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Research Paper

Correlations between Oxidative DNA Damage, Oxidative Stress and Coenzyme Q10 in Patients with Coronary Artery Disease

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Abstract

The correlation of coronary artery disease (CAD) with pro-oxidant/antioxidant balance and oxidative DNA damage was investigated.

Seventy-seven patients with CAD and 44 healthy individuals as control were included in this study. The comparative ratios of ubiquinol-10/ubiquinone-10, 8-hydroxy-2′-deoxyguanosine/ deoxyguanosine and the level of MDA measured by HPLC and the activities of GPX and SOD by colorimetric approach in blood samples obtained from patients with CAD were unraveled.

8-OHdG/dG ratios, serum MDA level and GPX activity were found significantly elevated level in serum of CAD patients compared to control group. The SOD activity was observed in stable levels in CAD patients. Ubiquinol-10/ubiquinone-10 ratio was significantly lower in patients with CAD than the controls.

The positive correlation was observed between 8-OHdG/dG ratios in both MDA levels and GPX activity, while the significant negative correlation was seemed between the ratio of 8-OHdG/dG and ubiquinol-10/ ubiquinone-10 as well as MDA levels and ubiquinol-10/ ubiquinone-10 ratio.

We conclude that, both the disruption of pro-oxidant/antioxidant balance and oxidative stress in DNA may play an important role in the pathogenesis of coronary artery disease.

Key words: CoenzymeQ10, 8-hydroxy-2′-deoxyguanosine, malondialdehyde, oxidative stress, antioxidants, coronary artery disease.

INTRODUCTION

Coronary artery disease (CAD) is one of the leading causes of morbidity and mortality in developed countries and is emerging as an epidemic in developing countries (1). It is well established that hypercholesterolemia is an important inducer of CAD (2). It has been known that there was a relation between the levels of DNA damage and the severity of the CAD (3). Published studies have demonstrated that DNA damage contributes significantly to the development and the progression of atherosclerosis (4,5).

Lipid peroxidation, which is mediated by free radicals, is considered to be the major mechanism of cell membrane destruction and cell damage. Free radicals are formed in both physiological and pathological conditions in mammalian tissues (6,7). The

recent studies showed that malondialdehyde (MDA) is an important marker of lipid peroxidation and progression of atherosclerosis is correlated with oxidative stress and can be followed up by MDA measurements (8).

Cells have an efficient antioxidant defence system, mainly composed of the enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX), which can scavenge the reactive oxygen species (ROS) produced by cellular metabolism, and make ROS level relatively stable under physiological conditions. Superoxide dismutase catalytically scavenges superoxide radicals (O_2^-), protecting against their potential cytotoxicity. It catalyzes the dismutation of highly reactive O_2^-, to O_2 and H_2O_2, which is a less reactive ROS (9).

Remnants of base and sugar groups, single-double strand breaks in the backbone and cross links to other molecules are produced as a consequence of DNA oxidation. Among the many types of oxidative DNA damage markers, 8-hydroxy-2′-deoxyguanosine (8-OHdG) is one of the most abundant oxidative products of cellular DNA damage. Since it can be detected by a high-performance liquid chromatography (HPLC) electrochemical detection method, it is sensitive and reliable (10, 11).

Coenzyme Q10 (CoQ10) is produced by the human body and is necessary for the basic functions of cells. CoQ10 is an important inhibitor of oxidative damage (12). The oxidized form of CoQ10 is called as ubiquinone-10, while the reduced form of CoQ10 is known as ubiquinol-10. It occurs in all cellular membranes, such as blood serum and serum lipoproteins. Ubiquinol offers efficient protection against the peroxidation of membrane phospholipids and serum low-density lipoproteins. Aside from its antioxidant role, CoQ10 is involved in electron transport and in adenine triphosphate synthesis associated with mitochondrial respiratory chain (13). Ubiquinol-10 is the first antioxidant to be oxidized when low-density lipoproteins are exposed to oxidants (14). Therefore, the ubiquinol-10/ubiquinone-10 ratio may be a sensitive marker for studying disturbances of the pro-oxidant-antioxidant balance in human blood (15). However, little is known about association of DNA damage with MDA, total antioxidant enzymes and CoQ10 in patients with CAD.

