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Effect of Losartan and Ramipril on Oxidative stress and Anti-oxidant Status in South Indian Hypertensive Patients

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Abstract: Over production of oxygen free radicals, which is mainly mediated by super oxide, occurs in human hypertension. Ramipril and losartan are the drugs which found to be more prescribed drugs for hypertension patients. In the present study, an attempt has been made to evaluate the levels of serum Glutathione, Total anti-oxidant status, lipid peroxidation (malondialdehyde) and liver function parameters like serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase. Total 75 subjects were selected for study. Out of 75 subjects 25 were normal healthy volunteers and 50 subjects were patients who are untreated with hypertension. The patients both male and female within the age group of 25-65 years were selected. Glutathione, total anti oxidant status, lipid peroxidation, serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase levels in plasma were estimated by using reported methods. Individual methods were standardized first and standard graphs were plotted and the parameters were measured significantly decreased values of glutathione, total anti oxidant status and malondialdehyde were observed ($p < 0.001$) in untreated hypertension patients as compared with normal healthy volunteers. After therapy there was significant ($p < 0.001$) increase in the glutathione, total oxidant status and malondialdehyde as compared with untreated patients. The results indicates that further extensive studies are required to understand the molecular level mechanism of losartan and ramipril in oxidative stress in hypertensive patients.

Key words: Hypertension, glutathione, total anti-oxidant status, oxidative stress, malondialdehyde, ramipril, losartan

INTRODUCTION

Hypertension is the leading cause of cardiovascular diseases worldwide. Cardiovascular diseases account for a large proportion of deaths and disability all over the world. It has been predicted that by the year 2020, there will be an increase by almost 75% in the global cardiovascular disease burden (Kearney *et al.*, 2005). Hypertension is an independent risk factor for both Coronary Heart Disease (CHD) and stroke. High blood pressure (BP) is an important public health problem in India (Gupta, 1996). Oxidative stress is mediated by Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) is a primary or secondary cause of many chronic diseases including hypertension. Over production of oxygen free radicals, which is mainly mediated by super oxide, occurs in human hypertension (Touzy, 2000). The reaction product between super oxide and NO,

peroxynitrite, constitutes a strong oxidant molecule, which is able to oxidize proteins, lipids and nucleic acids, causing cell damage, these pathological process are associated with hypertension because they contribute to the narrowing of arterial lumen, consequently to increase peripheral resistance and increase blood pressure (BP), in addition to mitochondrial sources of ROS, superoxide can be derived from xanthine oxidase, NADPH oxidase, cyclooxygenase, lipooxygenases and uncoupled NOS (Mueller *et al.*, 2005). Superoxide levels are controlled through endogenous antioxidant systems. Superoxide dismutase is the primary antioxidant in the vascular system (Landmesser and Harrison, 2001), which is often associated with endothelial dysfunction and hypertension.

Ramipril (1.25 mg once daily) is a prodrug, comparatively long-acting members of ACEI. Following oral administration peak plasma concentration of ramipril

are reached within one hour. The extent of absorption is at least 50-60%, not influenced by the presence of food in the GIT. Losartan is the first orally available angiotensin-receptor antagonist with out agonist properties. Following oral administration, losartan rapidly absorbed, reaching maximum concentrations 1-2 h post administration (Domenic *et al.*, 2006). The above facts are saying that the oxidative stress parameters like GSH, TAS and MDA plays very important role in the pathophysiology of the hypertension. By considering the facts, we have studied the status of the oxidative stress in hypertensive patients before and after treatment compare to the normal healthy persons. We studied the effect of ramipril and losartan on systolic blood pressure, diastolic blood pressure, endogeneous antioxidant Glutathione(GSH), lipid peroxidase (malonaldehyde/MDA), the Total Antioxidant Status (TAS) and the liver function parameters Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) in South Indian hypertension patients

MATERIALS AND METHODS

The present study was carried out in the Department of Pharmacology, University College of Pharmaceutical Sciences (UCPSc), Kakatiya University; Mahatma Gandhi Memorial (MGM) hospital, Waranagal (AP, India), in the year 2010.

