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Cardiovascular diseases: oxidative damage and antioxidant protection

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Abstract. – Atherosclerosis, the hardening of arteries under oxidative stress is related to oxidative changes of low density lipoproteins (LDL). The antioxidants prevent the formation of oxidized LDL during atherogenesis. Perhaps more than one mechanism is involved in the atherosclerosis disease where LDL is oxidized in all the cells of arterial wall during the development of this disease. The oxidation of LDL produces lipid peroxidation products such as isoprostans from arachidonic, eicosapentaenoic and docosahexaenoic acids, oxysterols from cholesterol, hydroxyl fatty acids, lipid peroxides and aldehydes. The lipid peroxidation bioassay can serve as a marker for the risk of cardiovascular. An *in vivo* test of levels of oxidative lipid damage is an early prediction of development of cardiovascular disease (CVD). Serum paraoxonase (PON) activity is correlated to severity of the coronary artery disease. The antioxidants level in the serum and serum paraoxonase activity provides information for the risk of CVD. The antioxidant enzyme superoxide dismutase is responsible for dismutation of superoxide, a free radical chain initiator. The subcellular changes in the equilibrium in favor of free radicals can cause increase in the oxidative stress which leads to cardiomyopathy, heart attack or cardiac dysfunction. The oxidative damage and defense of heart disease has been reported where dietary antioxidants protect the free radical damage to DNA, proteins and lipids. The ascorbic acid, vitamin C is an effective antioxidant and high vitamin E intake can reduce the risk of coronary heart disease (CHD) by inhibition of atherogenic forms of oxidized LDL. The vitamin A and beta-carotene protect lipid peroxidation and provitamin-A activity. It has been recently suggested that the protection of oxidative damage and related CVD is best served by antioxidants found in the fruits and vegetables. The oxidative damage and antioxidant protection of CVD have been described here.

Introduction

During atherogenesis, antioxidants prevent the formation of oxidized LDL[1]. It has been reported that serum paraoxonase (PON) activity is correlated to severity of the coronary artery disease[2]. The changes in free radicals can cause an increase in the oxidative stress[3], and antioxidants protect the free radicals damage to DNA, proteins and lipids[4].

Cardiovascular diseases (CVD) are the leading cause of death as reported by World Health Organization (WHO). About 30% of all deaths occurred in 2005 were due to heart diseases[5] and in USA alone, CVD care costs were more than $531 billion[6] due to high incidence of heart diseases and mortality rate. Good clinical practice guidelines are needed to prevent cardiovascular diseases and to evaluate patients for the risk of heart attacks and heart dysfunction. A number of multivariate risk predictions obtained by cohort studies or randomized trials have been reported in the literature for CVD risks. The patient's medical history and results of laboratory tests, and computer based data are important for the heart care. In USA, the commonly used CVD risk prediction models are based upon Framingham cohort study of men and women aged 30 to 74 years, and validated in multiple diverse populations. There are many causes of heart disease such as high blood pressure, hypertension, diabetes mellitus, high cholesterol level, stress, depression, family history, myocardial infarctions, heart arrhythmias, shock, stroke, smoking and alcoholism. Acute coronary syndrome (ACS) is a common complication of coronary heart disease caused by

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rupture of atherosclerotic plaque in coronary artery which results in the formation of a thrombus restricting the blood flow to the heart muscle. The electrocardiogram (ECG) and cardiac troponins are used in the diagnosis of ACS[7]. A heart attack usually occurs when the blood flow to a part of the heart is blocked by a blood clot. The ischemic stroke happens when blood vessel that feeds the brain gets blocked usually from blood clot formation and a part of blood supply is shut-off and brain cells start to die. A hemorrhagic stroke occurs when a blood vessel within the brain is ruptured which is usually due to hypertension and high blood pressure. The brain cells do not get enough oxygen supply due to blockade by blood clot in the artery and after a stroke, these cells are never replaced. However, some injured cells do not die and can be repaired by themselves resulting in the improvement of body functions[9].