The aim of the present study was to investigate the relationship between oxidative DNA damage, pro-oxidant/antioxidant balance, lipid peroxidation and antioxidant enzymes in CAD. For this reason, we determined the 8-OHdG levels in leukocyte DNA as a specific marker of oxidative damage of DNA, serum MDA levels as the index of lipid peroxidation, the serum GPX and SOD activities as antioxidant enzymes, ubiquinol-10/ ubiquinone-10 ratio as pro-oxidant-antioxidant balance in patients with CAD.

PATIENTS AND METHODS

Patients

Subjects who were older than 18 years and admitted to institutional outpatient clinic with a symptom compatible with chronic coronary artery disease and had an indication for coronary angiography were subsequently enrolled to this study. Patients who had 50% or more coronary stenosis in at least one major coronary artery were included in coronary artery disease group, while those with angiographically-normal appearing coronary arteries (without stenosis or ectasia) served as healthy controls. Subjects who had other cardiac diseases including cardiomyopathy, left ventricular systolic dysfunction or severe heart failure (New York Heart Association class III or IV symptoms), valvular heart disease, experienced acute coronary syndrome in the preceding 30 days before angiography or those with a history of percutenous coronary intervention were excluded. Other criteria for exclusion included acute or chronic inflammatory disorders, immunological diseases, renal or liver failure, and previous or current neoplastic disease. A total of 77 patients with coronary artery disease and 44 healthy controls that fulfilled criteria were enrolled.

Data regarding to medical history, including smoking habits, presence of hypertension, diabetes mellitus and hyperlipidemia were recorded for all subjects. Our study was approved by local ethics committee of Yuzuncu Yil University Medical Faculty and all subjects gave their written consent before enrollment. This study was in accordance with second Declaration of Helsinki.

Samples

Venous blood samples were drawn from each patient before coronary angiography process to avoid oxidative stress caused by X-rays, following an overnight fasting period. Serum was obtained by centrifugation at 2500 rpm for 15 minutes and kept in deep freeze at -20°C (without thawing) until the study day.

Isolation and hydrolization of DNA

DNA isolation from blood was performed with some modifications (16). Two mL of blood with ethylene diamine tetraacetic acid (EDTA) was mixed with 3mL of erythrocyte lysis buffer, and incubation

for 10 min in ice was followed by centrifugation (10 min at 3500 rpm). The supernatant was decanted, and the pellet was thoroughly resuspended in sodium dodecyl sulphate (10%, v/v), proteinase K (20 mg/mL) and 1.9 mL leukocyte lysis buffer. The mixture was incubated at 65 °C for 1 h and then mixed with 0.8 mL of 9.5 M ammonium acetate. After centrifugation at 3500 rpm for 25 min, the clear supernatant (2 mL) was transferred to a new sterile tube, and DNA was precipitated by addition of 4 mL of ice-cold absolute ethanol. DNA samples were dissolved in Tris EDTA buffer (10 mM, pH 7.4), and then were hydrolyzed (17).

Analysis of 8-OHdG and dG by the HPLC method

In the hydrolyzed DNA samples, 8-OHdG and dG levels were measured respectively by electrochemical HPLC (HPLC-ECD) and variable wavelength detector HPLC (HPLC-UV) systems as previously described (18). Twenty µL of final hydrolysate were analysed by HPLC-ECD (HP, Agilent 1100 modular systems with HP 1049A ECD detector, Germany): Column, reverse phase-C18 (RP-C18) analytical column (250 mm × 4.6 mm × 4.0 µm, Phenomenex, CA). The mobile phase consisted of 0.05 M potassium phosphate buffer [pH 5.5] containing acetonitrile (97: 3, v/v) with a flow rate of 1 mL/min. The dG concentration was monitored based on absorbance (245 nm) and 8-OHdG based on the electrochemical reading (600 mV). Levels of dG and 8-OHdG were quantified using the standards of dG and 8-OHdG from sigma; the level of 8-OHdG was expressed as the number of 8-OHdG molecules per 106 dG.

