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Research Article

EFFECT OF ACE INHIBITORS ON ANTIOXIDANT STATUS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Aim: To compare the effects of different ACEI with those acted by their blockade of ROS by sulfhydryl group and non sulfhydryl groups in Streptozotocin induced diabetic rats.
Method: In this study we determined the effect of ACEI on TOS, TAS and TOSI. The body weight measurement and blood glucose levels were checked for every week until the end of the study by using the GOD/POD method. The parameters are found by using the eral method, by observing the absorbance at 560nm for TOS, 444nm for TAS. The TOSI was found by taking the percentage ratio of TOS and TAS.
Results: The TOS value of Captopril was less (15.18 ± 2.16) when compared to other ACEI [Lisinopril (20.87 ± 1.69) and Enalapril (19.03 ± 3.14)] which indicate that the level of free radicals was low in case of Captopril treated group than other ACEI. In contrast the TAS value of Captopril was more (2.57 ± 2.16) when compared to other ACEI [Lisinopril (1.39 ± 1.69) and Enalapril (1.73 ± 3.14)]. All these values predict that the captopril has more antioxidant activity than the non sulfhydril angiotensin-converting enzyme inhibitors Enalapril and Lisinopril.
Conclusion: The present study revealed the Captopril is having more potent action of reducing the TOS in the serum levels and has the higher extent of the TAS than the other two ACEI, Enalapril and Lisinopril but when compared to Enalapril and Lisinopril Enalapril is having the more potent action than the Lisinopril in daily recommended doses.

Key words: ACE inhibitors, Reactive oxygen species, Superoxide, Nitric oxide, Oxidative stress, Total oxidative stress index.

INTRODUCTION

Hyperglycemia occuring in diabetes is the crucial factor that is responsible for the development of oxidative stress and reactive oxygen species (ROS) are the main mediators of cellular damage in diabetes. Increased lipid peroxidation and reduced antioxidant enzyme activity found to be associated with progression of albuminuria in diabetes. Angiotensin-II blockage by the angiotensin converting enzyme inhibitors (ACEI's) shows to increase the activity of antioxidant enzymes during the diabetes[1,2]. There are beneficial actions of ACEI's involved in limitation of angiotensin-II stimulated NADPH oxidation, formation of superoxide ions; and also these inhibitors augment NOS activity via bradykinin, this is due to the modulation of protein kinase-C. During the diabetic conditions due to oxidative stress there are severe complications that occur.

Common pathogenic mechanism in several complications of diabetes such as diabetic nephropathy, diabetic retinopathy, and atherosclerosis is due to excessive oxidative stress which arises as a result of an imbalanced cellular level between generation and elimination of ROS. There are several sources of ROS in diabetes, including defective mitochondrial metabolism[3], glucose autoxidation[4], NADPH oxidation and synthesis of advanced glycation in product. ROS may also acts as transduction signal for angiotensin-II in different cell types, like smooth muscle cells, endothelial cells and human ventricular myocytes.

ROLE OF ACEI'S AS AN ANTIOXIDANT STRATEGY

On the basis of the link between ACE action and vascular NAD(P)H oxidase activity, we propose that ACE inhibitors represent a novel antioxidant strategy that targets oxidative stress at its source. ACE inhibition limits the stimulation of vascular NAD(P)H oxidase, thereby preventing the increased superoxide flux associated with activation of the renin-angiotensin system, superoxide reacts with NO. The NO is known to inhibit the activity of NAD(P)H oxidase[5], another predictable effect of ACE inhibitors would be to reduce the ambient levels of superoxide in the vascular wall. ACE inhibition should also inhibit lipid peroxidation through reduced formation of peroxynitrite; this notion is consistent with observations that angiotensin II induces lipid peroxidation in experimental animals[6]. Because superoxide is the principal source of hydrogen peroxide (H_2O_2), ACE inhibitors should limit smooth muscle proliferation, and this prediction is consistent with observations that ACE inhibition limits the progression of carotid intimal thickening because, ACEI's will limit the production of H_2O_2, the formation of $H_2O^{-}{}_2$ derived oxidants such as hydroxyl radical and hypochlorous acid (HOCl) is also reduced by ACE inhibitors.

The vascular NAD(P)H oxidase isoforms and their regulation is relatively immature. Vascular isoforms of NAD(P)H oxidase is activated through a variety of pathways, including receptor tyrosine kinases, cytokines, thrombin, and mechanical forces. Antioxidant therapy with vitamin E is limited to scavenging lipid-soluble oxidants and may therefore be considered a more "symptomatic" rather than a causal treatment for vascular oxidative stress.

