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Biochemical Evaluation of Captopril on Oxidative Status, Membrane Electrolytes and Hemodynamics

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Abstract

We aimed to assess the oxidative status, renal function and membrane electrolytes of angiotensin converting enzyme inhibitor captopril. A total of 12 male Albino Wistar rats (200-250 g) were taken and divided into 2 groups (n=6); group I remained untreated; group II treated with 1 mg/kg b.w. of captopril intraperintonealy for 1 day. The effects of captopril on renal function were analyzed in terms of urea and creatinine; oxidative status by tissue and plasma Malonyldialdehyde (MDA) and tissue catalase activity; and electrolytes homeostasis by plasma sodium, potassium, intra-erythrocyte sodium, potassium and membrane Na^+-K^+-ATPase activity. Increased tissue Malonyldialdehyde (MDA), tissue catalase (*P<0.01*), plasma urea (*P<0.05*), creatinine (*P<0.01*), sodium (*P<0.01*), potassium (*P<0.05*), intra-erythrocyte Na^+-K^+-ATPase (*P<0.01*), while decreased level of intra- erythrocyte sodium (P<0.05), potassium and magnesium level was observed in Captropril- treated rats. The findings explain the antioxidant property of captopril with altered renal function and membrane electrolytes.

Key words: Captopril, catalase , electrolytes

Introduction

Angiotensin converting enzyme (ACE) is a dipeptidyl carboxypeptidase which cleaves angiotensin I (Ang-I) to angiotensin II (Ang-II), a potent vasoconstrictor agent and are well established drugs in the treatment of hypertension (Nagano, 2006). Although the pharmacological mechanisms of ACE inhibitors are not fully understood, various studies suggest that these agents may, except inhibition of Angiotensin II production, stimulate synthesis of prostaglandins and bradykinin, inhibit production of superoxides, scavenge free radicals, increased expression of endothelial nitric oxide synthase (Zicha *et al.,* 2006) and help in the protection of cardiovascular system during congestive heart failure (Sladkove *et al.,* 2007).

Captopril reduces blood pressure in patients with primary hypertension, as well as those with renal vascular problem. It mediates its effects through inhibiting the activity of ACE (Peng *et al.,* 2007). It improves the prognosis of congestive heart failure by increasing cardiac out put (Zhang *et al.,* 2008). It also has proven clinical efficacy in the treatment of ischemic heart (Simko *et al.,* 2003) and renal disease (Aznauridis *et al.,* 2007). Multiple atherogenic effects of Ang-II, collagen-induced platelet aggression also reduced by captopril (Kojsova *et al.,*2006). In addition, captopril can inhibit the free-radical production in atherogenesis and seems to have an antioxidant properties (Skowasch *et al.,* 2006).

In views of above findings the proposed study , thus, was designed to evaluate the estimation of oxidative status, renal function and membrane electrolytes of captopril – treated rats.

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Materials and Methods

Twelve Albino Wistar rats of male sex (200–250g b.w.), were purchased from the animal house of Aga Khan University Hospital (Karachi, Pakistan) for the study. Animals were acclimatized to the laboratory conditions one week before the start of experiment and caged in a quite temperature controlled room (23±4 ^oC). Rats had free access to water and standard rat diet. The experiments were conducted in accordance with ethical guidelines for investigations on laboratory animals.

Study Design

The animals were divided into two experimental groups (n=6).

Each group received the following treatment:

Group I: Control group remained untreated

Group II: Received captopril i.p. (1 mg / kg b.w.) for 1 day

After an hour of the captopril administration, animals were anesthetized, decapitated and blood was sampled from head wounds in lithium heparin coated tubes. A portion of blood was used to get plasma. Kidneys were excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination,

dried by blotting with filter paper and weighed. The tissues were then kept in freezer at -70 ^{o} C till analysed.

Kidneys were perfused with saline and homogenized in chilled potassium chloride (1.17%) using a homogenizer. The homogenates were centrifuged at 800 g for 5 minutes at 4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 g for 20 minutes at 4°C to get the post mitochondrial supernatant which was used to assay catalase and Malonyldialdehyde activities.