Analysis of MDA by HPLC method

Measurement of serum MDA concentration was performed according to Khoschsorur et al. (19). Briefly, 50µL of plasma sample was mixed with 0.44 M H_3PO4 and 42 mM tiobarbituric acid (TBA), and incubated for 30 min in a boiling water bath. After rapidly cooling on ice, an equal volume of alkaline methanol was added to the sample, vigorously shaken, centrifuged (3000 rpm for 3 min), and the aqueous layer was removed. Then, 20 µL supernatant was analysed by HPLC (HP, Agilent 1100 modular systems with FLD detector, Germany): Column, RP-C18 (5 µm, 4.6 x 150 mm, Eclipse VDB- C18. Agilent); elution, methanol (40:60, v/v) containing 50 mM KH_2PO4 buffer (pH 6.8); flow rate, 0.8 mL/min. Fluorometric detection was performed with excitation at 527 nm and emission at 551 nm. The peak of the MDA-TBA adduct was calibrated as a 1,1,3,3- tetraethoxypropane standard solution carried out in exactly the same process as with the plasma sample.

Analysis of SOD activity by spectrophotometric method

Measurement of SOD enzyme activation was measured with Randox -Ransod enzyme kit and it was measured with autoanalyzer at 505 nm and 37 ^oC. Ten µl was taken from erythrocyte suspension and mixed with 2500 µl 0.01 M phosphate (pH = 7.0). Later, it was diluted 251 times with water (F = 251). Inhibition was obtained between 30-60% (20).

Analysis of GPX activity by spectrophotometric method

Measurement of GPX enzyme activation was performed according to Paglia and Valentina (21). GPX enzyme catalyzes oxidation of glutathione. When the oxidized glutathione is reduced, NADPH is oxidized and it is turned into NADP. This change was observed at 340 nm wave and activation of GPX was measured.

Analysis of Coenzyme Q10 by the HPLC method

Analyses of ubiquinol-10 and ubiquinon-10 were performed according to Mosca et al (22). This method is performed by forcing the oxidation of CoQ10 in the sample by treating it with para-benzoquinone followed by extraction with 1-propanol and direct injection into the HPLC apparatus. Pre-oxidation of the sample ensures quantification of total CoQ10 by UV detection. This method achieves a linear detector response for peak area measurements over the concentration range of 0.05 to 3.47 µmol/L. Diode array analysis of the peak level was consistent with the CoQ10 spectrum. Supplementation of the samples with known amounts of CoQ10 yielded a quantitative recovery of 96% to 98.5%; the method showed a level of quantitation of 1.23 nmol per HPLC injection (200 µL of propanol extract containing 33.3 µL of plasma). A good correlation was found with a reference electrochemical detection method (r =0.99, P b .0001). Within-run precision showed a coefficient of variation of 1.6 for samples approaching normal values (1.02 µmol/L). Day-to-day precision was also close to 2%. The reference values of CoQ10 are 0.7 to 1 µg/mL (23). Moreover, CoQ10 values were compared to plasma cholesterol concentration, measured by a cholesterol-oxidase enzymatic test.

Statistics

Data were presented as mean ± standard deviation (SD). A non-parametric Mann Witney U-test was used to evaluate the significance of differences.

Spearman's correlation coefficients were used to calculate the association between the changes of groups.

RESULTS

The demographic characteristics of the groups are listed in Table 1. The mean age, gender, body mass index, smoking, hypertension and diabetes mellitus were similar in both groups. There was no difference in total, LDL, HDL cholesterol, triglyceride and creatinine values between patients and controls.

It was observed that there was a significant increase in the ratio of 8-OHdG/dG, the level of MDA and GPX activity in patients with CAD when compared to controls ($p<0.001$). Otherwise, the ratio of ubiquinol-10/ubiquinone-10 was significantly lower in patients with CAD when compared to controls ($p<0.001$) (Table 2). However, SOD activity was found similar in both groups ($p=0.779$) (Table 2).