Role of ACE inhibitors during Diabetes

Increased oxygen free radical activity, coupled with reduced protection against oxidative stress, could play a role in the etiology of neurovascular abnormalities in experimental diabetes mellitus[7]. Production of reactive oxygen species is increased in diabetic patients, especially in those with poor glycemic control.

ROS affect vascular smooth muscle cell growth and migration, endothelial function, including abnormal endothelium-dependent relaxation and expression of a proinflammatory phenotype, and modification of the extracellular matrix. All of these events contribute to the development of diabetic macrovascular and microvascular complications.

High blood glucose level determines over production of ROS by the mitochondria electron transport chain. High reactivity of ROS determines chemical changes in virtually all cellular components, leading to DNA and protein modification and lipid peroxidation. [8,9]

MATERIAL AND METHODS

Male albino rats of Wistar strain (weighing 145 - 170 g) are used for the study. Rats used for the study obtained from the animal house stock of the Department of Pharmacology, SRM College of Pharmacy, Kattankulathur, India and handle in accordance with the guidelines as per the "Institutional Animal Ethical Committee"(IACE) (Regd.No.662/2/C/CPCSEA) and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) rules(IAEC 92/2009). Animals allowed to free access of tap water and standard chow diet up to the end of the experimental period and divided in to the 5 groups; each group consists of 8 rats.

Table -1: Effect of ACE inhibitors Captopril, Lisinopril, Enalapril on body weight of Streptozotocin induced diabetic rats

S.No	Groups	Initial Body Weight(g)	1st week (g)	2nd week (g)	3 rd week (g)	4 th week (g)	5 th week (g)	6 th week (g)	7 th Week (g)
1	Normal	147.27±1.87	151.36±1.30**	154.87±2.69**	159.81±1.62**	165.03±1.41**	172.32±2.12**	174.77±1.92**	177.49 ±3.59**
2	Diabetic control	155.2 ± 2.34	140.11 ± 1.16	132.71 ± 2.61	126.13 ± 2.30	119 ± 1.06	112.37 ± 2.41	107 ± 1.12	102.91 ± 0.19
3	Diabetic + Captopril	167 ± 2.76	158.33 ± 2.01**	152.16 ± 0.32**	148.06 ± 1.18**	149.96 ± 2.12**	148 ± 1.40**	149.56 ± 2.39**	153.04 ± 2.16**
4	Diabetes + Enalapril	153.47 ± 2.12	147.17 ± 1.52**	136.33 ± 2.71ns	132.47 ± 0.38*	130.07 ± 3.45**	131.65 ± 1.96**	132.76 ± 0.08**	134.81 ± 2.26**
5	Diabetes + Lisinopril	157.18 ± 3.13	151.92 ± 0.92**	139.08 ± 1.17ns	136.01 ± 3.41**	134.61 ± 3.17**	134.04 ± 2.01**	133.17 ± 3.04**	133.99 ± 0.03**

Each value is represented as Mean ± SEM, Number of animals (n) = 8,*P< 0.05, **P< 0.01, nsP> 0.05 vs Diabetic control group. One way ANOVA followed by Dunnett test.

Table -2: Effect of ACE inhibitors Captopril, Lisinopril, Enalapril on Blood Glucose Levels BGL (mg/dl) of Streptozotocin induced diabetic rats

S. No.	Groups	0 week (mg/dl)	1st week (mg/dl)	2nd week (mg/dl)	3 rd week (mg/dl)	4 th week (mg/dl)	5 th week (mg/dl)	6rt week (mg/dl)	7 th week (mg/dl)
1	Normal	126 ± 2.98	128.57± 1.35**	127.37 ±1.70**	127.85 ± 1.04**	129 ± 1.68**	128.02 ± 1.67**	127.29±0.90**	128.11 ±1.44**
2	Diabetic control	355.01 ± 1.60	380.60 ± 3.01	385.9 ±2.43	389.62 ± 0.14	391.45 ± 0.09	392.02 ± 2.76	394.89 ± 1.66	395.11 ± 3.89
3	Diabetic + Captopril	369.74 ± 5.98	365.95 ± 1.12**	348.11 ± 0.66**	327.02 ± 4.34**	295.88 ± 2.07**	287.91 ± 0.83**	281 ± 1.02**	279.30 ±1.14**
4	Diabetes + Enalapril	352.4 ± 3.69	361.48 ± 0.40**	367.11 ± 3.01**	369.32 ± 2.18**	372.91 ± 1.88**	370.52 ± 1.14**	368.92 ±4.19**	366.12 ±0.62**
5	Diabetes + Lisinopril	357.19 ± 1.85	369.76 ± 2.07**	375.59 ± 1.86**	379.88 ± 3.59*	387.53 ± 0.32**	392.97 ± 1.79ns	397.59 ±2.83ns	406.63 ±3.16**

Each value is represented as Mean ± SEM, Number of animals (n) = 8, *P< 0.05, **P< 0.01, nsP> 0.05 vs Normal control group. One way ANOVA followed by Dunnett test.

