RESEARCH COMMUNICATION

Lipid Peroxidation, DNA Damage and CoenzymeQ10 in Lung Cancer Patients - Markers for Risk Assessment?

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Abstract

　　Objectives: Early diagnosis and prevention is very important for lung cancer patients. Previous studies have emphasized that the level of coenzyme Q10 (CoQ10), present primarily in mitochondria, decreases with age and is low in patients with chronic diseases. Our goal was to find out if there is any relationship between lung cancer and CoQ10 and lipid peroxidation levels. **Design and Methods**: Blood samples from lung cancer patients were collected. Total and oxide CoQ10 levels, 8-OHdG (product of DNA damage), and malondialdehyde (MDA) levels (lipid peroxidation) were analyzed with high performance liquid chromatography (HPLC). **Results**: The MDA level (P<0.001) and DNA damage rate (8-OHdG) (P<0.001) was higher in cancer patients than in the control group; in contrast, theCoQ10 enzyme level was significantly lower (P<0.001). **Conclusion**: The results suggest that the aforementioned parameters can be useful for lung cancer risk assessment.

Keywords: Lung cancer - coenzyme Q10 - MDA - DNA damage - 8-OHdG - HPLC

Asian Pacific J Cancer Prev, 12, 1399-1403

Introduction

　　Although lung cancer was a rare disease at the beginning of the 20th century, it has risen in parallel with an increase in cigarette smoking, becoming the most common type of cancer globally (Spiro and Porter, 2002). Murray and colleagues reported that lung cancer ranks as the tenth cause of death worldwide in 1997 and has led to 1 million human deaths per year. It is predicted that it will rise to the fifth cause of death by 2020 (Murray and Lopez, 2007). Recently, lung cancer prevalence has risen to 12.8% of all cancer cases and is responsible for 17.8% of all cancer deaths (Parkin et al., 2005). Lung cancer falls into various categories and is a significant cause of cancer deaths for men and women. The main cause of lung cancer is cigarette smoking, yet radioactive gases, heredity, and breathing in toxic chemicals can also lead to the disease.

　　Known factors like age, race, gender, occupation, air pollution, radiation, diet, viral infections, genetics, and immunology are just 6% of the factors for developing lung cancer (Spiro and Porter, 2002). There are four main types of the disease: small cell lung cancer, squamous cell carcinoma, large cell carcinoma, and adenocarcinoma. Moreover, the incidence of lung cancer is around 5-10% for those under age 50 (Spiro and Porter, 2002, Radzikowska et al., 2001).
A collapse in the sensitive balance between free radicals and the antioxidant defense system can lead to oxidative stress (Cross et al., 1987). Oxidative stress is known to cause cell damage by creating changes in lipids, proteins, and biological macromolecules such as DNA. Reactive oxygen species (ROS) modifies DNA through different mechanisms and induces lesions on bases and sugars, single and double strand DNA breaks, abasic regions, and DNA protein cross-links (Dandona et al., 1996, 7 Dizdaroglu, 1994).

　　Oxidative damage on the DNA by ROS seems to be as an indicator and chief cause of aging, cancer, cardiovascular diseases, immune system diseases, degenerative diseases, emerging diseases, and diseases caused by disruption in tissue function. Extensive purine and pyrimidine lesion detection and quantification analysis were done on the oxidative damaged cells and tissue.□8-hydroxy-2-deoxyguanosine (8-OHdG) has the highest mutagenic effects and is one of the most frequently encountered oxidative DNA base damage products of the 23 ROS-DNA products. Other DNA base damage products are less likely to be mutagenic (Shigenaga et al., 1989, Loft and Poulsen, 1999).

　　In nuclear and mitochondrial DNA, 8-OHdG is one of the predominant forms of free radical-induced oxidative lesions. In 1984, 8-OHdG was identified for the first time by Kasai and Nishimura as a marker of oxidative DNA damage (Kasai and Nishimura, 1984). As a result, 8-OHdG

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has been widely used as a biomarker for oxidative stress on DNA and carcinogenesis assessments.

