LIPID PEROXIDATION IN RELATION TO VITAMIN C AND VITAMIN E LEVELS

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SUMMARY

Oxidative stress plays an important contributory role in the pathogenesis of age-related chronic diseases. Increased lipid peroxidation process is caused by an enhanced free radical formation together with a higher supply of substrates and by an insufficient defense by antioxidants as well. Levels of malondialdehyde to content of lipid peroxidation substrates (polyunsaturated fatty acids), promoters (homocysteine – hydroxyl radical producer) and inhibitors (essential vitamins C and E) were estimated in a group (n=92) of subjective healthy adults randomly selected from general population. The relationship of malondialdehyde levels to values of peroxidisability index of fatty acids as well as to levels of homocysteine is significantly positive linear. A significant inverse linear correlation between malondialdehyde levels and natural antioxidant levels (vitamin C, vitamin E) was recorded. Lipid peroxidation products (conjugated dienes of fatty acids – initial, malondialdehyde – secondary) are significantly increased in groups of subjects with deficient levels of vitamin C (below the limit from antioxidative point of view), vitamin E and both vitamins, if compared to group with normal vitamin levels (over limit in accordance with antioxidative criterion). The results document that the deficiency in two key antioxidants for lipid peroxidation inhibition means the insufficient defense against free radicals and the increased lipid peroxidation.

INTRODUCTION

Oxidative stress can play an important contributory role in the pathogenesis of numerous degenerative or chronic diseases, such as atherosclerosis and cancer (1, 2). Reactive oxygen species are capable of chemically altering virtually all classes of biomolecules (lipids, proteins, nucleic acids) changing their structure and function. Lipid peroxidation of polyunsaturated fatty acids can proceed as a chain reaction producing hydroperoxides and aldehydes which once can decompose into a variety of breakdown products with cytotoxic, mutagenic and genotoxic properties (3). Humans have evolved a variety of mechanisms to protect from deleterious effects of free radicals. These include enzymes, nonessential endogenous antioxidants or essential exogenous radical scavengers – water and lipid soluble natural antioxidants such as ascorbate (vitamin C), α-tocopherol (vitamin E) and β-carotene (4).

Oxidative damage to lipids has been assessed by the measurement of thiobarbituric acid reactive substances, which are thought to reflect the production of malondialdehyde, a non-lipophilic peroxidation product of polyunsaturated fatty acids (5). In presented study we evaluated relationships of malondialdehyde levels to values of lipid peroxidation substrates, promoters and inhibitors in the group of healthy adult population.

SUBJECTS AND METHODS

The study group selected from general population consisted of 92 subjective healthy adult persons on a traditional mixed diet (43 men, 49 women; average age 41.1±0.8 years; body mass index 23.8±0.4 kg/m^2; smokers 14%). The probands were randomly selected from the group examined during the epidemiological study on health and nutrition of the general Slovak population (districts Bratislava and Senica).

Blood samples were collected in the standard way. Ethylenediamine tetraacetic acid (EDTA) was used as an anticoagulant. The plasma malondialdehyde concentration was determined according to the method of Wong et al. (6). Fatty acid content in plasma was measured by gas chromatography (7). Values of conjugated dienes of fatty acids were estimated by spectrophotometric method (8). Total homocysteine in plasma was assessed by HPLC method with fluorescence detection and SBD-F as derivation agent (9). Vitamins C and E in plasma were estimated by high performance liquid chromatography (HPLC) methods (10,11). The survey was carried out in spring. Intake of vitamins, mineral and trace elements in natural form was considered only (no supplementation). The Student's t-test and regression analysis were used for final evaluation.

RESULTS AND DISCUSSION

Lipid peroxidation is thought to proceed by hydroxyl radical mediated abstraction of a hydrogen atom from a methylene carbon of polyunsaturated fatty acids. The resulting carbon centered radical (lipid diene radical) may then undergo molecular rearrangement followed by interaction with molecular oxygen to form a peroxyl radical, which is capable to prolong a lipid
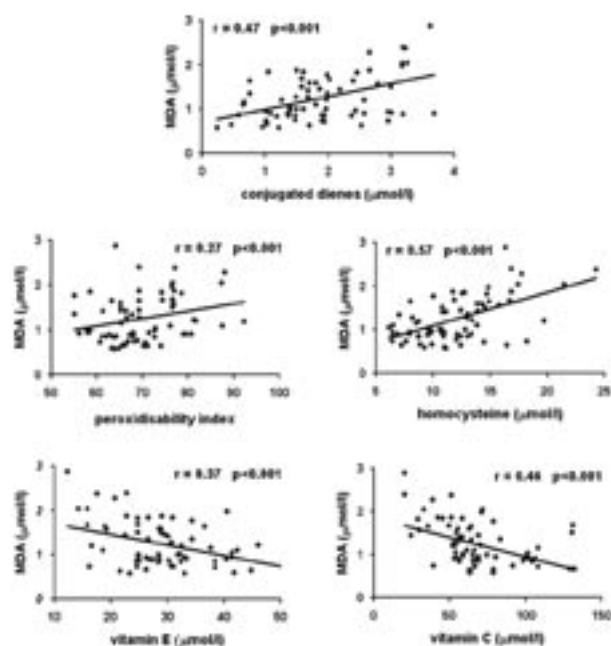
Fig. 1. *Relationship of malondialdehyde (MDA) levels to levels of conjugated dienes of fatty acids, to values of peroxidisability index of fatty acids, and to levels of homocysteine, vitamin E and vitamin C*

