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Plasma Antioxidant Status and Nitrate Levels in Patients With Hypertension and Coronary Heart Disease

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Abstract: In this study, we investigated first; the possible involvement of nitric oxide and antioxidant enzyme level in the pathogenesis of essential hypertension and second we investigated serum nitrite-nitrate and antioxidant enzyme activities and lipid profiles (total, HDL and LDL cholesterol, trygliceride) to determine the relationship between these parameters in atherosclerosis. 13 patients with coronary heart disease, 18 patients with essential hypertension and 16 age-matched health subjects were taken into this study. Plasma nitrite, nitrate levels were measured by Griess reaction, erythrocyter superoxide dismutase and catalase activities were determined by the methods of Misra&Fridovich and Aebi, respectively. Serum lipid analysis were performed in the autoanalyser. A decrease in superoxide dismutase activity and nitrite&nitrate levels, along with an increase in total cholesterol, LDL-cholesterol and triglyceride values were found in patients with hypertension. There was a positive correlation between nitrite and HDL-cholesterol levels in the same patient group. A decrease in superoxide dismutase activity and nitrate levels and no significant difference in nitrate, LDL-cholesterol, total cholesterol, triglyceride levels were detected in patients with coronary heart disease. On the other hand, catalase activities in both of the groups did not show any significant difference. These findings focus the attention to interactions between nitric oxide, antioxidant status and atherogenesis and propose that nitric oxide may be important physiopathologically in essential hypertension and atherosclerotic heart failure.

Key Words: Nitric oxide, antioxidant enzymes, hypertension, coronary heart disease.

Introduction

Nowadays, nitric oxide (NO) is a current molecule of interest because of its effects on vascular endothelium. Vascular endothelium produces NO continuously and the roles of NO in various diseases are still being studies. Studies using ^{15}N-labelled L-Arginine have clearly demonstrated that L-Arginin is a substrate for the generation of NO(1). It's synthesized in the cells by the enzyme NO-synthase and it's inactivated by free oxygen or superoxide anion and eliminated as nitrite and nitrate. The suggested process, the formation of nitrate and metHb from NO or nitrite together with HbO_2, yields products that are either eliminated via renal excretion (2) or reversed with some endogenous mechanisms (3, 4).

NO (EDRF) released by endothelial cells stimulates the enzyme solubl guanylate cyclase to mediate muscle relataxion and maintain a vasodilator role in the cardiovascular system. The biological profile of EDRF is nearly identical to that of NO (5) and experimental evidence indicates that EDRF is either NO or a nitroso compound that releases NO (6, 7). Specifically NO exerts potent vasodilator actions (8, 9). Numerous studies have been carried out to investigate the use of L-Arginine which is the precursor of NO for reducing the blood pressure in hypertension (10). In several diseases which manifest with hypertension like essential hypertension and chronic renal failure, the reduction of NO production may be important physiopathologically (10).

To date, a number of cardiovascular risc factors have been identified that may affect endothelial function and in turn mediate vascular disease and its complications. Hyperçholesterolemia and oxidized LDL are factors which are well known in this pathogenesis. It was suggested that oxygen derived free radicalls and NO can initiate lipid

peroxidation in LDL and so contribute to the pathogenesis of atherosclerosis.

Since it is known that antioxidant enzymes and NO metabolism may be important factors in the pathophysiology of coronary heart diseases and essential hypertension, plasma levels of nitrite and nitrate which are end-products of NO, and erythrocyter superoxide dismutase (SOD) and catalase activities in patients with coronary heart disease and essential hypertension have been determined in this study to investigate the possimle involvement of NO and antioxidant enzyme levels in the pathogenesis of these diseases.

Materials and Methods

Patients:

18 patients with essential hypertension (group 1) and 13 patients with coronary heart disease (group 2) and 16 healthy, age matched controls (group 3) composed our study population. All of them were informed about this study and consent was provided as recommended in the Declaration of Helsinki Ethical Guidelines.

Hypertensive patients (12 women and 6 men, mean age of 54.0±12.1) This group consisted of patients who have been diagnosed as essential hypertension for the first time or patients who were not normotensive although they have been using antihypertensive drugs for several months. All patients had outpatient casual measurements of BP>140/90 mm/Hg on three separate occassions. The criteria for inclusion were a) Left ventricule hypertrophy proved by echocardiographic measurements (septal thickness and posterior wall thickness in enddiastole>11mm), b) no evidence of heart failure, myocardial infarction, angina pectoris or of complicating congenital or valvular heart disease, c) no concomitant therapy.