Table -3: Biochemical Parameters

Sr. No.	Groups	TOS (µmol H_2O_2 equiv/L)	TAS(mmol Trolox equiv/L)	TOSI (AU)
1	Normal	12.19 ± 3.26	2.53 ± 0.15	0.596
2	Diabetic control	28.27± 3.21	1.15 ± 0.21	2.451
3	Diabetes + Captopril	15.18 ± 2.16	2.57 ± 2.16	0.587
4	Diabetes + Enalapril	19.03 ± 3.14	1.73 ± 3.14	1.098
5	Diabetes + Lisinopril	20.87 ± 1.69	1.39 ± 1.69	1.510

INDUCTION OF DIABETES AND DOSING

Diabetes was induced to the all the groups of animals except the normal group Streptozotocin(STZ) 60mg/kg was given through intraperitonial route by dissolving STZ in citrate buffer. The normal group(group-1), STZ control group(group-2) diabetic rats receiving no treatment which were given tap water, Captopril receiving group which were given captopril ad libitum in drinking water at a concentration of 50 mg/kg i.p (group-3), Enalapril given 20mg/kg i.p(group- 4), Lisinopril 5 mg/kg i.p (Group -5).

After animals are considered to be diabetic if they had serum glucose level >130mg/dl, and decrease in the body weight were considered as diabetic. Upto the seventh week ie, end of the experiment blood samples from all the rats in the study collected once in a week, collected by retro orbital puncture. The photometric estimation of glucose in plasma based on GOD/POD method done as described in the manufacturers (Merck Specilist Pvt Ltd) instruction manual.

At the end of the experiment the blood samples were collected and were measured for the Total plasma peroxide concentration, Total Antioxidant status, and for total oxidative stress index.

BIOCHEMICAL ANALYSIS

At the end of the experimental period, all the animals are sacrificed. Blood sample is collected in heparinised tubes and centrifuged at 1600 x g for 15 min. Plasma used for the estimation of total antioxidant status (TAS), total oxidative status (TOS) and the ratio percentage of the TOS to the TAS potential gives the oxidative stress index, an indicator of the degree of oxidative stress[10].

Both TOS and TAS are measured by the using a novel automated colorimetric measurement method by Erel et al[11].

STATISTICAL ANALYSIS

Results of all parameters were expressed as mean ± standard deviation for each group. One way ANOVA followed by Dunnett test.

RESULTS

Estimation of body weight

Table 1 shows the change in the body weight of the diabetic induced animals and the diabetic + drug treated animals. It has been observed that there was a decrease in the diabetic control group, due to the potent antioxidant activity of captopril than the other ACEI there was an increase in the body weight in captopril treated rat.

Estimation of blood glucose levels

From table 2 the captopril treated group is having the decrease in the blood glucose levels due to its high antioxidant activity than the other two ACEI's.

BIOCHEMICAL PARAMETERS

From the table 3 the TOS value of Captopril was less (15.18 ± 2.16) when compared to other ACEI [Lisinopril (20.87 ± 1.69) and Enalapril (19.03 ± 3.14)] which indicate that the level of free radicals was low in case of Captopril treated group than other ACEI. In contrast the TAS value of Captopril was more (2.57 ± 2.16) when compared to other ACEI [Lisinopril (1.39 ± 1.69) and Enalapril (1.73 ± 3.14)]. And at the same time the TAS value is more (2.75 ± 2.16) All these values predict that the captopril has more antioxidant activity than the non sulfhydril angiotensin-converting enzyme inhibitors Enalapril and Lisinopril.

The TOSI of Captopril (0.587) was nearly equal to normal (0.596) group of rats and this indicates that Captopril was more effective in maintaining the homeostasis of redox balance between oxidation and antioxidation.

DISCUSSION

Oxidative stress plays an important role in chronic complications of diabetes and is postulated to be associated with increased lipid peroxidation. The present study was to examine the involvement of free radicals in diabetes and the role of these toxic species in lipid peroxidation and the antioxidant defence system can be estimated by the changes in the level of extracellular total antioxidants, total oxidant status, and oxidative stress index in diabetic patients.

