Modulation of oxidative stress by enalapril and valsartan in adrenaline treated rats: a comparative study

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Abstract

Angiotensin (Ang II) II is known to promote oxidative stress in acute myocardial infarction (AMI). Inhibition of renin angiotensin system (RAS) or blockade of Ang II receptors may therefore be effective in reducing oxidative stress during AMI. The study evaluates and compares the protective effect of Angiotensin Converting Enzyme (ACE) inhibitor and AT_1 receptor blocker in adrenaline induced oxidative stress in rats. Rats were treated with two successive injections of adrenaline subcutaneously at a dose of 2 mg/kg administered 24 hours apart. In other two groups of rats enalapril (30 mg/kg) or valsartan (30 mg/kg) were given orally once daily through intragastric tube for 2 weeks and then two injections of adrenaline were administered 24 hours apart. Serum Aspertate Transaminase (AST), plasma Malonde Aldehyde (MDA), erythrocyte GSH and serum vitamin E levels were measured 24 hours after the 2nd injection of adrenaline in all the groups. Administration of adrenaline caused significant increase ($p<0.001$) in serum AST and plasma MDA levels and decrease ($p<0.001$) in erythrocyte GSH and serum vitamin E levels. Pre-treatment of enalapril or valsartan for 14 days reduced ($p<0.001$) serum AST and plasma MDA levels and increased the concentration of erythrocyte GSH in enalapril pre-treated group ($p<0.01$) and in valsartan pre-treated group ($p<0.05$). Pre-treatment of enalapril or valsartan also increased ($p<0.01$) serum vitamin E levels in adrenaline treated rats. However, no significant difference was noted between the effect of enalapril and valsartan on serum AST, plasma MDA, erythrocyte GSH and serum vitamin E levels. It may be concluded that both enalapril and valsartan offered cardioprotection in adrenaline induced oxidative stress, but the protection afforded by valsartan was not superior to enalapril.

Introduction

Myocardial infarction (MI) results from the prolonged myocardial ischemia with necrosis of myocytes due to interruption of blood supply to an area of heart[1]. The metabolic disarrangements that occur during ischemia predisposes for the formation of free radicals[2]. So, oxidative/antioxidative balance is disturbed early during the ischemic phase of acute myocardial ischemia (AMI). MI is associated with accumulation of reactive oxygen metabolites, which may play an important role in the pathogenesis of post ischemic dysfunction[3]. ROS are capable of reacting with unsaturated lipids and of initiating the self–perpetuating chain reactions of lipid peroxidation in the membranes. Malondialdehyde is a known stable product of lipid peroxidation[4]. Lipid peroxidation is thought to be involved in various pathological conditions, such as, platelet activation[5], tissue destruction[6]. Since acute MI may be related to a thromboembolic process[7], to destruction of tissue, so, a raised MDA concentration in the serum is expected[6].

Antioxidant status is an important marker of oxidative stress and may predict cardiovascular complications. GSH plays an essential protective role against oxygen reactive species. It detoxifies oxygen radicals and therefore prevents cellular damage from oxidative stress[8]. So, it may be considered a sensitive and specific index of myocardial oxidative stress. Vitamin E, present in cellular and sub cellular membranes, provides a defense against peroxidation of vital phospholipids. So, the biochemical actions of vitamin E, are concerned with the prevention of peroxidative damage to cells and sub cellular elements[9].

The activity of the adrenergic system is increased in AMI which is reflected by raised plasma catecholamine concentration[10]. A renin-angiotensin system (RAS) is present in the heart[11,12], activation of the RAS in myocardial ischemia, leads to increased formation of local Ang II[13]. Reactive oxygen species (ROS) are formed in excessive amounts and are considered important to myocardial reperfusion injury[14]. The levels of

