



Modulation of Oxidative Stress, Serum Lipids and Renal Dysfunction by Benazepril in Diabetes Mellitus

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Abstract

Present study assessed the effect of benazepril on oxidative stress, serum lipids and renal dysfunction in alloxan induced diabetic rabbits. Benazepril reversed the increase in level of malondialdehyde and decrease in level of glutathione and superoxide dismutase activity caused by induction of diabetes. It also had a beneficial effect on diabetic dyslipidemia as manifested by elevation in serum HDL cholesterol. However, it had no effect on serum LDL, total cholesterol or triglycerides. Benazepril also attenuated the renal dysfunction induced by diabetes. It resulted in significant reduction in blood urea, serum creatinine and urine albumin excretion as compared to diabetic control rabbits. Further, kidney weight was significantly less in benazepril treated rabbits as compared to diabetic rabbits. To conclude, benazepril was found to be effective in preventing the oxidative stress and renal dysfunction as well as beneficial on serum lipids in experimentally-induced diabetes mellitus.

Key Words

Benazepril, Oxidative Stress, Renal Dysfunction, Serum Lipids, Diabetes

Introduction

A variety of risk factors for development of atherosclerosis including diabetes mellitus (DM), hypercholesterolemia, hypertension have been identified. Endothelial dysfunction is the central pathophysiologic denominator for all complications of DM including nephropathy. The generation of oxygen free radicals (OFR) is supposed to be involved in the damage to endothelium, which is responsible for the progression of atherosclerosis(1). Diabetic nephropathy (DN) occurs as a result of the damage to microvasculature of the kidney. Further diabetic nephropathy may result in hypertension, which is also an independent risk factor for CAD. There has been accumulating evidence that lipid peroxidation caused by free radical induced oxidation of lipids in low-density lipoproteins (LDL) may be an important event in pathogenesis of CAD (2, 3). Thus, agents that inhibit lipid peroxidation or improve enzymatic antioxidant defenses may limit atherosclerosis and its clinical manifestations like stroke and myocardial infarction (MI). Intrarenal renin-angiotensin system plays an important role in diabetic nephropathy by initiation and progression of albuminuria as well as podocyte abnormalities in type-2 DM (4) thereby affecting the onset, progression and outcome of atherosclerosis (5). It has

also been noted that angiotensin generates free radicals and inhibition of renin angiotensin system may decrease oxidative stress (6). The ACE inhibitors are known to affect intrarenal hemodynamics, BP & may prevent the onset of DN or slow the decline in renal function independent of their effect on BP (7, 8). ACE inhibitors also slow the progression of DN in people who have poorly controlled hyperglycemia. Benazepril is a second-generation non-sulfhydryl ACE inhibitor and it is used extensively in the treatment of hypertension. It is effective in improving renal function and slowing the progression of renal diseases of various origins (9), however its effect has not been studied in modulating oxidative stress and renal dysfunction in diabetes. Further, the role of ACE inhibitors in modulating serum lipids is not clearly known as contrary reports are available (10,11). Taking the aforementioned factors into account, this study is an attempt to assess the role of benazepril in modulating oxidative stress, serum lipids and renal dysfunction in experimentally induced DM.

Material and Methods

Male albino rabbits, weighing between 1.5 to 2.5 kg, were used. All the animals were housed, maintained & feed as per the guidelines for the Care and Use of Animals

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in Scientific Research. Permission was taken from Institutional Animal Ethics Committee. The rabbits were administered various drugs in the specified dose and a volume of 5 ml.