This study comprised of three groups, one group of healthy volunteers and two groups of essential hypertensive patients. The present study included total 75 subjects. Out of 75 subjects 25 were normal human volunteers (NHVs) and 50 subjects were patients who are untreated with hypertension. Out of 50 patients 25 were treated with ramipril and 25 were treated with losartan. The patients within the age group of 25-65 years were selected. They were clinically, histopathologically and newly diagnosed for hypertension. Male patients with normal blood sugar levels, patients with only essential hypertension with out any prior antihypertensive therapy. Twenty five healthy volunteers having no history of smoking, alcoholism, any type of diseases were considered as controls. The subjects having age less than 25 years and above 65 years, secondary hypertension, diabetic patients and patients suffering from renal disease were excluded from the study. Study was conducted after getting the approval by the Institutional Ethics Committee. (Ref No.: 25 \KMC\KU\2009). The patients were treated with either losartan 50 mg or ramipril 2.5 mg for at least 10 days. After 10 days of treatment by taking the prior consent, venous blood was collected from the subjects under aseptic condition by venipuncture using 5 mL sterile disposable syringe and needle. About 3-4 mL of blood was collected. Serum was separated by centrifugation at 3000 rpm for

10 min at room temperature. The samples were stored at 4°C before analysis and all the samples were analyzed on the same day of collection (Naidu *et al.*, 2007).

All the methods were standardized first and standard graphs were obtained. Serum glutathione total anti oxidant status, lipid peroxides, SGOT and SGPT were measured by using standard methods.

Glutathione in blood: 0.5 mL of 5% TCA solution was added to 0.5 mL of citrated blood to precipitate the proteins and centrifuged at 3000 rpm for 20 min. To 0.1 mL of supernatant, 1 mL of sodium phosphate buffer (pH 8) and 0.5 mL of DTNB (39.6 mg in 100 mL of 1% sodium citrate solution to give a concentration of 1 mM were added. The absorbance of the yellow color developed was measured at 412 nm (Beutler *et al.*, 1963).

Total anti-oxidant status: Total anti-oxidant status in serum was determined by the method of Blois (1958) using a stable, free radical, α , α -diphenyl- β -picrylhydrazyl (DPPH) (Sigma Aldrich, USA), at a concentration of 0.2 mM in methanol.

All the results were expressed in mean \pm SD. Statistical analysis was done by using student's t-test. $p < 0.05$ was considered as significant whereas $p < 0.001$ was considered as highly significant.

Blood Pressure (BP): The patient should be sitting or lying at ease. Both the systolic and diastolic blood pressure was measured by using the digital B.P apparatus which is purchased from Omron, USA.

Lipid peroxides: The amount of lipid peroxidation products present in the serum samples were estimated by the thiobarbituric acid reactive substances (TBARS) method, which measures the malondialdehyde (MDA) reactive products by using spectrophotometer method (Moore and Robert, 1998).

SGOT and SGPT: Both SGOT and SGPT in human serum or plasma are usually assayed by Reitman and Frankel (Reitman and Frankel, 1957) colorimetric method.

Statistical analysis: Each group comprised of 25 hypertensives ($n = 25$). All the values were expressed as Mean \pm SD. The data was analyzed using ANOVA followed by Student Newman Kuel method. In tests, the criteria for statistical significance was * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

RESULTS

Demographic parameters like Age, weight, height, were not significantly different among the three groups (Table 1).

Table 1: Demographic data of ramipril and losartan pretreatment groups

Parameter	NHV	Ramipril	Losartan
Age (years)	30±6.0	48.36±10.68	48.68±8.2
Height (cm)	150±4.5	147.36±7.72	151.56±5.6
Weight (kg)	66±5.2	51.76±12	55.80±10.9

Table 2: Effect of ramipril and losartan on the blood pressure, SGOT and SGPT of hypertensive subjects

Parameters	NHV	Ramipril		Losartan	
		Pre	After	Pre	After
SBP (mmHg)	122.6±5.9	165±14.14	147±15.2***	153±4	140±10.9*
DSB (mmHg)	80.2±1.9	94.2±11.7	85.43.84***	94±11.9	91±10.5*
SGOT (IU L ⁻¹)	24.8±4.5	33.12±6.9	29±6.3	31.8±8.65	28.76±7.42
SGPT (IU L ⁻¹)	25.2±4.9	33.52±6.8	27.32±5.1	31.8±9.4	25.24±4.9