Patients with coronary heart disease (7 women and 6 men, mean age of 63.8±8.4) This group consisted of patients who have been diagnosed clinically and with laboratory (ECG, Echocardiography,). All of them had typical angina (retrosternal chest pain on exercise, decreasing of the pain in ten minutes on rest, disappearing of the pain by sublingual nitrite in five minutes) and a significant ST-segment depression in the precordial leads on their ECG during exercise or at rest. When left ventriculer motion on echocardiography was evaluated, segmental hypokinesia or akinesia was determined. All of them had normal ejection fraction and IVS/PW ratio eco-cardiographically. Coronary heart patients with hypertension were excluded. None of the patients had a history of prior myocardial infarction or previous cardiac surgery. There was no important concomitant disease.

Controls: Sixteen normal subjects (eight women and eight men, mean age of 50.4±6.0)with no history of cardiac or coronary disease, with a normal ECG and physical examination were served as controls.

Blood parameters:

All patients stopped using durgs and feeding foods including nitrite, 5 days prior to the drawing of their fasting blood samples into tubes including heparin. Nitrite and nitrate that were the end products of NO in plasma, SOD and Catalase in erythrocytes and their lipid profile (Total Cholesterol, LDL, Triglyceride, HDL, VLDL) in serum were detected.

Nitrite-nitrate assay: Plasma nitrite levels were determined by a colorimetric method based on the Griess reaction (11). Nitrate and nitrite levels were measured by the same assay after enzymatic reduction of nitrate to nitrite with nitrate reductase from Escherichia coli. The nitrate concentration was calculated from the difference between total nitrite concentrations after and before the bacterial reduction. After enzymatic reduction to nitrite, we also compared total nitrite levels in group 1 and 2 with controls.

SOD activity: After separation of plasma, the packed erythrocytes were washed two times 9 g/l NaCl solution and haemolysed with ice-cold water. SOD and Catalase activities were determined immediately in haemolysates. The hemoglobin values of these haemolystates were determined with Drabkin's method. SOD activities were measured according to Misra & Fridovich based on the inhibition of autoxidation of epinehrine by SOD at 480 nm in a LKB Ultraspec-2 spectrophotometer. The activity of enzyme that causes 50% inhibition of epinephrine autoxidation is defined as 1 unit (12, 13).

Catalase activity: Catalase levels were determined as described by Aebi. The Catalase mediated decomposition of H_2O_2 was followed directly at 240 nm. One unit of Catalase activity is defined as the level of enzyme required to decompose 1 Micromal H_2O_2 in 1 minute (14-16).

Total cholesterol, tryglyceride and HLD-cholesterol assay: Serum total Cholesterol, HDL and Tryglyceride levels were measured in RA-Xt auto-analyzer by using Biotrol kit. LDL-cholesterol was calculated using the Fridewald formula.

Statistical analysis:

Table 1. Results of patients with hypertension, coronary heart disease and control subjects.

	GROUP 1	GROUP 2	GROUP 3
	n=18	n=13	n=16
BMI kg/m2	29.0±4.6*	26.6±3.9*	23.7±1.5
SBPmmHg	192.7±22*	130.0±14	124.3±16
DBPmmHg	115.0±7*	76.0±8	79.6±6
LDLmg/dl	166±4**	145±43	123±16
T.Cholesterol mg/dl	252±53*	226±41	197±23
Triglyceride mg/dl	197±70*	141±45	130±17
HDLmg/dl	43,5±8,9	37±7,8**	44±6,9

Data were given as Mean±SEM.*p<0.01, **p<0.05, treatment versus control.
Hypertensive patients, patients with coronary heart disease and controls were seen in Group-1, Group-2 and Group-3 respectively.
SBP: Sistolic Blood Pressure and DBP: Diastolic Blood Pressure

Table 2. The findings of women and men in group-1, group-2 and group-3.

	GROUP 1	GROUP 2	GROUP 3
Women SOD (U/g Hb)	1576.6±766	1843.4±866	2919.7±1162
Men	1664.1±626.7	1953.8±942	3322.5±923.9
Women Catalese (U/g Hb)	10334.1±1416.6	10708.1±2086	10117.7±1602.9
Men	10807.5±1803.4	12547.8±2915	9879.2±1071
Women Nitrite (µmol/l)	5.2±2.6	8.7±4.5	7.5±2.5
Men	3.7±1.6	7.7±5.0	8.8±3.5
Women Nitrate (µmol/l)	8.6±3.5	8.9±4.3	13.3±2.7
Men	7.1±3.9	8.1±4.6	11.1±1.1

Data were given as Mean ±SEM.*p<0.01, **p<0.05, treatment versus control.
Hypertensive patients, patients with coronary heart disease and controls were seen in Group-1, Group-2 and Group-3 respectively.