Captopril, Lisinopril, Enalapril, have been reported to possess free radical scavenging activity so these drugs were used for the present in estimation of the oxidative stress, antioxidant status and the degree of oxidative stress was assessed by measuring the blood serum levels by spectrophotometrically by eral method[11].

The present study was under taken to compare the potent antioxidant activity of the different angiotensin converting enzymes such as Captopril, Lisinopril, and Enalapril during the diabetic conditions. This study also helps to verify the possible interaction on hyperglycaemic, total oxidant status, and total antioxidant status in diabetic rats.

The beneficial action of angiotensin-converting enzyme inhibition is here broadened to the promotion of health. Indeed, the Prevention of Atherosclerosis with Ramipril-2 collaborative research group[12] suggested that beneficial effect of angiotensin-converting enzyme inhibitors on major coronary events may be due to reversal of endothelial dysfunction.

The present study demonstrates that administration of the sulfhydryl angiotensin-converting enzyme inhibitor such as Captopril has the more antioxidant activity than the non sulfhydril angiotensin-converting enzyme inhibitors Enalapril, Lisinopril.

Sulfhydryl compounds as a class have antioxidant effects[13], being able to neutralize oxygen radicals by either a hydrogen donating or electron transferring mechanism[12,13]. The mechanism of oxygen radical scavenging mediated by sulfhydryl compounds may also involve carbon-centred radical production. It also appears that the protective effects of sulfhydryl agents correlate better with their direct hydroxyl radical scavenging abilities than with their antiperoxidative potency[14,15].

These considerations are further supported by the fact that in the present study the non-sulfhydryl angiotensin-converting enzyme inhibitor Enalapril did not exhibit any antioxidant effects. The antioxidant effects of Zofenopril and captopril, but not Enalapril, were also reported in vivo in apolipoprotein E-deficient mice[16]. In addition, it has previously been reported that captopril is very effective in scavenging free radicals, in a manner similar to glutathione, N-2-mercaptoproppionylglycine, and N-acetylcysteine, but this effect was not mimicked by Enalapril[17].

Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. The levels of these defense mechanisms are altered in diabetes and, therefore, the ineffective scavenging of free radicals plays a crucial role in determining the extent of tissue injury[18]. SOD and CAT are considered primary enzymes since they are involved in the direct elimination of ROS. SOD scavenges the superoxide radical by converting it to H_2O_2 and hence reduces the toxic effects due to this radical or other free radicals derived from secondary reactions. The activity of SOD was found to be lower in diabetic subjects. The observed decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of the enzyme, which have been reported to occur in diabetes [19].

It is well known that, the effects of various antioxidants in plasma are additive and the cooperation of antioxidants in human serum provides protection of the organism against attacks by free radicals. Therefore, the measurement of TOSI may reflect accurately the antioxidant status of the organism[11,20].

Although determination of either oxidants or antioxidant components alone may give information about the oxidative stress, determination of oxidants along with antioxidants is more useful in this context. Therefore, oxidants and antioxidant capacity should be measured simultaneously to assess oxidative stress more exactly. In addition, the ratio percentage of the total plasma peroxide level to TOSI, regarded as an indicator of oxidative stress, reflects the redox balance between oxidation and antioxidation[11,21].

From the earlier reports it was noted that ACEI has increased the blood glucose levels by decreasing serum potassium levels[22] there by inhibiting insulin release and overall inhibition of glucose uptake by peripheral tissues in normal rats and our results are similar with the reports. Several studies have shown that ACE inhibitors decrease the incidence of new-onset type 2 diabetes by their possible protective effect on the pancreatic beta cell through inhibiting the vasoconstrictive effect of angiotensin II in the pancreas and increasing islet blood flow, which could improve insulin release by beta cells.

In the novel assay, most potent free radicals are produced and they oxidize o-dianisidine molecules to dianisidyl. The potent free radical reactions, starting with $OH^{\cdot}$, do not end in only a one-step reaction; generally, they continue, even forming a free radical chain reaction. Antioxidants prevent the prolongation of these oxidation reactions in various steps.

Recent studies have shown that Captopril is a potent antioxidant than the Enalapril and Lisinopril that may provide important protection against atherosclerosis, coronary artery disease, inflammation and other complications associated with the diabetes.

CONCLUSION

Increasing evidence in experimental studies suggest that oxidative stress plays a major role in the pathogenesis of diabetes mellitus. The present study revealed the Captopril is having more potent action of reducing the total oxidant status in the serum levels and has the higher extent of the total antioxidant status than the other two angiotensin converting enzyme inhibitors, Enalapril and Lisinopril but when compared to both Enalapril and Lisinopril Enalapril is having more potent action than in lowering oxidative stress Lisinopril in daily recommended doses.

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