peroxidation by reaction with new lipid molecules to form lipid hydroperoxides. This propagating step of lipid peroxidation significantly directly depends on a number of double bonds (5). Primary lipid hydroperoxides are unstable and a wide spectrum of secondary products is thus formed. The most representative secondary product is malondialdehyde. Lipid conjugated diene is an initial product. We recorded a significant positive linear correlation of these two lipid peroxidation products (Fig.1).

Increased lipid peroxidation process is on one hand caused by higher free radical formation together with higher supply of substrates (polyunsaturated fatty acids) and by insufficient protection as a consequence of natural antioxidant deficiency or deficient cofactors for endogenous synthesis of antioxidants (5,12) on the other hand. We recorded a significant positive linear correlation between malondialdehyde levels and peroxidisability index of polyunsaturated fatty acids (Fig.1). A significantly higher content of lipid peroxidation substrates was found in vegetarians (in comparison to general population) as a consequence of plant oil

consumption (13). On the other hand vegetarians have a significantly higher antioxidative status (14) due to higher and frequent consumption of fruit, vegetables, oil seeds, grain sprouts, whole grain products, over limit plasma essential vitamin levels (15), resulting in the low incidence of risk lipid peroxidation values.

The hydroxyl radical is one of the most reactive chemical species known. In contrast to superoxide radical and hydrogen peroxide there exist no enzymatic protection against hydroxyl radical (16). The hydroxyl radicals can be produced also by elevated homocysteine. This amino acid with prooxidative activity may cause atherosclerosis by damaging the endothelium either directly or by altering oxidative status (17). It has been suggested that hyperhomocysteinemia may promote the production of hydroxyl radicals through homocysteine autooxidation and thiolactone formation (17,18). In study of Young and co-workers (19), tissue concentrations of malondialdehyde were significantly increased and the contribution of linoleic and linolenic acids to the total fatty acid content of heart was decreased in hyperhomocysteinemic animals. Figure 1 expresses a significant positive linear correlation between malondialdehyde levels and homocysteine levels.

Imbalance in prooxidant-antioxidant status results in oxidative damage of lipids, proteins and nucleic acids. Improved antioxidant status helps to minimise oxidative damage and this may delay or prevent pathological changes (4, 15, 20). A significant inverse linear correlation between malondialdehyde and vitamin C or vitamin E levels was recorded (Fig.1). In accordance with antioxidative criterion (below limit or deficient plasma value of antioxidant vitamins – low antioxidative defence; over limit or normal plasma value – high antioxidative defence) (15), the probands were distributed into groups. Table 1 shows the significantly increased levels of malondialdehyde and conjugated dienes in group with deficient vitamin C levels, in group with deficient vitamin E levels and in group with values of both vitamins below the limit levels, if compared to group with normal levels of both vitamins (over limit). Values of prooxidants between groups with only below limit of one vitamin are non-significantly changed. The highest increase of prooxidants was recorded in group with below limit levels of both vitamins. The results document that an insufficient antioxidative protection leads to prevalence of injurious prooxidative process.

Vitamin C and vitamin E are diet-derived antioxidants of major physiological importance. The main sources of vitamin E are whole-grain or dark grain products, oil seeds, grain sprouts, soy and other pulses whereas animal products are generally poor sources

Table 1. *Levels of conjugated dienes of fatty acids and malondialdehyde in dependence to vitamin C and vitamin E levels*

Vitamin C	>50	<50	<50	>50
Vitamin E	>30	<30	>30	<30
n	29	27	5	31
Vitamin C (µmol/l)	76.9±3.6	30.5±1.8[c]	40.9±3.4[c]	70.7±4.4
Vitamin E (µmol/l)	36.2±1.0	21.8±0.9[c]	34.1±2.0	25.0±0.7[c]
Conjugated dienes of fatty acids (µmol/l)	1.46±0.12	2.54±0.15[c]	2.32±0.30[a]	1.94±0.13[a]
Malondialdehyde (µmol/l)	0.95±0.06	1.63±0.14[c]	1.51±0.20[a]	1.26±0.08[b]

Results are expressed as mean±SEM (standard error mean)

[a] p<0.05   [b] p<0.01   [c] p<0.001   (vitamin C and vitamin E below limit groups vs. group with over limit both vitamin levels)
limit values for free radical disease risk (15)

of this vitamin. Vitamins C and β-carotene are derived from fruit and vegetables. Vitamin C, a water-soluble antioxidant, react directly with superoxide, hydroxyl radical and singlet oxygen, and it reduces the tocopheryl radical back to α-tocopherol (21). Vitamin E, a lipid-soluble chain breaking antioxidant converts the peroxyl radical to the much less reactive hydroperoxide, thus inhibiting the propagating step in lipid peroxidation (3, 5).

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