The tendency to mutate is increased with incremental ROS during DNA replication (Senturker and Dizdaroglu, 1999, Fraga et al., 1990, Shigenaga and Ames, 1991, Dizdaroglu et al., 2001). The guanine base, which has the lowest ionization potential of any DNA constituent, is the main target of ROS (Hirano et al., 1996). Therefore, the measurement of 8-OhdG is considered to be a direct indicator for oxidative DNA damage and is the most commonly used method for determining oxidative DNA damage (Dandona et al., 1996, Halliwell 1999, Halliwell 1997). Another significant result of excessive ROS production is lipid peroxidation (Ames 1983, Guyton and Kensler, 1993). When free radical levels exceed the antioxidant capacity of the cells, lipid peroxidation occurs. Lipid peroxidation is terminated by the conversion of lipid hydroperoxide to aldehydes and other carbonyl compounds. One of these compounds is malondialdehyde (MDA), a compound that is frequently used to determine the degree of lipid peroxide levels (Dormandy 1983, Gutteridge 1995). Aldehyde structured compounds have a long life span; thus, they can pass through the cell membranes so that the effects of lipid peroxidation can appear in organs and tissues.

Despite all efforts, most patients die within the first year of a lung cancer diagnosis. Early diagnostic methods can be helpful for life quality and survival of lung cancer patients. Still, as of yet, no blood test has made a significant impact on early lung cancer diagnosis (Duncan, 2009). Nonetheless, recent findings have encouraged researchers to explore the biomarkers for diagnosing lung cancer. Lee et al. studied the CTAP III/NAP-2 rate for predicting lung cancer. They found that only a CTAP II/NAP-2 rate is somewhat effective but is still not sufficient for fast and early diagnosis of lung cancer. Several parameters should be taken into consideration like age, smoking status, breathing, and other blood biochemical parameters (Yee et al., 2009) Oil-soluble, vitamin-like Coenzyme Q10 (CoQ10) is present in most eukaryotic cells, primarily in the mitochondria. CoQ10 is a part of the electron transport chain (ETC) and is involved in aerobic cellular respiration and generation of energy in the form of ATP. Previous studies emphasized that CoQ10 level decreases with age, and it is particularly low in patients with chronic diseases such as heart conditions, muscular dystrophy, Parkinson's disease, cancer, diabetes, and HIV/AIDS (Lockwood et al., 1995). As such, free radicals can cause lipid, protein peroxidation, and DNA damage. For this reason, the CoQ10 enzyme in the ETC has an important role in protecting DNA from oxidative damage.

Some researchers have indicated that CoQ10 supplement diets can prevent age-related DNA double-strand breaks and can increase lifespan (Quiles et al., 2004). In this respect, all results suggested that lung cancer also could have been connected to low CoQ10 levels that affect the progression rate of cancer.

In this research, we intended to reveal changes in CoQ10, MDA (lipid peroxidation), and the DNA damage rate (8-OHdG products) in lung cancer patients. We are

expecting that these results will shed light on solving the problem of the resistance of lung cancer to therapy as well as providing useful parameters for risk assessment of the disease.

Materials and Methods

Blood samples from lung cancer patients were used to study their CoQ10, 8-OHdG, and MDA levels. These samples were obtained from patients who were hospitalized in the oncology clinics of Yuzuncu Yıl University (YYU) Research Hospital in the Department of Thoracic Surgery. Sixty people participated in the study (30 lung cancer patients and 30 healthy people). These volunteer patients were informed about the study conducted according to the Declaration of Helsinki and approved by the Ethics Committee of the YYU School of Medicine.