Statistical analysis were performed with the Mann Whitney nonparametric test and Spearman's rank correlation in the ANOVA statistical program.

Results

General characteristics and serum cholesterol, trygliceride and HDL-cholesterol levels of patients are shown in Table-1. Total cholesterol in group 1 was higher than group 3 (p<0.05). Serum LDL (p<0,05) and Triglyceride (p<0,01) levels were also higher in group 1 than group 3. We couldn't find a significant difference in HDL values between group 1 and group 3. There weren't any difference in LDL, cholesterol and triglyceride levels between group 2 and group 3.

The data of antioxidant enzymes and nitrite,Nitrate levels both in men and women are given in Table-2. There was no significant difference in antioxidant enzymes and nitrite, nitrate levels between men and women in all three groups.

As it's shown in Table-3, there was significant difference in the SOD activities between Group 1 and group 3 (p<0.01). The means of the catalase activities in

Table 3. Antioxidant enzyme activities and nitrite-nitrate levels of patients and controls.

	GROUP 1	GROUP 2	GROUP 3
SOD U/g Hb	1605±745*	1902±868*	3121±1035
Catalase U/g Hb	10491±1519	11057±1919	9998±1322
Nitrite µmol/L	4,7±2,4**	7,5±3,8	8,1±3,0
Nitrate µmol/L	8,3±3,6**	7,6±2,9*	12,2±2,3
Total Nitrit µmol/L	13,0±5,3*	15,2±6,5**	20,4±3,0

Data were given as Mean ±SEM.*p<0.01, **p<0.05, treatment versus control.
Hypertensive patients, patients with coronary heart disease and controls were seen in Group-1, Group-2 and Group-3 respectively.

group 1 and group 3 were 10491±1519 U/gr Hb and 9998±1322 U/gr Hb and there wasn't any significant difference between them. Plasma nitrite level in group 1 was 4.7±2,4 µmol/L and 8,1±3 µmol/L in controls. It was significantly lower in group 1 than controls (p<0,05). Plasma nitrate levels were also lower in group 1 than group 3. Total nitrite levels of group 1 were also lower than group 3 (p<0.01). When we compared the group 2 with controls, we observed that SOD activities in group 2 showed a decrease compared to group 3 (p<0.01). There was not a difference in mean catalase activities between group 2 and group 3. Plasma nitrate levels were low in group 2 (p<0.01).

There was a significant difference in total nitrite levels between group 2 and group 3 and it was lower in group 2 (p<0.05). No correlation was observed between SOD and nitrite, nitrate and lipids. Only HDL levels of the group 2 was lower than group 3 (p<0,05). We didn't also find any correlation in group 2 between nitrite, nitrate and other lipid parameters. We found a positive correlation between nitrite and HDL values (r=0,4, p<0.05) and between catalase and HDL (r=0,4, p<0.03) in group 1.

Discussion

In recent papers, measurement of erythrocyter SOD and catalase activities was used as a marker showing the intracellular antioxidant status of the organism. In our study, we also determined the activities of these enzymes and found that the activity of SOD as an antioxidant enzyme, was low in group 1, but catalase activity was not found to be different between groups significantly. Because of the decreased level of SOD in hemolyzates in patients with essential hypertension, we assumed that oxidative stress was increased in these patients Kumar et al have suggested that an increase in free radical generation and a simulatenous decrease in the production of NO and antioxidants such as SOD and vitamine E occurs in essential hypertension (17). This increase in free radical generation can inactivate prostacyclin and NO and decrease their half life which can lead to an increase in peripheral vascular resistance and hypertension. Increased oxidative stress and the generation of the free oxygen radicals can also result in modification of LDL to oxide LDL that could lead to the formation of the type of fat filled cells in atherosclerotic lesions.

We found that the levels of nitrate and nitrite in plasma with hypertensive patients were significantly lower compared with controls, that's why we suggested that the production of NO or plasma levels of NO were deficient in the hypertensive group. NO which has local vasodilator effects on vascular endothelium, is a tonus regulator (18). Inhibitors of the L-Arginine pathway such as L-NNA or L-NAME cause endothelium dependent contractions in isolated vessels (19, 20) or hypertension in the intact organism (21). Radomsky et al have shown that the role of NO in blood vessel function is not restricted to regulation of vascular tone, but extends to vessel repair and maintenance (22).

