Endothelin-1 induced alterations in oxidative-nitrosative stress markers

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ABSTRACT

Objective: The aim of this preliminary study is to investigate the effects of exogenous Endothelin-1 (ET-1) on systolic blood pressure and heart rate as well as on plasma nitric oxide metabolites, malondialdehyde, copper and zinc concentrations and red cell superoxide dismutase and catalase activities.

Methods: Thirty Wistar-Albino male rats, 8-10 weeks old, with a mean body weight of 285 gm were used in the study. Daily systolic blood pressures were measured by tail plethysmography. Following exogenous administration of ET-1 (1 nmol/kg) systolic arterial blood pressures were recorded and blood samples of control and experimental groups were drawn. Nitric oxide metabolites (nitrite, nitrate), malondialdehyde, copper, zinc concentrations in plasma, superoxide dismutase and catalase activities and copper, zinc concentrations in red cell were determined both in control and experimental groups. All laboratory procedures were performed at the Department of Pathophysiology, School of Medicine, Ankara University, Ankara, Turkey in 2003.

Results: There were statistically significant increases in plasma nitrate, red cell superoxide dismutase activity, systolic arterial blood pressure and statistically significant decreases in red cell catalase activity, plasma copper, red cell zinc concentrations in experimental group due to exogenous ET-1 administration compared to controls.

Conclusion: There appears an important interaction between exogenous ET-1 and oxidative-nitrosative stress markers which may affect the progression of hypertension.

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Vascular endothelium plays a crucial role in the maintenance of circulatory homeostasis and blood pressure. Imbalance between synthesis, release and effect of endothelial factors capable of vasodilation and vasoconstriction causes an endothelial dysfunction.[1-3] Regulation of vascular smooth muscle tone is induced by either vasoconstriction or vasodilation by means of Endothelin-1 (ET-1) and nitric oxide (NO).[2,4,5] Alterations in NO synthesis may be the most widely used indicator to evaluate the degree of endothelial dysfunction in the last decade.[2,6] Among many factors that lead to endothelial dysfunction, reactive oxygen species (ROS) seems to play an important role by reacting with NO. Virtually all cells of the vascular wall are potential sources for production of ROS. Low concentrations of superoxide anions (O_2^-) are continuously formed, and remain low in physiological situations. Antioxidant enzyme red cell copper-zinc superoxide dismutase (Cu/Zn-SOD) dismutates these anions to hydrogen peroxide, which needs trace elements zinc and copper for its stability and activity. However, in pathological situations, in which large amounts of superoxide anions are produced, NO will combine rapidly with this anion to form peroxynitrite radical. Peroxynitrite causes intense cellular damage, as it is rapidly protonated to yield the highly reactive

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peroxynitrous acid generating hydroxyl radical (OH^-).[2,7] This cascade of events will finally cause attenuation of NO mediated vasodilatation. Thus, any factor which enhance vascular O_2^- formation may significantly contribute to endothelial dysfunction and finally to vasoconstruction.[7-10] Endothelin-1, which is a potent stimulator of protein kinase C (PKC) in vascular smooth muscle cells, increases O_2^- formation in vascular wall.[7]

Following the ET-1 administration, the high production of ROS; NO, lipid peroxides, and ROS scavenging systems particularly intracellular antioxidant enzymes might be affected. Considering the inter-relationship between these factors, the purpose of this study was to investigate the effects of ET-1 administration on mean arterial pressure elevation, NO metabolites, malondialdehyde (MDA) concentration, Cu/Zn-SOD and catalase activities, and also on zinc, copper concentrations.

Methods. Thirty Wistar Albino male rats, 8-10 weeks old, were housed in individual cages in a climate-controlled room with an ambient temperature of 22 ± 1^{0}C and were in a light regulated space for 15 days. The rats in control group were given standard diet (10 g/day) and water (20 g/day) as experimental group. At the beginning of the study, the mean body weights of control group was 281 gm and experimental group was 289 gm. After 10 days of training, the mean body weights of control group was 288 gm and experimental group was 295 gm.