angiotensin II in cardiac tissue are several times the levels of angiotensin II in circulating blood. Recent evidence suggests that most of the angiotensin II in the heart is not derived from angiotensin I in the circulation, and that most of the angiotensin I in cardiac tissue is generated in the tissue itself[15]. Angiotensin II (Ang II) has been shown to exert a direct vasoconstrictor effects on the coronary arteries[16] and also has a direct positive chronotropic and ionotropic effect on myocardium[17]. It is also regarded as a pro-oxidant because it can stimulate the production of ROS. Therefore, blockade of renin angiotensin system (RAS) by angiotensin converting enzyme inhibitor (ACEI) and angiotensin receptor blocker (ARB) could reduce oxidative stress and could be beneficial for prevention of MI. Ang I may be converted to Ang II by endothelial or extra endothelial ACE, or by chymostatin sensitive Ang II generating pathway[18]. ACE inhibitors may not totally block the vascular formation of Ang II. As Ang II receptor antagonist acts more distally by blocking the AT_1 receptor, so it might exert a better effect. Enalapril maleate, a class of ACE inhibitor, is inactive and becomes active enalaprilic acid when it is absorbed and de-esterified in the liver. It is a potent ACE inhibitor, with a long duration of action[19]. Valsartan is a new potent and specific Ang II receptor blocker (ARB)[20]. Among the ARBs, valsartan has 30,000 times higher affinity for AT_1 than for Ang II-type 2 receptors-AT_2, that selectively inhibits the biological effect of Ang II at the AT_1 receptor[21]. Pre-treatment with this drug may reduce ROS generation. Therefore, the present study aims at deciding whether enalapril or valsartan, which one is the definitive winner to prevent oxidative stress in MI.

Materials and Methods

Drugs: Adrenaline, ampoule containing 1mg/ml (1:1000) - (GacoBangladesh) was purchased from local market. Enalapril Maleate was provided by Beximco Pharmaceuticals and Valsartan by Orion Pharmaceuticals as gifts.

Animals: The experiment was carried out on 36 healthy rats of Long Evans Norwegian strain, aged between 2-3 months of both sexes, weighing between 170-210 gm, fed on standard rat diet and allowed to drink water ad libitum.

Experimental design: Experimental animals were divided into six groups each group containing six rats and were treated accordingly. Group I received distilled water (1 ml) subcutaneously daily in a single dose 24 hours apart for 2 consecutive days and served as control. Group II received only enalapril through intragastric tube for 14 days; group III received only valsartan through intragastric tube for 14 consecutive days. Group IV received adrenaline (2mg/kg body weight)[22] subcutaneously daily in a single dose 24 hours apart for 2 consecutive days. Group V received enalapril (30mg/kg bw)[23] orally through intragastric tube daily for 14 consecutive days, then adrenaline was given subcutaneously in a single dose 24 hours apart for 2 consecutive days from 15th day. Group VI was treated with valsartan (30mg/kg bw)[24], daily orally through intragastric tube for 14 days, on 15th day 1st injection of adrenaline and after 24 hours the 2nd injection were given. All the rats were sacrificed 24 hours after the last dose under light anesthesia by chloroform. About 2 ml of blood from each rat was collected in a clean and dry test tube by cervical decapitation. The serum was separated by ultra-centrifugation (4000 rpm for 5 minutes) and collected by micropipette, transferred to labeled test tubes for biochemical study.

Methods for studying bio-chemical parameters:
Estimation of Serum AST levels: Serum AST levels was estimated by kinetic test UV-optimized IFCC method (J. Clin. Chem. ClinBiochem 1986; 24:497). The concentration of enzyme was measured in spectrophotometer (Micro Flow Cell Photometer AE-100F ERMA Inc) at wavelength 340 nm.

Estimation of GSH concentration in erythrocyte: GSH concentration in erythrocyte was estimated by Ellman's method[25]. The color intensity was measured by the spectrophotometer (UV–VIS spectrophotometer) at 412 nm wavelength.

Estimation of Plasma MDA level: Plasma MDA level was estimated by Thiobarbituric acid method[26]. The absorbance was measured using spectrophotometer at 532 nm.

Estimation of Serum vitamin E level: Serum vitamin E level was estimated by colorimetric method[27]. Tocopherols and carotenes are first extracted into xylene and the absorbance is read at 460 nm to measure the carotenes. A correction for the carotenes is made after adding ferric chloride and reading at 520nm.

The quantitative variables were expressed as mean±SD. ANOVA (multiple comparisons) was done for statistical analysis. Post hoc analyses of differences were done by Bonferroni test.