Preparation of Drugs Administered

Benazepril salt was received from Novartis India Ltd. It was suspended in distilled water (DW) and a dose of 4 mg/kg/day was administered per orally for 12 weeks. Diabetes mellitus was induced in the rabbits by intravenous injection of a single dose of 150 mg/kg alloxan hydrochloride (sigma chemical company, USA) in the marginal ear vein. One group (n=8 rabbits) received normal saline intravenously and this group served as normal control group. Rest of the animals were treated with alloxan and divided into two groups of eight rabbits each. One group received normal saline and served as diabetic control whereas the other group was treated with benazepril (4 mg/kg, p.o.) daily from day one through 12 weeks. At the end of the study, the rabbits were sacrificed using light ether anaesthesia and kidney was dissected out. After removing the fat, kidney weight was recorded. Body weight expressed in kilograms (kg) for all rabbits was recorded initially and at weekly intervals till the end of the study, i.e. 12 weeks. Blood samples were drawn from the marginal ear vein of overnight fasted rabbits at the end of study i.e. 12 weeks. Apart from it, at weekly intervals, a drop of blood was obtained from each animal for measurement of blood sugar by pricking the marginal ear vein. Blood sugar was estimated using glucose oxidase method with the help of glucometer and glucostrix. Estimation of blood urea was done by enzymatic kinetic method and serum creatinine levels were measured by picric acid method using standard kits obtained from Merck, India. Serum malondialdehyde (MDA) levels are a quantitative measure of serum lipid peroxides. The thiobarbituric acid (TBA) method (12). Erythrocyte superoxide dismutase (SOD) activity was determined by the pyrogallol auto-oxidation method (13,14). The level of reduced glutathione (GSH) in whole blood was estimated as protein-free sulfhydryl content using Ellman's reagent. Total cholesterol, HDL-cholesterol and LDL-cholesterol and serum triglyceride levels were estimated using standard kits obtained from Randox laboratories, UK. Urine was collected by using metabolic cages and measured for albumin excretion using ELISA kits (Biochrome, India) at the end of the study i.e. at 12 weeks.

Statistical Analysis

The data was analyzed statistically by carrying out repeated measure analysis of variance followed by post-hoc Tukey's test for inter-group comparisons. A value of $p < 0.05$ was considered significant for comparison.

Results

Body Weight and Blood Sugar: There was gradual increase in blood sugar and fall in body weight in diabetic rabbits. Benazepril failed to prevent the weight loss (Fig. 1) but partially prevented the hyperglycemia induced by diabetes (Fig. 2).

Oxidative Stress Parameters: Serum MDA levels were significantly elevated whereas red cell SOD activity and whole blood GSH levels were significantly reduced in diabetic control group ($p < 0.05$ as compared with normal control). Benazepril reversed these oxidative stress parameters, albeit partially ($p < 0.001$ as compared to both normal and diabetic control groups). (Table 1)

Serum Lipids: Diabetic rabbits demonstrated dyslipidemia manifested as a significant increase in total cholesterol, triglycerides and LDL-cholesterol whereas fall in HDL-cholesterol levels. Benazepril treatment elevated the HDL-cholesterol levels even above the normal rabbits ($p < 0.001$), whereas serum triglycerides ($p < 0.01$ as compared to diabetic control rabbits) and total cholesterol ($p < 0.001$ as compared to diabetic control rabbits) was reduced. At a dose of 4mg/kg/d, benazepril had no effect on LDL-cholesterol. (Table 2)

Parameters of Renal Function

Diabetic control group demonstrated microalbuminuria and elevated blood urea and serum creatinine levels at 12 weeks period ($p < 0.001$ as compared to normal control). Benazepril treatment reduced the urinary albumin excretion and prevented the rise in blood urea and serum creatinine (Table 3). Further kidney weight and the ratio of kidney weight to body weight was significantly higher in diabetic control rabbits, both of these parameters were also reversed by benazepril treatment (Table 3).