During 10 days training, daily measurements of blood pressure by tail-cuff plethysmography were performed. Before arterial blood pressure measurements rats were taken to a heated cage (maximum 32^{0}C) and allowed to rest inside the cage for 15 minutes. A minimum of 4 consecutive measurements were taken and were recorded on the computer. The heart rates were also measured and recorded. In this study, non-ischemic hearts were first perfused under baseline conditions for 30 minutes. Subsequently, a bolus of 1 nmol/kg ET-1 was administered. For this administration, exogenous ET-1 was diluted with isotonic saline solution in a 1:1 ratio. Following exogenous administration of ET-1 (1 nmol/kg) systolic arterial blood pressures were recorded and blood samples of control (n=10) and experimental (n=20) groups were drawn and put in the heparinized tubes. Plasma kept in polypropylene tubes and each sample was studied rapidly. Red cell Cu/Zn-SOD[11] and catalase[12] activities, plasma MDA concentration[13] and plasma total nitrite, nitrite, nitrate concentrations[14,15] were determined spectrophotometrically. For NO metabolites measurement, first total nitrite concentration and endogenous nitrite concentration were determined, then the nitrate concentration was calculated by subtracting the endogenous nitrite concentration from the total nitrite concentration. Also, in this study, plasma and red cell zinc and copper concentrations were determined by atomic absorption spectrophotometry, according to Perkin-Elmer's principles.[16,17] All laboratory procedures were performed at the Department of Pathophysiology, School of Medicine, Ankara University, Ankara, Turkey in 2003.

For statistical analysis of data, Student's t-test, Spearman Rank Correlation Analysis were used. The statistical analyses were performed in the Department of Biostatistics, School of Medicine, Ankara University. All data were expressed as mean ± SD.

Results. All results are shown on **Table 1** After ET-1 administration, a progressive rise in systolic blood pressure and a progressive decline in heart rate was observed in experimental group compared to controls. Also, in experimental group, red cell Cu/Zn-SOD activity was significantly higher while red cell catalase activity was significantly lower than controls. Nitric oxide metabolite, nitrate concentration in plasma, was higher in experimental group. Plasma copper and red cell zinc concentrations of experimental group were found to be lower compared to controls.

Discussion. Endothelin activates phospholipase C, which leads to an accumulation of inositol triphosphate and intracellular calcium and, in turn, to long-lasting vasoconstriction.[2,18] Once, it was released into the bloodstream, endothelin rapidly attaches itself to the tissue, and is quickly broken down by an enzyme in the plasma. For these reasons, it always has a very low plasmatic concentration.[2] Thus, endothelins' plasma levels are usually normal in hypertension but treatment of hypertensive patients with ET antagonists causes a decrease in blood pressure, suggesting that ET-1 contributes to endothelial dysfunction in hypertension.[19,20] As Ikeda et al[21] reported before, exogenous ET-1 causes an initial transient decrease followed by a sustained increase in systolic blood pressure in rats. In the studies that endothelial cell monolayers were investigated for preproET-1 messenger RNA, a modest increase were seen at 30 minutes.[5,22] In our study, the maximal increase in systolic arterial blood pressure was recorded at 30 minutes, which was the time that exogenous ET-1 showed the maximum biological effect ($p<0.001$) and a decrease in the heart rate was accompanied ($p<0.05$). As mentioned in various studies, systemic doses of ET-1 have a greater pressor effect on experimental models of arterial hypertension.[2] Infusion of ET-1 with high salt intake caused hypertension in rats.[5,23] Similarly, in the

salt-sensitive volume-loaded type of hypertension, the hypertensive rats, showed increased expression of preproET-1 mRNA. In one study, it was also found that the increased level of preproET-1 mRNA was linearly correlated with an increase in blood pressure levels.[21] Hence, ET-1 induces significant increase in arterial blood pressure.[20]