Chemicals, equipment, and manufacturers
Ethylene diamine tetraacetic acid (EDTA), proteinase, ammonium acetate, electrochemical (HPLC-ECD), coomassie brillant blue R-250, Tris EDTA, wavelength detector (HPLC-UV), sodium dodesilsulfate (SDS), NaOH (Sigma-Aldrich Ltd. Poole, Dorset, UK). Sodium carbonate, sodium potassium phosphate, bicarbonate, 1,1,3,3-tetraethoxypropane standard solution, trihydroxymethyl amino methane (Tris), NaCl, sodium citrate dehydrate, HCl, acetic acid, propanol, and tiobarbituric acid (TBA) (Merck AG, Darmstatd, Germany). Analytical column (250 mm × 4.6 mm × 4.0 μm, Phenomenex, CA, Merck AG, Darmstatd, Germany). Methanol (34885), ethanol (34870), benzoquinon (B10358), lithium perchlorate (20,528-1), pure Coenzyme Q10 (C9538) (Sigma–Aldrich, Taufkirchen, Germany), n-propanol (24135) (Riedel-de-Haën, Germany) were used as supplemental chemicals.

Sample collection
Blood samples were collected into coagulant blood tubes. Blood serums were separated by centrifugation at 600 g and stored at −80 °C for assay.

Analysis of 8-OHdG and dG by the HPLC Method
To compare the DNA damage rate between lung cancer patients and healthy people, DNA samples were isolated from whole blood samples, according to Miller et al., with some modifications (Miller et al., 1988). Two ml of blood with Ethylene Diamine Tetraacetic acid (EDTA) were mixed with 3 ml of erythrocyte lyses buffer and incubation (10 min on ice) was followed by centrifugation (10 min at 3500 rpm). The supernatant was decanted and the pellets were thoroughly resuspended in the sodium dodecyl sulfate (10%, v/v), proteinase K (20 mg/ml) and 1.9 ml leukocyte lyses buffer. The mixture was incubated at 65 0C for 1 h and then mixed with 0.8 ml of 9.5 M ammonium acetate. After centrifugation (3500 rpm for 25 min), the clear supernatant (2 ml) was transferred to a new sterile tube, and DNA was precipitated by adding 4 ml of ice-cold absolute ethanol. Finally, the DNA samples were dissolved in the Tris EDTA buffer (10 mM, pH 7.4) and hydrolyzed.

8-OHdG and dG levels were measured in the hydrolyzed DNA samples by HPLC with electrochemical (HPLC-ECD) and variable wavelength detector (HPLC-UV) systems, respectively, as previously described (Donald et al., 1997). Twenty μL of final hydrolysates were analyzed 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 readings (8600 mV). Levels of dG and 8-OHdG were quantified using dG and 8-OHdG standards from sigma; the 8-OHdG level was expressed as the number of 8-OHdG molecules per 106 dG.

Measurement of MDA

Malondialdehyde (MDA; $CH2(CHO)_2$) is one of the end products of lipid peroxidation and is a marker for oxidative stress. Plasma MDA concentration was measured according to Auer T. et al (Khoschsorur et al., 2000). Briefly; 50μL of a plasma sample were mixed with 0.44 M H3PO4 and 42 mM tiobarbituric acid (TBA) and incubated for 30 min in a boiling water bath. As it cooled rapidly on the ice, an equal volume of alkaline methanol was added to the samples, the samples were vigorously shaken and centrifuged (3000 rpm for 3 min), and then the aqueous layers were removed. Next, the 20μL supernatant was analyzed 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 KH2PO4 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 by exactly the same process as had been done with the plasma sample.

Measurement of CoQ10

Coenzyme Q10 is also known as ubiquinone, ubidecarenone, coenzyme Q and is sometimes abbreviated as CoQ10. Ubiquinol is an electron-rich (reduced) form of Coenzyme Q10. Ubiquinol-10 and ubiquinone-10 were measured simultaneously with high-performance liquid chromatography by an electrochemical detection method and defined standards.