In the hypertensive group (group I), therewas a significant difference in the level of cholesterol,

triglyceride, LDL, HDL compared with control group. Cholesterol, LDL and triglyceride were higher and HDL were lower than controls. In group I, we didn't find any significant correlation between cholesterol and nitrate, nitrite and we also didn't find a correlation between sistolic, diastolic tension and nitrite, nitrate values.

In this study, a decrease in SOD activity of patients with coronary heart disease when compared to controls were observed. Dubois Rande et al (23) and Mc Murray et al (24) have found also a decrease in antioxidant enzyme activities and increase in reactive oxygen products (MDA, TBARS) in patients with unstable angina and chronic heart failure, respectively. Buczynski et al have also showed an increase in platelet MDA and Thromboxane A2 and decrease in platelet SOD and catalase activities (25). This decrease in SOD activity as we also observed may be explained with the effect of increased oxygen-derived free radicals on SOD. It's known that, lower O_2^- concentrations induce the SOD activity while higher O_2^- concentrations inhibit. Furthermore catalase is activated in higher H_2O_2 concentrations while SOD is inhibited (26).

We assumed that the plasma NO values of patients with coronary heart disease were deficient too because of the nitrate levels in plasma of group 2 have been determined low. Although there was not any difference in the nitrite levels between group 2 and group 3, this observation was attributed to the fact that nitrite has a shorter half life than nitrate. Total nitrite levels of the group 2 were also significantly lower than group 3 but this deficency was mild compared with the hypertensive patients. Some recent papers indicate decreased production of NO in hypertensive patients, while others pointed an increase (27, 28, 29). Since impaired NO production accelarates the activation and aggregation of platelets and leucocytes in the lesion region, atherogenic pathway can be extended (8, 9, 30-32). On the other hand, NO is also a reactive oxygen compound and can react with other free radicalls such as superoxide anion (33) and may cause the poroduction of more destructive compounds. If the concentrations of SO and NO are each increased 10 fold, peroxynitrite will be formed at a 100 fold greater rate. Thus many pathological states including ischemia, inflammation can lead to the formation of large amounts of peroxynitrite (34). Peroxynitrite directly oxidizes important biological molecules (34) which result in modification of LDL to a form that could lead to the formation of the type of fat filled çells that accumulate in atherosclerotic lesions (35). Although there were some differences between group 2 and group 3 in LDL and total cholesterol and triglyceride values, these differences weren't statistically significant. Only HDL values of the group 2 were significantly lower than controls. Hypercholesterolemia causes a decrease in plasma NO level in according to NO synthase inhibitors (27, 28). Lefer AM and Max L investigated the relationship between the hypercholesterolemia and NO production by addition of L-arginine to isolated coronary arteries and they showed NO is an important protective agent produced by endothelium and may protect it against atherogenesis (28).

NO also exerts potent antiplatelet activity and inhibition of both neutrophil aggregation and adhesion (32, 36). The importance of NO as an inhibitor of neutrophil and endothelial cell interaction has been demonstrated by Kubes (37). Kubes et al showed that infusion of the NO Synthase antagonist L-N-Methylarginin induced a 10 fold increase in neutrophil adherence to the vessel wall (37).

Therefore, it has been known that the basal formation of NO maintains a moderate but significant vasodilation in the systemic vessels and counteracts platelet activity. Failure of the vascular endothelium to elicit NO-mediated vasodilation may be due to decreased formation, increased degradation, decreased sensitivity to the NO formed or a mixture of these factors. Irrespective of the mechanism behind, this is referred to as endothelial dysfunction. Endothelial dysfunction occurs in several cardiovascular settigns, like atherosclerosis, hypercholesterolemia, diabetes and essential hypertension. Endothelial dysfunction leads to an impaired tissue perfusion, increased local vascular resistance, decreased defense against thrombus formation. Occurence of oxygen derived free radicals may increase due to impaired tissue perfusion so endothelial dysfunction may be an aggravating factor in the atherosclerotic processes.

In conclusion, we observed that the levels of SOD, nitrite and nitrate in the essential hypertension and coronary heart disease groups were lower than controls. But we didn't observe any correlation between cholesterol, SOD, catalase and nitrite, nitrate. We suggest that oxidant stress and NO may have multipl effects on the initiation and progression of atherogenesis and may

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