The arrhythmia is an abnormal rhythm of heart, and results in either very fast or very slow heart beating. The bradycardia is when heart rate is less than 60 beats per minute and trachycardia is when the heart rate is more than 100 beats per minute. The heart may not be able to pump up enough blood to meet the need of the body and causes arrhythmias. In case of stenosis, the heart valves do not open enough to allow the precise amount of blood to flow. When heart valves do not close properly, the blood leaks through and this condition is called regurgitation. The mitral valve prolapsed is the condition of heart when the valve leaflets bulge or prolapsed back into the upper chamber of heart. This condition allows the blood to flow backward in the heart. The other CVD include congenital heart disease from birth, cardiomyopathy, pericardial disease, Aorta disease and Marfan syndrome, and blood vessel disease as vascular disease[10].

In this review article the oxidative damage of heart due to lipid peroxidation and its antioxidant protection with vitamins, Ascorbic acid, (vitamin C), beta-Carotene (vitamin A), and Tocopherols (vitamin E) have been described.

A study on the oxidative damage and antioxidant protection related to cardiovascular diseases has been described where oxidative modifications of low density lipoprotein (LDL) under oxidative stress and atherosclerosis are mentioned (1). It depends on the capability of body to inhibit LDL oxidation and to remove or neutralize atherogenic Ox-LDL when formed which can be done by enrichment of LDL and arterial cells with potent antioxidants and vitamins that can prevent oxidative damage to the arterial walls and heart muscle. The LDL oxidation products after lipid peroxidation such as isoprostanes from arachidonic, eicosapentaenoic and docosahexaenoic acids, oxysterols from unesterified and esterified cholesterol, hydroxy fatty acids, lipid peroxides and aldehydes are responsible for the oxidative damage of the heart. There is a need of an efficient bioassay of lipid peroxidation which can serve as a marker to understand the risk of CVD. The use of various biomarkers will provide scientific basis for the trials of antioxidants and to validate biomarker concept. The biomarkers will help to know the early events of oxidative lipid damage to heart and to predict subsequent development of CVD. The presence of specific antioxidants in the serum and serum paraoxonase enzyme activity can provide important information about the risk of damage to heart for CVD. The other bioassays to include are biomarkers for endothelial dysfunction, monocyte adhesion, macrophage uptake of lipoproteins, thrombotic and inflammatory processes.

Paraoxonase Activity

J. Loscalzo[8] has described the paraoxonase and coronary heart disease risk. The paraoxonase enzyme hydrolyses the toxic metabolite of parathion paraoxan and indicates vascular oxidant stress. It hydrolyses synthetic organophosphates, insecticides in mammals. The paraoxonase enzyme comprises three isoforms, PON1, PON2 and PON3, which attenuate oxidant stress. The PON1 protects LDL from oxidation; it binds to high density lipoprotein (HDL) in a calcium dependent manner and inhibits its oxidation[11]. In clinical studies of serum paraoxonase activity the organophosphates are used as substrates to determine enzyme activity. The paraoxonase (PON1) activity of serum hydrolyses oxidized lipids in LDL which retard the development of atherosclerosis[12]. The PON1 activities were significantly lower in the subjects with coronary heart disease than control subjects (activity to paraoxon 122.8 vs. 214.6 nmol/min/ml). The PON1 activity as a predictor of CVD in type 2 diabetes subjects has been observed earlier[13].

Blood Sampling for Paraoxonase Activity

The blood sampling for paraoxonase activity is performed as follows: the venous blood is ob-

tained under sterile conditions from healthy and CHD subjects. The blood is collected in vacutainers for lipid profile and enzyme activities. The plasma for enzyme activity is obtained after centrifugation of blood at 500 g for 5 min in a table top centrifuge within 3 hrs of sampling. The cholesterol, triglycerides and HDL lipoproteins are determined. The LDL can be calculated using Friedewald formula.