The results for CoQ10 (Ubiquinol-10 and ubiquinone-10) were expressed as a molar concentration (nmol/l). Lipophilic antioxidants are carried by circulating lipoproteins in plasma. The positive correlation between plasma lipids and plasma level of lipophilic antioxidants is well established (Murray and Lopez, 2007). Therefore, the CoQ10 results were related to plasma cholesterol concentration (micromoles per mole cholesterol). The CoQ10 redox status was calculated as the percentage of ubiquinone-10 within the total concentration of CoQ10 (Menke et al., 2000, Artuch et al., 1999). An Agilent 1100 Series system HPLC (Agilent Technologies, Waldbronn, German) was used to analyze oxide coenzyme Q10 and total coenzyme Q10 levels in lung cancer patients and control group blood samples.

For HPLC measurement, an ODS reversed phase supercosil LC 18 (15 x 0.46 cm i.d. 3 μm) colon was used. 50 μL 1,4 benzoquinon (2 mg/ml) was added into a 200 μL blood plasma sample and the mixture vortexed. After 10 minute incubation at room temperature, 1 ml n-propanole was added and vortexed for 10 seconds. The mixture was centrifuged at 600 g for 2 minutes. 200 μl of supernatant was collected in vials to be used in the HPLC.
In the spectral analysis, the UV detector settled at 275 nm and the mobile phase flow rate of ethanol-methanol (%65-35) settled at 1 ml/min. Regarding the Ubiquinol-10 (CoE) and ubiquinone-10 (T.CoE) distinction, 50 mM sodium per chloride, methanol-ethanol (80:20) mixture was poured at a 1 ml/min flow rate in the electrochemical detector in the mobile phase. The samples were added to the system after the system became stable. Oxidized and reduced CoenzymeQ10 was measured by an electrochemical detector at 0.35 V.

Statistical Analysis

Data between the groups for continuous variables were compared with a student t test and a chi-square t-test. A two-tailed P-value of <0.05 was considered significant. The Pearson correlation coefficient was calculated to define relationships between the parameters.

Results

In this research, control group participants and cancer patients were chosen from similar ages, genders and social class profiles. However, 77% of cancer patients were smokers as compared to only 37% of controls.

Excessive reactive oxygen species production is a natural result of destruction of the electron transport chain mechanism in the mitochondria. Having excessive ROS products leads to oxidation of lipid sand proteins, and these oxide products may be the cause of cell death or cancer. MDA measurement is a common technical method to evaluate lipid peroxidation. As for the MDA measurement results, the lipid peroxidation rate was increased among the lung cancer patients (p<0.001) (Tables 1,2). Excessive increment of 8-OHdG products was detected (p<0.001), which is a natural result of ROS increment over DNA.

CoQ10 has a central role in the ETC which transduces electrons to reactive oxygen via Cytocrom C from NADH. Low level CoQ10 would lead to excessive ROS products which we observed in our results of MDA and 8-OHdG levels.

Table 1. Descriptive Statistics and Comparison Results for Variables

Parameters	Control n=30	Patient's n=30	P Value
MDA (μmol/L)	4.07 ± 1.28	10.4 ± 1.37	0.001***
8-OHdG	0.93 ± 0.31	2.32 ± 0.46	0.001***
Ubiquinol-10	3.33 ± 0.64	1.29 ± 0.38	0.001***
Ubiquinone-10	4.19 ± 0.66	4.48 ± 0.61	0.088

Data are Mean ± SD

Table 2. Pearson Correlation Coefficients among Variables

	Control Group				Patient Group			
	MDA	8-OHdG	Ubiquinol-10	Ubiquinone-10	MDA	8-OHdG	Ubiquinol-10	Ubiquinone-10
MDA	1				1			
8-OHdG	-0.218	1			0.503**	1		
Ubiquinol-10	0.453*	0.119	1		0.434*	0.415*	1	
Ubiquinone-10	0.448*	0.070	0.841**	1	0.143	0.277	0.462*	1

*$p<0.05$; **$p<0.01$

To approve MDA and 8-OHdG results, Ubiquinol-10 and ubiquinone-10 levels were studied in the lung cancer patients and healthy people's blood samples. As for the HPLC results, low level Ubiquinol-10 ($p<0.001$) was detected in the control group.