In addition multivariate linear regression analysis showed a positive correlation between 8-OHdG/dG ratio and MDA levels ($r=0.306$, $p<0.01$) and GPX activity ($r=0.563$, $p<0.01$), while there was a significant negative correlation between the ratio of 8-OHdG/dG and the ratio ubiquinol-10/ ubiquinone-10 ($r= -0.514$, $p<0.01$) (Table 3). However, there was a negative correlation between MDA levels and ubiquinol-10/ ubiquinone-10 ratio ($r=-0.190$, $p<0.05$) (Table 3).

Table 1. Clinical characteristics of the study population.

	Patients with CAD (n=77)	Control group (n=44)	P value
Age (year)	56.0 ± 7.8	54.3 ± 10.2	0.307
Sex (male)	51 (% 66.2)	31 (% 66.0)	0.975
Body mass index (kg/m²)	27.5 ± 3.8	26.9 ± 3.3	0.314
Smoking (n)	12 (% 15.6)	8 (% 17.0)	0.833
Hypertension (n)	28 (% 36.4)	12 (% 25.5)	0.584
Diabetes mellitus (n)	10 (% 13.0)	4 (% 8.5)	0.445
Total cholesterole (mg/dl)	190.5 ± 38.5	200.0 ± 41.4	0.208
Triglyceride (mg/dl)	183.2 ± 87.8	192.7 ± 125.2	0.650
LDL cholesterol (mg/dl)	115.5 ± 31.4	124.9 ± 44.5	0.206
HDL cholesterol (mg/dl)	41.3 ± 9.8	44.2 ± 10.3	0.124
Creatinine (mg/dl)	0.90 ± 0.17	0.87 ± 0.13	0.292

Table 2. Comparison of the plasma levels of 8-OHdG/dG and the serum malondialdehyde (MDA), the serum glutathione peroxidase (GPX), superoxide dismutase (SOD), ubiqinol-10/ubiquinone-10 between patients with coronary artery disease (*CAD*) and control group.

	Patients with CAD (n=77) Mean±SD (min-max)	Control (n=44) Mean±SD (min-max)	P value
8-OHdG/dG	3.01 ± 1.77 (0.95-8.98)	0.28 ± 0.14 (0.12-0.96)	0.000
MDA (umol)	11.27 ± 9.05 (0.64-38.88)	4.86 ± 2.75 (0.14-12.34)	0.000
GPX (U/mL)	26.75 ± 10.78 (7.58-50.74)	10.46 ± 6.07 (2.04-22.90)	0.000
SOD (U/mL)	6.32 ± 4.00 (0.3-17.9)	6.54 ± 4.44 (0.2-18.2)	0.779
Ubiquinol-10/Ubiquinon-10	0.49±0.34 (0.11-1.89)	1.23±0.84 (0.45-3.92)	0.000

Table 3. Spearman's correlation coefficients among variable.

	8OHdG/dG	MDA (µmol/l)	Ubiquinol-10/ Ubiquinone-10	GPX (EU/ml)	SOD (EU/ml)
8OHdG/dG	1				
MDA (µmol/l)	0.306**	1			
Ubiquinol-10/ Ubiquinone-10	-0.514**	-0.190*	1		
GPX(EU/ml)	0.563**	0.106	-0.471	1	
SOD(EU/ml)	0.007	-0.031	-0.151	-0.006	1

Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed). 8-OHdG/dG = 8-hydroxy-2'-deoxyguanosine/deoxyguanosine; MDA = malondialdehyde; GPX = glutathione peroxidase; SOD = superoxide dismutase.

DISCUSSION

In the present study, DNA damage marker 8-OHdG/dG was significantly increased and the ratio of ubiqinol-10/ubiquinone-10 as a pro-oxidant/ oxidant marker was significantly decreased in patients with CAD when compared with controls. Also, the antioxidant GPX activity was increased in CAD patients.