Discussion

The results revealed that administration of alloxan resulted in hyperglycemia, polyuria, loss of body weight and increase in oxidative stress. After 12 weeks of treatment with benazepril, there was no effect on body weight of rabbits i.e. it failed to prevent the loss of body weight. As benazepril an ACE inhibitor, has no direct effect on insulin secretion or blood sugar and as such it is not expected to have any effect on body weight. Since, benazepril treatment resulted in slight reduction in blood sugar levels and these were still very high as compared to control group. Diabetic animals developed increased oxidative stress manifested by significantly elevated serum MDA levels, reduced red cell SOD activity and decreased GSH levels ($p < 0.05$ as compared to control group). Serum MDA is a marker of lipid peroxidation and both GSH and SOD are antioxidants. There are various mechanisms that may be involved in the genesis of oxidative stress like glucose autooxidation, formation of advanced glycation end products, the polyol pathway and

protein glycation. According to Inouye *et al.* (15), increased blood glucose concentration may also lead to depression of natural antioxidant defenses like vitamin C and glutathione (15). Our results are consistent with the various previous studies that also demonstrated the increase in oxidative stress in diabetes (16). Treatment with benazepril resulted in significant decrease in serum MDA level and increase in SOD activity and GSH levels ($p < 0.05$ as compared to diabetic control group). These findings suggest its potent antioxidant role. In a previous study in apo-E deficient mice, another ACE inhibitor, captopril increased the resistance of LDL to copper sulphate induced oxidative stress (17), suggesting that the free radical scavenging capacity of captopril may be related to its sulphhydryl group. In our study, a non-sulphhydryl ACE inhibitor, benazepril also inhibited lipid peroxidation. This is in accordance with another study that showed fosinopril to be effective in inhibiting LDL oxidation (18). One study on ACE inhibitors demonstrated no effect on serum lipid profile (11) but other studies indicated their role in decreasing LDL/HDL ratio (9, 19). In a recent study, telmisartan decreased LDL and total cholesterol levels in blood (20). In the present study, benazepril had no effect on serum LDL cholesterol levels but it resulted in increase in HDL and reduction of TG and total cholesterol levels. Increased HDL levels may also be due to improvement of urinary albumin excretion.

Table 1. Effect of Alloxan Induced Diabetes and its Modulation by Benazepril on Oxidative Stress

Group T/t	MDA (nmol/ml)	SOD(Unit/g Hb)	GSH(mg/g Hb)
Control	1.48 ± 0.02	1923.0 ± 21.3	1.17 ± 0.03
DM Control	2.69 ± 0.03 ^a	1009.2 ± 32.2 ^a	0.64 ± 0.02 ^a
Benazepril	2.08 ± 0.04 ^{*,a}	1579.6 ± 30.7 ^{*,a}	0.96 ± 0.01 [*]

(Values are mean ± SD from 8 animals in each group)
P values < 0.001; ^a when compared with a normal control group, ^{*} diabetic control group

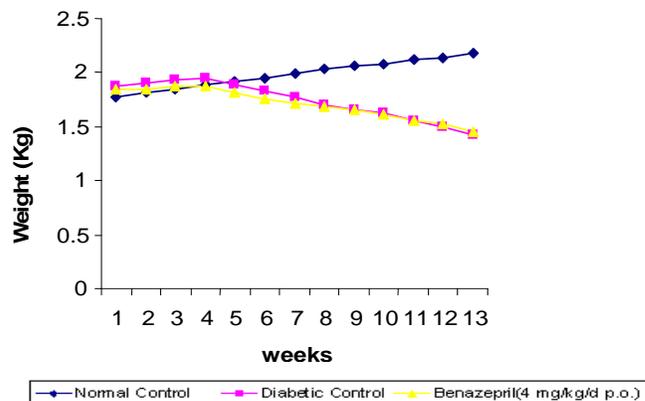


Fig. 1. Effect of Alloxan Induced Diabetes on BW & its Modulation by Benazepril at various time intervals

Table 2. Effect of Alloxan Induced Diabetes and its Modulation by Benazepril on Serum Lipids

