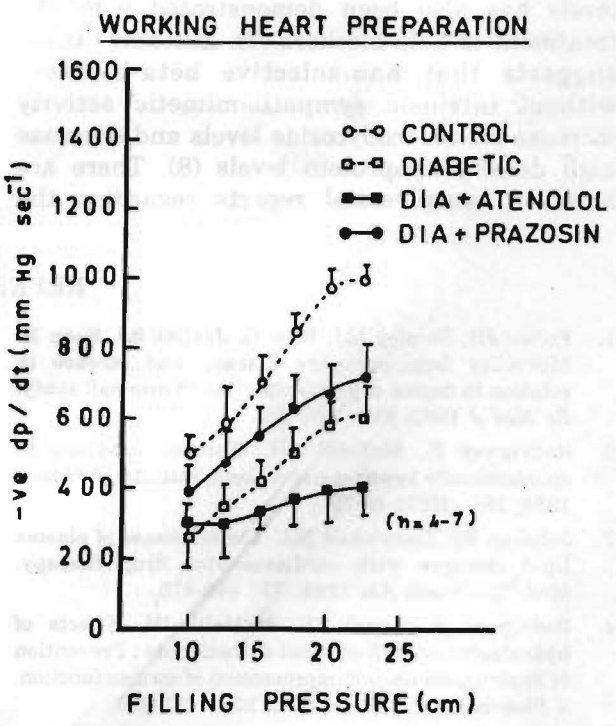

Fig. 3 : Effects of 6 weeks treatment of atenolol and prazosin on rate of fall (-ve dp/dt) of control and steptozotocin induced diabetic rat heart.

disrupted intercalation. Vacuole formation is also there. Hearts from atenolol treated animals also showed distorted fibres with clustered nuclei, extensive vacuolation and disrupted intercalation myocardium from prazosin treated rats did not show any signs of cardiomyopathy.

DISCUSSION

Atenolol prevented diabetes induced increase in blood pressure but failed to improve the cardiac performance, or decrease the lipid levels. This indicates that the beneficial effect obtained from reduced blood pressure might have been masked due to the predominance of other deteriorating factors like, hypercholesterolemia, increased ACE levels, etc. It is also possible that with the blockade of beta-adrenoceptors the unopposed alpha-activity might result in the increase in low-density lipoproteins. An increase in LDL-cholesterol levels has also been demonstrated with the treatment of beta-blockers (7). Extensive trials suggests that non-selective beta-blockers without intrinsic sympathomimetic activity increase serum triglyceride levels and decrease high density lipoprotein levels (8). There are however, controversial reports regarding the effects of selective beta-adrenoceptor blockers metoprolol and atenolol (9). In the present investigation cholesterol levels were found to remain high in diabetic animals even after 6 weeks of treatment with atenolol. This may be one of the important reasons of the ineffectiveness of atenolol in preventing cardiac dysfunctions. It has been previously demonstrated that prazosin treatment has prevented progression of cardiomyopathy and left ventricular hypertrophy (10). Further, prazosin has also been reported to reduce total cholesterol and triglyceride levels (11). In the presence study, prazosin treatment in diabetic rats controlled the elevated blood pressure with reduction in cholesterol levels. It also produced regression in diabetes induced left ventricular hypertrophy.

In conclusion our data suggest that prazosin may be a preferred drug as compared to atenolol in hypertension associated with diabetes mellitus.

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