***p<0.001; **p<0.01; *p<0.05

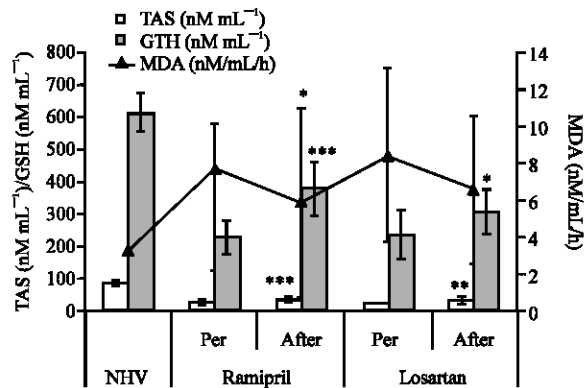


Fig. 1: Effect of ramipril and losartan on the oxidative stress parameters of hypertensive subjects

Blood pressure: The mean±SD levels of BP are shown in Table 2, significant decrease in BP is observed in both ramipril and losartan groups as compared to pretreatment values. Ramipril (p<0.01) reduced BP more effectively than losartan (p<0.05). It was observed that the both drug treatment groups having the BP values higher than the NHVs/control group.

Glutathione: The mean±SD levels of glutathione expressed as μM are shown in Fig. 1. GSH levels were significantly increased in both ramipril (p<0.001) and losartan (p<0.05) treatment groups as compared to their pretreatment values. Ramipril was comparatively more effective in increasing GSH levels as compared to losartan group. But in both groups the GSH levels are lower than the NHVs (Fig. 1).

Total serum anti-oxidant status: Total antioxidant levels were significantly increased with ramipril (p<0.001) and losartan (p<0.01) treatment as compared to pretreatment values. Ramipril seems to be more effective in increasing total Antioxidant levels as compared to losartan group. But in both drug treatment groups the TAS levels are lower than the NHVs/control group (Fig. 1).

Lipid peroxidation: MDA levels were decreased in ramipril (p<0.05) as compared to pretreatment values. MDA levels were not significantly decreased with losartan as compared to pretreatment values (Table 2).

SGOT and SGPT: No significant changes in SGOT and SGPT were observed in both ramipril and losartan group as compared with control group (Table 2).

DISCUSSION

Free radicals are highly reactive compounds that activate and alter the cellular antioxidant defense system. This includes enzymatic and non-enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), ascorbic acid (vitamin C) and alfa-tocopherol (vitamin E). Under conditions of excessive oxidative stress, however, cellular antioxidants are depleted.

The people who exposed to repeated psychogenic stress may develop hypertension more frequently eg: Air traffic controllers annually developed hypertension at a rate 5-6 times greater than that of non professional pilots. Among healthy employed men, job strain is associated with higher awake ambulatory BP. An increased risk for developing hypertension is increased heart rate in response to stress. Exposure to major catastrophes such as earth quakes and massive explosions leads to higher levels of blood pressure (George, 1996).

High blood pressure (BP) is one of the important public health problems in India and world wide (Gupta, 1996) Oxidative stress mediated by free radicals ROS, RNS is a primary or secondary cause of many chronic diseases (Ferrira *et al.*, 2005), heart failure, stroke, coronary heart disease along with consequences and the impairment of renal function . The serious complications are not only the consequences of increased blood pressure but are also related to the arterial endothelial dysfunction, abnormal endothelial dysfunction and accelerate the process of hypertension.

The results of the present study showing that the systolic BP levels were significantly higher (Table 2) in hypertensive patients without Antihypertensive treatment compared with healthy subjects. Ramipril significantly reduced SBP than losartan treated subjects due to its antioxidant property and dual mode of action by release of NO. Losartan has also shown significant decrease in SBP but it is less effective than ramipril in reducing SBP.

Diastolic BP is controlled by the peripheral resistance. In the overall population mean DBP increases progressively throughout adult life in men and women.

Through out the adult life men have a slightly higher mean DBP than women.