Discussion

Lung cancer is one of the most frequent cancer types around the world and a continuously growing health problem (Spiro and Porter, 2002). Cancers can grow in any part of the lung, but 90%-95% of cancers of the lung are thought to arise from the epithelial, or lining cells of the larger and smaller airways (bronchi and bronchioles); for this reason, lung cancers are sometimes called bronchogenic carcinomas or bronchogenic cancers.

The structure, physicochemical properties, cellular origins, reactions, and impacts of free radicals have been implicated as important mediators in many clinical disorders. Many recent studies have proved the role of oxidative stress in carcinogenesis. Malondialdehyde (MDA) is an end-product of lipid peroxidation of membrane polyunsaturated fatty acids by free radicals and is an indicator of oxidative damage (Gutteridge, 1995, Wiseman et al., 1996, Moriya et al., 2001, Esme et al., 2008, Demir et al., 2010).

The results of the present study have shown that erythrocyte MDA concentrations in lung cancer patients were significantly higher than for the control group. Furthermore, it was observed erythrocyte 8-OHdG levels increases significantly in patients with lung cancer when compared to those in control groups.

Coenzyme Q10 (CoQ10; Ubiquinone-10 and Ubiquinol-10) is a key enzyme in the ATP synthesis and electron transport chain. (Overvad, 1999). Coenzyme Q10 (CoQ10) is a redox molecule which is found both in oxide forms (Ubikinon-10) and reduced forms (Ubikinol-10) in the mitochondria. CoQ10 is related with oxygen sourced free radicals and singlet oxygen levels, thus CoQ10 inhibits lipid peroxidation and prevents damage of biomolecules (Bonakdar et al., 2005). Free radicals act as inter reaction products and are exposed to electron reduction reactions. Unstable free radicals get electrons from ubikinon and become stable in the ETC system. Coenzyme Q is an important antioxidant with this feature (Turunen et al., 2004). In this study, we found significantly lower erythrocyte coenzyme Q10 levels in patients with lung cancer.

If compared with other membrane antioxidants such as α-tokoferol, Ubikinol-10 is at low concentrations; however, it first reacts as an antioxidant when plasma is exposed to oxidants. Coenzyme Q10 is also involved in the regeneration of other antioxidants (38 Bhagavan et al. 2007). In addition, as indicated in previous studies, coenzyme Q10 has the role of ensuring membrane stability, cell signaling, gene expression, control of cell growth, and apoptosis (Ruiz-Jimenez et al., 2007, Crane 2001). Coenzyme Q10 has been claimed to have a protective effect against cancer. Folkers et al. reported that coenzyme Q10 has shown macrophage-potentiating activity in cancer patients with some evidence of increased survival (Folkers et al., 1993).

In a Danish trial, 32 women were given routine chemotherapy, radiotherapy, surgery, vitamins, minerals, and coenzyme Q10. Six of the women showed partial or complete cancer regression. The researchers concluded that these six women would normally have died, but during the two years of the trial no deaths occurred. However, the multiplicity of nutritional supplements used in the study prevented the identification of coenzyme Q10 as the dominant factor. In two patients with metastatic breast cancer whose coenzyme Q10 dose was increased from 90 to 360 mg daily, liver metastases and pleural cavity metastases apparently disappeared (Lockwood et al., 1995). Considering all of the data, there is some evidence that shows a correlation between cancerous conditions and low levels of CoQ10.

According to our results, MDA level ($P<0.001$) and DNA damage rate (8-OHdG) ($P<0.001$) were increased; however, the oxide CoQ10 enzyme level was significantly decreased ($P<0.001$) in the lung cancer patients. Our MDA and 8-OHdG results support previous studies (Erhola et al., 1997, Honda et al., 2000).

Moreover, increased MDA level ($P<0.001$), increased DNA damage rate (level of 8-OHdG) ($P<0.001$), and decreased oxide CoQ10 enzyme level (CoE, $P<0.001$) in the lung cancer patients may be useful parameters for lung cancer risk assessment. Still, more detailed studies are needed on therapeutic uses of CoQ10 for lung cancer prevention.

Acknowledgements

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