Result

Results showed that administration of enalapril or valsartan did not cause any significant change in serum AST, plasma MDA, erythrocyte GSH and

serum vitamin E levels when compared with control (Table I).

Serum AST level in the adrenaline-treated rats and control group were 116.86±21.49 U/L and 25.46±3.51 U/L respectively. The rise in serum AST level was significant (p<0.001). Twenty four hours after the 2nd injection of adrenaline, serum AST level in enalapril and valsartan pre-treated group was 57.6±6.88 U/L and 54.82±8.08 U/L respectively. The decrease in serum AST level in both the pre-treated group as compared to only adrenaline treated group was significant (p<0.001) (Table I). Enalapril and valsartan pre-treatment decreased the serum AST level by 50.71% and 53.09% respectively (Table II).

Twenty four hours after the 2nd injection of adrenaline, plasma MDA level in the adrenaline treated rats and control group were 1.22±0.12 µmol/L and 0.39 ± 0.09 µmol/L respectively. The rise in the plasma MDA level was significant (p<0.001). Plasma MDA level in enalapril pre-treated group was 0.74±0.12 and in valsartan pre-treated group was 0.91±0.08 µmol/L after the 2nd injection of adrenaline. In the pre-treated groups, the decrease in plasma MDA level as compared to only adrenaline treated group was significant (p<0.001) (Table I). Pre-treatment with enalapril and valsartan reduced plasma MDA level by 39.34% and 25.41% respectively (Table II).

The concentration of erythrocyte GSH in adrenaline treated group and control group were 0.57±0.17 mg/gmHb and 1.26±0.13 mg/gmHb 24 hours after the 2nd injection of adrenaline respectively. The reduction in concentration of erythrocyte GSH concentration was significant (p<0.001). After the 2nd injection of adrenaline, the concentration of erythrocyte GSH in enalapril and valsartan pre-treated groups were 1.09±0.26 and 0.98 ± 0.14 mg/gmHb respectively. The change in the concentration of erythrocyte GSH in enalapril pre-treated group (p<0.01) and in valsartan pre-treated group (p<0.05) (Table I) as compared to only adrenaline treated group was significant. Enalapril pre-treatment increased 42.11% and valsartan pre-treatment increased 40.35% erythrocyte GSH concentration in adrenaline treated rats (Table II).

Serum vitamin E level in the adrenaline-treated rats and in the control group 24 hours after the 2nd injection of adrenaline were 6.20±1.33 mg/L and 11.22 ± 0.96 mg/L respectively. The decrease in the serum vitamin E level was significant (p<0.001). Serum vitamin E level in enalapril pre-treated group was 9.27±0.9 mg/L and in valsartan pre-treated group was 8.95±1.27 mg/L after the 2nd

injection of adrenaline. The attenuation in serum vitamin E levels by enalapril and valsartan pre-treatment as compared to only adrenaline treated group was significant (p<0.01) (Table I). Treatment with enalapril and valsartan increased serum vitamin E level by 49.52% and 44.34% respectively (Table II).

The change in serum AST level when compared between enalapril and valsartan pre-treated groups, the change was not significant (p>0.05). Also the changes in plasma MDA, erythrocyte GSH and serum vitamin E levels in enalapril and valsartan pre-treated groups when compared with each other, they were not significant (p>0.05) (Table II).

Plasma MDA to serum vitamin E ratio (Plasma MDA/serum vitamin E) is calculated. The unit of serum vitamin E is converted to µmol/L from mg/L (Table III).

Table I: Serum AST, plasma MDA, erythrocyte GSH and serum vitamin E levels after adrenaline administration in enalapril and valsartan pre-treated rats