Determination of PON1 Activity

The paraoxonase (POase) and diazoxonase (DZOase) activities can be determined by a UV-Vis. spectrophotometer. Such enzyme study can be done in diabetes subjects with CHD. Paraoxon (1.2 M) (Sigma Chemical, St Louis, MO, USA) and Diazoxon (1.0 M) (Chemical Service, West Chester, PA, USA) are used as substrates, respectively, in a Tris buffer (0.1 M, pH 8.5) containing 2 M NaCl and 2 mM CaCl2[14]. A blank tube determination of basal assay mixture without plasma is performed to observe the hydrolysis of paraoxon and diazoxon solutions[15]. One ml of substrate (paraoxon or diazoxon) is placed in a cuvette. Upon addition of plasma to substrate solutions (10 µl for paraoxonase and 5 µl for diazoxonase), the reaction can be monitored for 2 minutes at 25°C in a spectrophotometer at 280 nm in UV and 405 nm in visible range. The enzyme activities can be determined after 1 and 2 minutes. The enzyme activities of paraoxonase and diazoxonase are expressed as 1 umol of substrate hydrolysed per minute per liter of plasma or serum. The extinction coefficients for p-nitrophenol (hydrolysis product of paraoxon) and 2-Isopropyl-4-methyl-6-hydroxypyrimidine (for diazoxon) are 18 mM and 3 mM-1 cm-1 at pH 8.5, respectively. A two-dimensional plot of initial rates of diazoxon hydrolysis on y axis versus rates of hydrolysis of paraoxon on the x axis is used for the simultaneous determinations of enzyme paraoxonase and diazoxonase activities genotyping in addition to phenotyping. The statistical analyses of the data of enzyme activities can be performed. Following are the results of enzyme paraoxonase and diazoxonase activities of healthy and CVD, CHD and diabetes subjects[13].

Healthy Serum POase = 140 and 162 U/L
Healthy Plasma POase = 685 U/L with 1 M NaCl
Vascular Disease POase = 546 U/L with 1 M
Healthy Serum POase = 226 U/L with NaCl
CHD Serum POase = 151 U/L.
(Unit = nmol/min/L).
Healthy Serum DZOase = 4.32 U/L
(Unit = µmol/min/L)
Healthy Plasma DZOase = 10.00 U/L
Vascular Disease DZOase = 8.49 U/L

Therefore, determination of enzyme paraoxonase activity (POase) in blood serum or plasma is a good indicator of early detection of coronary heart disease (CHD) and other cardiovascular diseases (CVD).

Antioxidants

The role of antioxidants and oxidative stress in cardiovascular diseases has been described[16]. It has been reported that increased intake of antioxidants such as vitamins C and E, protects cardiovascular diseases. However, irrational or excessive use of antioxidants may produce risk of potential toxicity. The highly reactive oxygen derived free radicals (ROS) of endogenous or environmental origin play a cognitive role in the genesis and progression of various cardiovascular diseases[17,18]. The free radicals are controlled by antioxidants levels and excessive free radical formation and insufficient removal by antioxidants leads to oxidative stress (OS) on heart[19]. The risk factors due to excess free radicals are use of tobacco, smoking, alcohol drinking, diet, pollution, heavy exercises and metabolic abnormalities lead to increased oxidative stress to heart[20]. The ROS can stimulate oxidation of LDL, low density lipoprotein, cholesterol, cholesterol derived species and modification of proteins which leads to foam cell formation and atherosclerotic plaques in arteries[21]. There is good evidence that vitamins C, ascorbic acid, and E, tocopherols, exert a protective effect on the heart against CVD by reducing oxidative stress (OS).

The present status of antioxidants in human CVD protection is that the use of vitamins is necessary which regulates endothelial nitric oxide levels as well as by inhibiting cardio-vascular inflammation, lipid peroxidation, platelet aggregation and LDL oxidation and to prevent endothelial dysfunction. The antioxidants also influence plaque stability. The antioxidants vitamins can reverse endothelial dysfunction induced by methionine and can restore endothelial function in hyperlipidemia children and young smokers. In patients of chronic heart failure, allopurinol, xanthine oxidase inhibitor, a potential antioxidant which reverses endothe-

lial dysfunction in heavy smokers, type 2 diabetes and mild hypertension. The antioxidants slow down the thickening of arteries, atherosclerosis, and progression in CHD. It is reported that increased glutathione-1 peroxidase activity lowers the risk of CVD. The catalase enzyme inactivates ROS, superoxide dismutase enzyme by regulating the availability of nitric oxide and selenium by increasing glutathione peroxidase activity and protects CVD[22]. The natural antioxidants present in fruits and vegetables are flavonoids and phenolic compounds which protect heart from cardiovascular diseases[23,24]. The dietary factors based on cereals, pulses, spices, green vegetables, citrus fruits, palm and soybean oil, cod liver oil, sprouts, green peppers, whole grains, honey, walnuts and tea can significantly increase the liver antioxidants enzymes and their supplementation which reduces the risk of coronary heart disease (CHD).