Endothelin has been shown to increase O_2^- production in various tissues. The stimulation of O_2^- production by ET-1 results in a strong attenuation of vasodilatation.[7] Increased production of superoxide radicals consequently leads to significant increase in dismutation reactions. Actually, red cell Cu/Zn-SOD activity in this study was significantly higher (p<0.001). Although, the effectiveness of red cell Cu/Zn-SOD enzyme activity was augmented significantly, red cell catalase enzyme activity was significantly low (p<0.001). The authors considered this as an inhibition of catalase enzyme by increased production of O_2^-. On the other hand, some investigators were determined that intravenous injection of SOD decreases arterial blood pressure in spontaneously hypertensive rats, this data also supports the hypothesis on the increased production of ROS in hypertension pathogenesis and our results confirm these findings.[2,24] One of lipid peroxidation markers, plasma MDA concentration was also measured in this study. Following ET-1 administration, in 30 minutes, MDA concentration was found only to be slightly increased. In different studies, plasma levels of nitrites/nitrates are increased in normotensive rats made hypertensive following clipping of one renal artery.[8] Nitric oxide production by endothelial cells is regulated by mechanical factors such as shear stress, cyclic strain and stretch. In spontanea hypertensive rats, it has been reported that mechanical factors induces NO production by augmenting endothelial NO synthase (eNOS), this suggests that eNOS has possible physiological adaptation to hemodynamic forces which are increased in hypertension.[6,8] In this study, plasma nitrate concentration of experimental group was found to be higher when compared to control group (p<0.05). Hence, increased nitrate concentration in our study suggested that there appears an augmentation in NO production after ET-1 induced elevation in arterial blood pressure.

In this study, the investigators found that red cell zinc concentration decreased (p<0.05). As zinc has an important role in red cell Cu/Zn-SOD enzyme stability, this finding could be considered as a result of increased activity of Cu/Zn-SOD enzyme. In this condition, decreased red cell zinc concentration might be the result of increased uptake and utilization of the element intracellularly. Indeed, zinc has been shown to antagonize the catalytic properties of the redox-active transition metals, such as copper, promoting formation of hydroxyl radical from hydrogen peroxide and superoxide. However, in this study with lower zinc concentration in the cell, metal catalyzed formation of hydroxyl radical

Table 1 - Systolic blood pressure, heart rate, oxidative-nitrosative stress markers, and zinc-copper concentrations.

Parameters	Controls (X ± SD)	Experimental group (X ± SD)	Statistical analysis
Systolic blood pressure (mm Hg) (initial)	130 ± 7	152 ± 11	p<0.001
Heart rate (beat/min) (initial)	349 ± 33	346 ± 68	p>0.05
Systolic blood pressure (mm Hg) (after ET-1)	125 ± 8	189 ± 20	p<0.001
Heart rate (beat/min) (after ET-1)	328 ± 19	305 ± 71	p<0.05
Red cell copper-zinc superoxide dismutase activity (U/g Hb)	4530 ± 387	5679 ±3 25	p<0.001
Red cell catalase activity (k/g Hb)	167 ± 2	161 ± 1	p<0.001
Plasma malondialdehyde concentration (nmol/mL)	10.203 ± 2.056	10.759 ± 2.160	p>0.05
Plasma total nitrite concentration (µmol/L)	53.225 ± 11.570	57.006 ± 12.276	p>0.05
Plasma nitrite concentration (µmol/L)	21.912 ± 3.910	20.850 ± 4.959	p>0.05
Plasma nitrate concentration (µmol/L)	28.573 ± 6.368	36.757 ± 9.286	p<0.05
Plasma zinc concentration (µg/dL)	111.729 ± 9.968	109.980 ± 11.898	p>0.05
Plasma copper concentration (µg/dL)	113.143 ± 7.198	101.600 ± 10.733	p<0.05
Red cell zinc concentration (µg/mL)	4.500 ± 0.258	4.125 ± 0.403	p<0.05
Red cell copper concentration (µg/mL)	0.259 ± 0.089	0.261 ± 0.081	p>0.05
Body weight (at the beginning) (g)	281 ± 13	289 ± 23	p>0.05
Body weight (at the end) (g)	288 ± 14	295 ± 26	p>0.05

may become accelerated.[25] Under such an oxidative stress circumstances, organism needs to increased defensive mechanisms and antioxidant enzymes' activity against the destructive factors. Under this condition, the concentration of the transition metal copper, have another important role in Cu/Zn-SOD activity, was expected to show a decrease in plasma and red cell due to increased utilization. As expected, our results showed that the plasma copper concentration was significantly decreased when compared with controls ($p<0.05$), but unfortunately red cell copper concentration did not show any statistical difference.

In conclusion, ET-1 induced marked increase in mean arterial blood pressure as well as an increase in nitrate concentration and significant alterations in antioxidant enzyme activities with corresponding changes in copper and zinc concentrations. These results suggested that ET-1 significantly alters oxidative and nitrosative stress markers.

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