Catalase activity was estimated by the method of Sinha (1972). Briefly, the assay mixture consisted of 1.96 ml phosphate buffer (0.01 M, pH 7.0), 1.0 ml hydrogen peroxide (0.2 M) and 0.04 ml PMS (10%) in a final volume of 3.0 ml. 2 ml dichromate acetic acid reagent was added in 1 ml of reaction mixture, boiled for 10 minutes and cooled. Changes in absorbance were recorded at 570 nm**.**

The Malonyldialdehyde (MDA) was determined spectrophotometrically according to the method of Ohkawa *et al.* (1979). Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium dodecyle sulphate, 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 ml of 10%(w/v) of PMS. The mixture was brought up to 4.0 ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0 ml distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm and compared with those obtained from MDA standards. The concentration values were calculated from absorption measurements as standard absorption.

urea was estimated spectrophotometrically by the Oxime method (Butler *et al.*, 1981). creatinine was estimated spectrophotometrically by the Modified Jeff's method (Spierto *et al.*, 1979).

Plasma was analyzed for the estimation of sodium and potassium by flame photometry (Corning 410) and magnesium by the method of Hallry and Skypeck (1964).

Heparinized blood was centrifuged and plasma was separated. Washed erythrocytes three times with magnesium chloride solution (112mmol/L) were then used for the estimation of intra -erythrocytes sodium and potassium (Fortes and Starkey, 1977).

ATPase activity was measured by the method of Denis *et al.*(1996) in a final volume of 1 ml as follows: Membrane (400 µg) were preincubated for 10 minutes at 37^{o} C in a mixture containing 92 mmol/L Tris-HCl (p H=7.4), 100 mmol/L NaCl, 20 mmol/L KCl, 5 mmol/L $MgSO_4.H_2O$ and 1 mmol/L EDTA. Assays were performed with or without 1 mmol/L Ouabain, a specific inhibitor of Na-K-ATPase activity was calculated as the difference between inorganic phosphate released during the 10 minute incubation with and with out ouabain. Activity was corrected to a nanomolar concentration of inorganic phosphate released mg protein/hour.

All assays were performed in duplicate, and blanks for substrate, membrane and incubation time were included to compensate for endogenous phosphate and non-enzyme related breakdown of ATP. Under these experimental conditions, the coefficient of variation was 7.5%.

Statistical analysis:

Results are presented as mean ± SE. Statistical significance and difference from control and test values evaluated by Student's t-test. Statistical probability of $P < 0.01, < 0.05$ were considered to be significant.

Results and Discussion

ACE inhibitors (Captopril), in addition to their hemodynamic activities, have advantageous vascular structural and functional effects. Several mechanisms could explain the beneficial effects of captopril on the functional reactivity of vascular system. It has been shown that it can not only decrease the Ang-II level, but also causes synthesis and release of a variety of vasodilators through endothelium such as prostacyclin, bradykinin and endothelium-derived nitric oxide (Lubas *et al.*,2008).

Table 1 showed decrease plasma MDA level and increased tissue MDA level but results were not significant, however, significant increased level of catalase was observed in captopril - treated rats ($P<0.01$) as compared to control. Previous study reported that a possible mechanism by which captopril improve vascular reactivity may be dependent on inhibiting the oxidative stress. It has been shown that ACE inhibitors could inhibit lipid peroxidation in sera , increase the antioxidant capacity in a number of tissues, ultimately leading to morphological and functional changes related to the aging process. Captopril treatment increases the antioxidant enzymes (catalase, superoxide dismutase), non-enzymatic antioxidant defenses and glutathione in several mouse tissues (Asgary *et al.*, 2007). Increased tissue catalase indicating it's antioxidant property.