Oxidative Stress

The oxidative stress (OS) is an excess formation and insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). The ROS include free radicals such as superoxide (•O2-), hydroxyl (•OH), peroxyl (•RO2), hydroperoxyl (•HRO2-6) and non radical species such as hydrogen peroxide (H2O2) and hydrochlorous acid (HOCl). The RNS include free radicals like nitric oxide (•NO), nitrogen dioxide (•NO2), and non radicals such as peroxynitrite (ONOO-), nitrousoxide (HNO2) and alkyl peroxynitrates (RONOO). Reactive molecules, •O2-, •NO and ONOO- all play important role in cardiovascular diseases (CVD). Oxidative stress is involved in many diseases, such as atherosclerosis, myocardial infarction, heart failure, Parkinson's disease, Alzheimer's disease, fragile X syndrome and chronic fatigue syndrome, CFS, but short-term oxidative stress is also important in prevention of aging by induction of a process named mitohormesis. The reactive oxygen species are beneficial, as they are used by immune system as a way to attack bacteria and viruses and kill pathogens. The ROS are also used in the cell signaling process[24].

Superoxide Dismutase Enzyme

The parameters of oxidative stress are enzyme superoxide dismutase, lipid peroxidation, TBARS, MDA, and enzyme catalase. The superoxide dismutase (SOD) enzyme catalyses the breakdown of superoxide into oxygen and hydrogen peroxide and present in all aerobic cells and in extracellular fluids[25]. SOD enzyme contains metal ions, copper, zinc, manganese or iron cofactors. The copper-zinc SOD presents in the cytosol, and manganese in the mitochondria in humans. The copper-zinc of SOD occurs in extracellular fluids of the body. The catalase enzyme catalyses the conversion of hydrogen peroxide to water and oxygen, using iron or manganese as cofactor. This enzyme is localized in the peroxisomes of eukaryotic cells[26,27].

Peroxiredoxins

The peroxiredoxins are peroxidases that catalyzes the reduction of hydrogen peroxide, organic hydroperoxides and peroxynitrite. They are divided into 3 classes: 1, typical 2-cysteine peroxiredoxins; 2, atypical 2-cysteine peroxiredoxins; and 3, 1-cysteine peroxiredoxins. These enzymes react in the same basic catalytic mechanism in which a redox-active cysteine (the peroxidatic cysteine) in the active site is oxidized to a sulfenic acid by the peroxide substrate. The oxidation of this cysteine residue in peroxiredoxins inactivates these enzymes, but this can be reversed by the action of sulfiredoxin. The peroxiredoxins are important in antioxidant metabolism[28].

The most common medications used in oxidative stress are digoxin for fatigue and pain, and furosemide for nocturnal dyspnea attacks. Several medications have been described for the oxidative stress, CHD, heart failure and CVD in the medical literature.

Antioxidants Enzymes and Smoking

Anti-oxidant enzymes GPx, glutathione reductase, and ECSOD activity in the serum is lower in the smokers than the non-smokers[29]. The serum vitamin C, ascorbic acid and folic acid concentrations also are lower in smokers as compared to non-smokers, but serum malondialdehyde and TBARS are higher[30]. The enzyme activities of SOD and catalase in erythrocytes are significantly lower in heavy, light and passive smokers than in non-smokers. The passive smokers are affected by the environmental smoke to the same extent as active smokers. The cessation of cigarette smoking increases plasma levels of several antioxidants micronutrients and

improves resistance towards oxidative damage. The administration of antioxidants, such as vitamins, ascorbic acid, C and tocopherols, E, suppresses increased smoking-related lipid peroxidation markers in cigarette smoking people[30].

Vitamins, A, C and E

At present there are several evidences which suggest that an increased intake of antioxidants, vitamins C, ascorbic acid, tocopherols, E, and beta-carotene, A, have some protective role in the coronary heart disease and cardiovascular diseases. The proper amounts of antioxidants, vitamins should be taken daily however; higher amounts when taken may increase the risk of toxicity of liver, kidney, and heart. Vitamins are good source of antioxidants and it has been suggested that the organic food products have higher antioxidant activity and bioactivity than conventional foods. Thus, consumption of these food products are good source for protection and prevention of chronic diseases[31]. The best recommendation is to increase daily intake of natural antioxidants, vitamins, fruits and vegetables in the diet which is good for the protection of heart attack and cardiovascular diseases.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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