Groups	Serum AST (U/L) (Mean±SD)	Plasma MDA(µmol/L) (Mean±SD)	Erythrocyte GSH (mg/gmHb) (Mean±SD)	Serum vitamin E (mg/l) (Mean±SD)
Control (DW) 1 ml (Gr – I)	25.46±3.51	0.39±0.09	1.26±0.13	11.22±0.96
Enalapril 30mg/kg bw (Gr – II)	34.03±4.03$^a_0$	0.38±0.09$^a_0$	1.26±0.14$^a_0$	12.46±1.32$^a_0$
Valsartan 30mg/kg bw (Gr – III)	35.77±9.21$^b_0$	0.42±0.08$^b_0$	1.24±0.21$^b_0$	11.87±1.61$^b_0$
Adrenaline 2mg/kg bw (Gr – IV)	116.86±21.49$^c_3$	1.22±0.12$^c_3$	0.57±0.17$^c_3$	6.20±1.33$^c_3$
Enalapril 30 mg/kg bw + Inj. Adrenaline 2mg/kg bw (Gr – V)	57.6±6.88$^d_3$	0.74±0.12$^d_3$	1.09±0.26 $^d_2$	9.27±0.9 $^d_2$
Valsartan 30 mg/kg bw + Inj. Adrenaline 2 mg/kg bw (Gr –VI)	54.82±8.08$^e_3$	0.91±0.08$^e_3$	0.98±0.14 $^e_1$	8.95±1.27 $^e_2$

Values were expressed as mean±SD

a_0 p> 0.05 Gr II versus Gr I	d_3 p< 0.001 Gr IV versus Gr V
b_0 p> 0.05 Gr III versus Gr I	e_1 p< 0.05 Gr IV versus Gr VI
c_3 p< 0.001 Gr IV versus Gr I	e_2 p< 0.01 Gr IV versus Gr VI
d_2 p< 0.01 Gr IV versus Gr V	e_3 p< 0.001 Gr IVversus Gr VI

Table II: Intergroup variation with percentage of increase or decrease

Variable	Enalapril+Adrenaline (Gr - V)	Valsartan+Adranaline (Gr – VI)	Level of Significance
Serum AST	50.71% ↓	53.09% ↓	1.000^{NS}
Plasma MDA	39.34% ↓	25.41% ↓	0.150^{NS}
Erythrocyte GSH	42.11% ↑	40.35% ↑	1.000^{NS}
Serum vitamin E	49.52% ↑	44.34% ↑	1.000^{NS}

↓ =Decrease NS= Not significant
↑ =Increase

Table III: Ratio of plasma MDA to serum vitamin E

Variable	(Enalapril + Adrenaline) Group V	(Valsartan + Adrenaline) Group VI
Plasma MDA/serum vitamin E ratio (µmol per liter/ µmol per liter)	0.019	0.024

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Discussion

Reactive oxygen species (ROS) generation during AMI are known to play a major role in causing necrosis of myocardial tissue and Ang II contributes in part to ROS production in heart via Ang II type 1 (AT_1) receptor mediated induction of NADPH oxidase which might result in myocardial damage by increasing oxidative stress[28]. This was known earlier that antioxidants and some cardioprotective drugs that eliminate pro-oxidants and scavange free radicals may provide protection against ischemic myocardial damage[29]. Ang II receptor blockers (ARBs) and ACE inhibitors can inhibit the source of ROS in the heart. Thus these agents may represent casual antioxidants that eliminate injurious oxidative stress in the heart. The aim of the present study is to evaluate and compare the protective effect of AT_1 receptor blocker and ACE inhibitor in adrenaline induced oxidative stress in experimental MI in rats. The results of this study showed that pretreatment of valsartan (30 mg/kg) or enalapril (30 mg/kg) for 2 weeks before administration of adrenaline (2 mg/kg in two subsequent doses, 24 hours apart) afforded cardioprotection as evidenced by significant reduction in serum AST level and markers of oxidative stress.

In this investigation, significant rise in serum AST level was found after 24 hours of adrenaline treatment. AST starts to rise about 12 hours after infarction and reaches a peak on the 1st or 2nd day[30]. The sera of rats with myocardial necrosis were worthy of particular attention because of the possibility that destruction of cardiac muscle, rich in transaminase activity, results in a release of this enzyme into blood stream and thus increase the serum transaminase activity. An increase in oxidative stress after adrenaline treatment was revealed by a significant increase in plasma MDA level and a significant decrease in erythrocyte GSH concentration and serum vitamin E level. The rise in MDA level indicates enhanced lipid peroxidation which is one of the important manifestations of oxidative damage initiated by ROS[31]. Couple of researchers[31], in their study showed that myocardial injury induced in rats with Isoproterenol for 2 days resulted in a marked elevation of lipid peroxidation, serum marker enzymes and a significant decrease in activities of endogenous antioxidants such as reduced GSH. Some others[32] demonstrated increased concentration of plasma MDA and decreased level of plasma GSH and vit E in AMI patients at the time of admittance to the hospital. Similarly significant rise in erythrocyte MDA level and significant fall in GSH level were observed in MI patients by some researchers. Our results