Group Treatment	Total cholesterol (mg /dl)	Triglycerides (mg /dl)	LDL cholesterol (mg /dl)	HDL cholesterol (mg/dl)
Normal Control	71.70 ± 1.88	68.65 ± 1.72	23.60 ± 0.97	32.71 ± 0.74
Diabetic Control	245.92 ± 7.32 ^a	237.35 ± 8.06 ^a	77.32 ± 2.05 ^a	21.78 ± 0.68 ^a
Benazepril	225.98 ± 9.39 ^{*,a}	226.00 ± 6.35 ^{a,£}	75.98 ± 2.08 ^a	44.08 ± .99 ^{*,a}

(Values are mean ± SD from 8 animals in each group)
P values < 0.001; when compared with ^a normal control group, ^{*} diabetic control group < 0.01; [£] as compared to diabetic control group

Table 3. Effect of Alloxan Induced Diabetes & its Modulation by Benazepril on Renal Function

Group	Blood urea (mg /dl)	Serum creatinine (mg /dl)	Urine albumin (µg/ml)	Kidney weight (g)	Kidney weight/body weight
Normal	28.77 ± 2.40	0.73 ± 0.04	18.87 ± 2.03	3.16 ± 0.12	1.45 ± 0.08
Diabetic	113.83 ± 8.01 ^a	2.46 ± 0.05 ^a	491.12 ± 27.01 ^a	6.88 ± 0.25 ^a	4.76 ± 0.24 ^a
enazepril	35.68 ± 1.86 [*]	0.75 ± 0.10 [*]	58.75 ± 11.29 [*]	3.80 ± 0.08 [*]	2.63 ± 0.18 [*]

(Values are mean ± SD from 8 animals in each group)
P values < 0.001; when compared with ^a normal control group, ^{*} diabetic control group

DN is one of the major complications of NIDDM, which is a common cause of death in diabetic patients. The severity of renal disease in diabetic patients correlates with the levels of blood urea and serum creatinine. DN accounts for considerable morbidity and mortality even in patients with well-controlled blood sugar. In our study we evaluated various parameters related to diabetic renal

dysfunction like urinary albumin excretion, blood urea, serum creatinine, kidney weight and kidney weight/body weight ratio. Benazepril treatment prevented the rise in urinary albumin excretion and blood urea and serum creatinine ($p < 0.05$ as compared to diabetic control group). It also reduced the kidney weight and renal weight/body weight ratio. Considerable evidence suggests that the

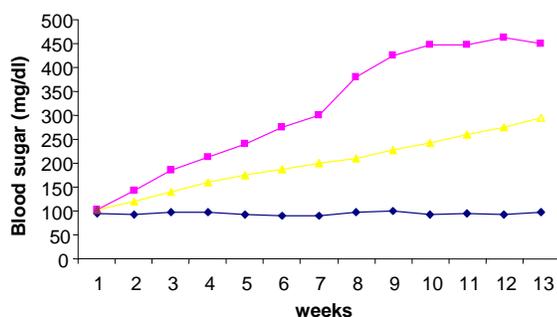


Fig. 2 Effect of Alloxan Induced Diabetes on B.Sugar & its Modulation by Benazepril at Various Time

intrarenal renin-angiotensin system plays an important role in diabetic nephropathy affect BP & intrarenal hemodynamics (21). ACE inhibitors delay the onset and slow the progression of DN in people with diabetes independent of their effect on blood pressure. They also slow the progression of DN in people with diabetes who have poorly controlled hyperglycemia (22). Dual blockade of the rennin angiotensin system (RAS) may offer additional renal and cardiovascular protection in type 1 diabetic patients with DN (22). Our results are similar to that obtained in other studies on ACE inhibitors and diabetic nephropathy in both humans and animal models (23). Various ACE inhibitors have been reported to lower the urine albumin excretion rate (23) and our findings are in accordance with these studies.

Conclusion

Benazepril can be a very good drug in diabetic patients due to its potent anti-oxidant role, favourable effect on lipid profile and ability to prevent the progression of nephropathic changes.

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