According to Table 1, urea level was increased ($P<0.05$), similarly, creatinine level was also increased in captopril-treated rats as compared to control ($P<0.01$). A well known side-effect of ACE inhibitor is acute worsening of renal function due to the predominant dilatation of the efferent arterioles, and consequent decrease in glomerular filtration. Renal impairment is a significant adverse effect of all

ACE inhibitors, and is associated with their effect on ang-II mediated homeostatic functions such as renal blood flow. ACE inhibitors can induce or exacerbate renal impairment in patients with renal artery stenosis (Shin *et al.*, 2009).

Intra-erythrocyte sodium level was decreased significantly ($P<0.05$) as compared to control, similarly intra-erythrocyte potassium level was also decreased but results were not significant. Increased Na^+-K^+-ATPase was observed significantly in captopril-treated rats ($P<0.01$). Similarly, plasma sodium and potassium level was significantly increased as compared to control while plasma Mg^{++} level was decreased but results were not significant. The most common cause of electrolyte disturbances is renal failure leading to depletion disorders like hypomagnesemia, hypokalemia and hyponatremia (Temel and Akyuz, 2007). Captopril alters the abnormal permeability to Na^+ of the vascular smooth muscle membrane, contributes to the antihypertensive effect. Inhibition of ACE by captopril reduced the stimulatory effect of Ang-I, suggested that intraluminal conversion of Ang- to Ang-II can occur in the cortical collecting duct, resulting in enhanced apical sodium entry. An acute increase in serum sodium produce an acute fall in serum osmolality, produce acute free water shifts into and out of the vascular space until osmolality equibilirates in these compartments. Similarly, rise in serum K^+ may consider the fall in serum pH as K^+ shifts from cellular to vascular space and captopril produce some K^+ retention by inhibiting aldosterone production (Kazerani *et al.*,2009).

Captopril involving thiol group, participating in formation of thiols and therefore, it may have stronger antioxidant effects than other ACE inhibitors. The mechanism of thiol group-related protective action of captopril, although presumed to be secondary to free radical scavenging and the subsequent decrease in reactive oxygen species (Guerrero and Rodriguez, 2009). Clearfield *et al.*(1994) documented that captopril increases the superoxide dismutase and glutathione activity in liver as well as Na^+-K^+-ATPase and Ca^{++}-ATPase activity in erythrocytes. Treatment with ACE inhibitors increases the apparent affinity of the Na^+-K^+ pump for Na^+, suggested that ang-II regulates the pump's selectivity for intracellular Na^+ at sites near the cytoplasmic surface. Decreased activity of Na^+-K^+-ATPase was also reported, considered only when high concentration of captopril was employed in physiological studies (Pechanova, 2007).

In present study the increased renal catalase enzyme level explains the antioxidant property of captopril while adverse effects occur from the therapeutic dose of the captopril. Increased urea and creatinine level indicates the renal impairment, which may be responsible for altered membrane electrolytes. The duration of the effects may be dose related and temporary and can go away with discontinuation of the treatment.

Table 1. Effect of Various parameters in Control and captopril-treated rats (n=6)

Variables	Control	Captopril-treated rats
Plasma MDA (nm/dl)	0.303±0.16	0.245±0.07
Tissue MDA (nm/g of tissue)	24.3±4.22	28.43±3.55
Tissue catalase (mmol/gm of tissue)	0.614±0.51	2.43**±1.79
urea (mg/dl)	52.93±4.54	58.00 *±5.91
creatinine (mg/dl)	2.05±0.74	17.51**±5.35
Intra-erythrocytes Na^+ (mmol/L)	12.73±2.43	10.34*±1.29
Intra-erythrocytes K^+ (mmol/L)	166.84±18.71	163.16±38.28
Intra-erythrocytes Na^+ - K^+ -ATPase (nm/mg/hr)	97.35 ±34.29	345.42**±173.96
Plasma Na^+ (mmol/L)	108.33±1.03	114.66**±4.50
Plasma K^+ (mmol/L)	4.8±1.31	6.68*±1.04
Plasma Mg (mg/dl)	1.81±0.09	1.37±0.13

Values are mean±SE

Significant difference between Control and captopril-treated rats by t-test ***P<0.01, *P<0.05*

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