indicate that adrenaline has got the ability to produce ROS which enhance lipid peroxidation, a detoriative process in myocardial cells that results in the increase in serum AST levels. Oxygen derived free radicals are generated particularly in the early stage of MI and GSH is involved in the reduction of hydrogen peroxide radicals, resulting in a decrease in GSH levels during that period[33]. Prolonged exposure to oxidative stress (as seen in MI), was found to result in depletion of the defense system which might decrease GSH activity[34]. The antioxidant vitamin E was found to be decreased in plasma of AMI patients in previous studies[35,36]. The finding of the present study correlates with those studies. The antioxidant vitamin E interrupts lipid peroxidation by scavenging peroxyl radical intermediately[37]. The decreased erythrocyte GSH and plasma vitamin E level may be associated with enhanced protective mechanism to oxidative stress in MI patients.

Ang II is regarded as a pro-oxidant because it induces $^{\cdot}O_2^{-}$ release via activation of membrane bound NADPH-oxidases[38]. A number of studies documented that ARBs have antioxidant properties most likely due to modulation of NADPH oxidase activity[39]. In our study pre-treatment with enalapril or valsartan caused significant decrease in the erythrocyte MDA level 24 hours after adrenaline administration. In a study by some researchers[40], it was seen that enalapril significantly decreased the level of plasma MDA in atherosclerotic rabbit compared to the control group. They explained that enalapril a nonsulfhydryl containing ACE inhibitor, reduces MDA level by inhibiting lipid peroxidation. Another group[41] investigated the vascular effects of ARB in hypertensive patients and found that compared with control, ARB significantly reduced plasma MDA level. Couple of researchers[42] examined the effect of AT_1 receptor blocker on atherosclerosis and found that MDA was reduced compared to control. Inhibition of lipid peroxidative effect in this study caused by enalapril and valsartan was 39.34% and 25.41% respectively. However, difference in antiperoxidative effect was not significant when compared between these two drugs. In this investigation, enalapril or valsartan pre-treatment for 14 days significantly increased erythrocyte GSH concentration and plasma vitamin E level in adrenaline treated rats. The effects of enalapril and valsartan on erythrocyte GSH level were 42.11% and 40.35% respectively and this difference was not significant. On the other hand, the effects of enalapril and valsartan on plasma vitamin E level were 49.52% and 44.34% respectively and the difference was not significant. Another group of researchers[43] investigated glutathione content of

various tissues and reported that enalapril administration was able to enhance total glutathione content in various tissues including erythrocytes. On the other hand, some others examined the changes in oxidative stress parameters by blocking the RAS at AT$_2$ receptor site and found that ARB significantly increased GSH and CAT activity and oxidative stress was markedly reduced. They concluded that ARB appears to modulate free radical production, increase antioxidants and reduce oxidative stress. In our study enalapril and valsartan pretreatment significantly increased plasma vitamin E level in adrenaline treated rats. The plasma MDA to serum vitamin E ratio was calculated and the ratio was found to be low and it indicates amelioration of oxidative stress in enalapril or valsartan pretreated groups. The possible mechanism of reduction of oxidative stress could be that both of these drugs act by inhibiting RAS pathway and resulting in a decrease in the activity of Ang II. Ang II is recognized to be one of the major contributors to the progression of heart disease through generation of ROS. By inhibiting this pathway these two drugs reduce ROS generation which ultimately raises the endogenous antioxidant vitamin E.

It may be concluded that ACE inhibitor and angiotensin II receptor blocker (ARB) both can afford cardioprotection by reducing oxidative stress as evidenced by a decrease in plasma MDA level, an increase in erythrocyte GSH concentration and serum vitamin E level and low MDA to vitamin E ratio. However, cardioprotective effect of ARB is not superior to ACE inhibitor.

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