DNA damage is caused by multiple factors including oxidative stress, Vitamin B_{12} deficiency and ischemia-reperfusion injury (3,4,6). The ratio of 8-OHdG/dG is correlated with the severity of the oxidative stress. The higher activity of an antioxidant

enzyme may be a compensatory regulation in response to increased oxidative stress. In our previous study, we demonstrated that 8-OHdG may be a good biomarker for risk assessment of subclinical cardiovascular disease in haemodialysis patients (24). DNA damage has been related with the development of cardiovascular pathologies in the general population, which is supported for the monoclonal origin of cells from human atherosclerotic plaques (25).

In the present study, the lipid peroxidation product i.e. MDA levels have been increased significantly in plasma of the patients with coronary artery disease as compared to the controls. Raharjo et al. (26) reported that MDA is an important marker of lipid peroxidation. Belch et al. (27) showed that progression of atherosclerosis is correlated with oxidative stress and can be followed up by MDA measurements. Results of the studies of Pezeshkian et al. (28) showed that, MDA levels increased significantly in heart diseases. Some other investigates have also reported increase of SOD, MDA and GPX levels in patients with CAD (7). In our study, similar with the literature, the serum levels of MDA were found significantly higher in patients than that of the controls ($p<0.001$). In our previous study, we showed that the serum levels of retinol, tocopherol and cholecalciferol concentrations were lower in CAD patients than that of the healthy control group (29).

Antioxidant enzymes (SOD, GPX) are compounds that dispose, scavenge, and suppress the formation of free radicals and oppose their actions. Two main categories of antioxidants prevent the generation of free radicals and those intercept any free radicals that are generated (7,30,31). SOD is an important antioxidant enzyme having antitoxic effect against superoxide anion. GPX, an oxidative stress inducible enzyme, plays a significant role in peroxyl scavenging mechanism, and in maintaining functional integration of the cell membranes. Alteration in the oxidant - antioxidant profile is known to occur in CAD (3,4). We have found that in the present study, GPX enzyme activation was significantly higher in patients with CAD than controls. However, SOD enzyme activation was similar in patients with CAD and in controls.

CoQ10, also known as ubiquinol-10, plays a central role in the mitochondrial respiratory system, in which it shuttles electrons from complexes I and II to complexes III. CoQ is an ubiquitous constituent of cellular membranes and functions as an antioxidant. It is also a modulator of the mitochondrial permeability transition pore (32). CoQ10 aids them by supplying energy for the function of cells with high metabolic demands, such as heart muscle. The correlation between oxidative damage and mitochondrial alterations is strong. In our study, the ratio of 8-OHdG/dG and MDA were significantly negatively correlated with the ratio of ubiquinol-10/ ubiquinone-10 in CAD. These observations propose that oxidative dysfunction occurs in the pathogenesis of CAD and are consistent with the process of lipid peroxidation and oxidative DNA damage. Blood levels of CoQ10 were significantly lower in patients with ischemic heart disease and in those with dilated cardiomyopathy as compared to the healthy controls (33). Mabuchi et al., (34) reported that serum levels of CoQ10 diminished during treatment with statin in patients with hypercholesterolemia. CoQ10 levels are reported to decrease in patients with some chronic diseases such as heart disease.

In a clinical study, the administration of CoQ10 to heart transplant candidates led to a significant improvement in functional status, clinical symptoms, and quality of life. However, there were no objective changes in echo measurements or ANF and TNF blood levels. Coenzyme Q10 may serve as an optional addition to the pharmacologic armamentarium of patients with end-stage heart failure (35). In a double-blind trial, 144 people who had recently experienced a heart attack were given either placebo or 120 mg of CoQ10 daily for 1 year, along with conventional treatment. The results showed that participants receiving CoQ10 experienced significantly fewer heart-related problems, such as episodes of angina pectoris or arrhythmia, or recurrent heart attacks (36,37). CoQ10's best-established use is for congestive heart failure, but the evidence is not compatible with proven benefit.

In conclusion, the present study revealed up-regulation of lipid peroxidation, oxidative DNA damage and deficiency of CoQ10 ratio in CAD. It causes to disrupt pro-oxidant/antioxidant balance and therefore CoQ10 supplementation may show cardioprotective effects in CAD.

Competing Interests

The authors have declared that no competing interest exists.

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