In the present study DBP levels were significantly higher in hypertensive patients with out antihypertensive treatment compared with healthy subjects. Ramipril treatment in hypertensive subjects has shown more significant results in reducing diastolic BP than losartan treated subjects.

Antioxidants decrease the incidence of diseases however more human studies are required to establish the efficacy and safety of these agents in various chronic or acute oxidative stress-related diseases (Rees *et al.*, 1989) e.g., cardiovascular diseases (CVDs). The role of antioxidants in CVDs is based on the premise that free radicals can injure arteries, can induce atherosclerosis by inducing fatty streaks resulting in atheroma. By oxidation of LDL can injure myocardium during reperfusion in MI, Hypertension occur due to deregulating of nitric oxide production. The antioxidants can prevent most of these above processes. Several factors such as low food intake, nutrients malabsorption and inadequate nutrient release from the liver, acute phase response and infection and an inadequate availability of carrier molecules may influence circulating antioxidant concentrations (Solzbach *et al.*, 1997). Ramiprilat protects the vascular endothelium against free radical induced functional injury (Gillis *et al.*, 1992).

In present study the total antioxidant levels were found to be significantly reduced (Fig. 1) in all hypertensive patients with out antihypertensive treatment compared with healthy subjects, Total antioxidant levels have been significantly increased with clinical improvement under treatment with ramipril ($p < 0.001$) as compared to losartan treatment due to its antioxidant property.

Glutathione peroxidase appears to have a major role in the prevention of oxidative stress; it may also be an important antiatherogenic antioxidant. Glutathione (GSH) is a tripeptide comprised of glutamate, cysteine and glycine. GSH is present in mast cells, where it functions as an antioxidant protecting cells from toxic effects of ROS (Arthur, 2000). Glutathione peroxidase deficiency has endothelial dysfunction combined with structural vascular abnormalities, such as increased periadventitial inflammation and collagen deposition surrounding the coronary arteries. Glutathione has been regulated by immune cell function. Glutathione peroxidase with 5-lipoxygenase might constitute a protective function of the enzyme, in addition to its antioxidant activity (Sies, 1999). Enalapril and captopril enhance glutathione dependent antioxidant defences (Elena de Cavanagh,

2000). In present study Glutathione levels were significantly low in all hypertensive patients, the decreased was more pronounced in untreated hypertensives (Fig. 1).

Hypertension is a state of increased free-radical activity which oxidatively stresses or injures the endothelium conjugated dienes and lipid peroxides are by products of the lipid Peroxidation of cellular structures induced by free radicals and can be conveniently measured as thiobarbituric-acid-reactive substances (Mollanauv *et al.*, 2005). In the our study the MDA levels were found to be higher in all hypertensive patients (Fig. 1) with out antihypertensive treatment compared with healthy subjects, MDA levels have been decreased with ramipril treatment as compared to losartan treatment. It may result in its beneficial effects on the restoration of no bioavailability and endothelial function.

SGOT is an enzyme found in heart muscle, liver cells, skeletal muscle and kidneys. Injury to these tissues results in the release of the enzyme in blood. No significant changes were observed in both the treated groups. SGPT is found in a variety of tissues but is mainly found in liver, increased levels are found in hepatitis, cirrhosis obstructive jaundice and other hepatic diseases. In the present study no significant change in SGOT and SGPT was observed with the both of the drugs (Table 2) In this study it was observed that the glutathione and total anti-oxidant status (both enzymatic and non enzymatic) was significantly decreased in hypertension patients as compared to healthy volunteers indicate that patients are unable to produce sufficient amounts of antioxidants to cope up with the increased oxidative stress in them. The decrease was more pronounced in untreated patients indicating that the anti-oxidants were nearly completely utilized to scavenge the superoxide free radicals and the increase in the anti-oxidants was due to the prevention of the free radical production after the drug treatment.

CONCLUSION

The strength of the present work lies in the fact that alterations in the levels of GSH, TAS and MDA before and after treatment. From the results it was clear that there was significant decrease in GSH and TAS in untreated patients and significant increase in the same after treatment. Hence, it appears that suitable anti oxidant supplementation is warranted to protect from free radical attack in hypertension patients and the drugs like ramipril and losartan were having the significant protection against oxidative stress in hypertensives.

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