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 LDL⁴. It has been reported that serum paraoxonase (PON) activity is correlated to severity of the coronary artery disease. The changes in free radicals can cause an increase in the oxidative stress, and antioxidants protect the free radicals damage to DNA, proteins and lipids⁴.

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 and in USA alone, CVD care costs were more than $531 billion 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...
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 enzymatic 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.

**Paraonxonase Activity**

J. Loscalzo 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. 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. 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.

**Blood Sampling for Paraonxonase 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 vacu-
tainers 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. Paraaxon (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 determina-
tion 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 coeffi-
cients 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 diazo-
oxon hydrolysis on y axis versus rates of hydroly-
sis of paraaxon on the x axis is used for the si-
multaneous determinations of enzyme paraox-
sonase and diazoxonase activities genotypeing in
addition to phenotyping. The statistical analy-
ses of the data of enzyme activities can be per-
formed. 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 paraox-
onase 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 an-
tioxidants 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 oxy-
gen derived free radicals (ROS) of endogenous
or environmental origin play a cognitive role in
the genesis and progression of various cardio-
vascular diseases[17,18]. The free radicals are con-
trolled 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 radi-
cals are use of tobacco, smoking, alcohol drink-
ing, diet, pollution, heavy exercises and meta-
bolic abnormalities lead to increased oxidative
stress to heart[20]. The ROS can stimulate oxida-
tion of LDL, low density lipoprotein, chole-
sterol, cholesterol derived species and modifica-
tion of proteins which leads to foam cell forma-
tion and atherosclerotic plaques in arteries[21].
There is good evidence that vitamins C, ascor-
bic 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 ox-
ide 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 vit-
amins can reverse endothelial dysfunction in-
duced by methionine and can restore endothe-
rial 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. The natural antioxidants present in fruits and vegetables are flavonoids and phenolic compounds which protect heart from cardiovascular diseases. 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 antioxidant 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), peroxy (•RO2), hydroperoxy (•HRO2-6) and non radical species such as hydrogen peroxide (H2O2) and hydrochloric acid (HClO). The RNS include free radicals like nitric oxide (•NO), nitrogen dioxide (•NO2), and non radicals such as peroxynitrite (ONOO-), nitrous oxide (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.

**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. 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.

**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.

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. 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. 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. Thus, consumption of these foods and antioxidants has been suggested that the organic food products have higher antioxidant activity and bioactivity than conventional foods. 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. 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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