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SHORT COMMUNICATION

Products of DNA, Protein and Lipid Oxidative Damage in Relation to Vitamin C Plasma Concentration

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Summary

Oxidative stress plays an important role in the pathogenesis of numerous chronic age-related free radical-induced diseases. Improved antioxidant status minimizes oxidative damage to DNA, proteins, lipids and other biomolecules. Diet-derived antioxidants such as vitamin C, vitamin E, carotenoids and related plant pigments are important in antioxidative defense and maintaining health. The results of long-term epidemiological and clinical studies suggest that protective vitamin C plasma concentration for minimum risk of free radical disease is higher than 50 μmol/l. Products of oxidative damage to DNA (DNA strand breaks with oxidized purines and pyrimidines), proteins (carbonyls) and lipids (conjugated dienes of fatty acids, malondialdehyde) were estimated in a group of apparently healthy adult non-smoking population in dependence on different vitamin C plasma concentrations. Under conditions of protective plasma vitamin C concentrations (>50 μmol/l) significantly lower values of DNA, protein and lipid oxidative damage were found in comparison with the vitamin C-deficient group (<50 μmol/l). The inhibitory effect of higher fruit and vegetable consumption (leading to higher vitamin C intake and higher vitamin C plasma concentrations) on oxidation of DNA, proteins and lipids is also expressed by an inverse significant correlation between plasma vitamin C and products of oxidative damage. The results suggest an important role of higher and frequent consumption of protective food (fruit, vegetables, vegetable oils, nuts, seeds and cereal grains) in prevention of free radical disease.

Key words

Vitamin C • DNA damage • Protein carbonyls • Lipid peroxidation

Free radicals, prooxidants and antioxidants are widely discussed in clinical and nutritional literature. Reactive oxygen and nitrogen species, which are generated *in vivo*, lead to either physiological concentrations required for normal cell function, or excessive quantities, which cause damage to nucleic acids, lipids, proteins and other biomolecules (Halliwell 1996, Stadtman and Levine 2000, Valko *et al.* 2004). Oxidative stress is also proposed to be involved in the process of aging both by inducing damage to

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mitochondrial DNA and by other mechanisms (Cadenas and Davies 2000). Antioxidants are needed to prevent the formation and oppose the actions of free radicals. Endogenous antioxidant defenses (superoxide dismutases, H_2O_2-removing enzymes, metal binding proteins) are inadequate to prevent damage completely, so that diet-derived antioxidants are important in maintaining health (Gey 1995, Halliwell 1996). There is evidence that a higher intake of vitamin C and other antioxidants is associated with reduced risk of chronic diseases such as cancer and cardiovascular disease, probably through antioxidant mechanisms (Padayatty *et al.* 2003).

Oxidative stress is thought to play an important contributory role in the pathogenesis of numerous degenerative or chronic diseases (Gey 1995, Halliwell 1996, Ray and Husain 2002). Improved antioxidant status helps to minimize oxidative damage, and thus can delay or prevent pathological changes. This suggests the possible utility of antioxidant-based dietary strategies for lowering the risk of chronic age-related, free radical induced diseases. Fruit, vegetables, vegetable oils, nuts, seeds and cereal grains are the main food constituents for vitamin C, vitamin E and β-carotene. Vegetarians consuming exclusively or predominantly plant food have a higher antioxidative status in comparison with the general population consuming a traditional mixed diet (Krajčovičová-Kudláčková *et al.* 2000, 2004a).

Vitamin C is the first line of defense against oxygen radicals in the water-soluble compartment (Nordberg and Arner 2001). This vitamin reacts directly with superoxide, hydroxyl radical and singlet oxygen. The vitamin C serves to prevent lipid hydroperoxide formation in plasma lipoproteins, e.g. LDL, by reducing α-tocopheryl radicals formed upon reaction with lipid peroxyl radicals and thus vitamin C has an important function in the prevention of atherosclerotic plaque formation. Free radicals are also involved in both cancer initiation and tumor promotion. Ascorbic acid may block some of these processes (Bendich and Langseth 1995). In addition, ascorbic acid, acting as a nitrite trap, inhibits the formation of carcinogenic nitrosamines from dietary precursors.

The main goal of this study was to assess the products of oxidative damage to DNA, lipids and proteins in the adult general population in conditions of different plasma vitamin C concentrations. If the antioxidant function of vitamin C is accepted and is important for human health, then the morbidity and mortality from two main free radical diseases – cancer and cardiovascular disease must be used as criteria for determining vitamin C requirements. The results of epidemiological and clinical long-term studies suggest that the protective plasma vitamin C concentration for minimum risk of free radical diseases is higher than 50 μmol/l (Gey 1995).

Table 1. Products of DNA, protein and lipid oxidative damage in relation to vitamin C plasma concentrations

	Vitamin C plasma concentrations	
	< 50 μmol/l	**> 50 μmol/l**
Number of subjects	21	27
Age range (years)	21-69	20-59
Age average (years)	40.4±2.6	39.7±2.4
Body mass index (kg/m^2)	23.7±0.7	23.2±0.7
Smokers	0	0
Vitamin C range (μmol/l)	12.8-49.1	52.2-86.8
Vitamin C average (μmol/l)	33.1±2.3	65.7±2.1 ***
Products of oxidative damage		
DNA breaks + oxidized purines (AU)	217±9	183±5 **
DNA breaks + oxidized pyrimidines (AU)	205±8	182±7 *
Protein carbonyls (μmol/l)	133±11	96±7 **
Conjugated dienes of fatty acids (μmol/l)	2.47±0.16	1.74±0.11 ***
Malondialdehyde (μmol/l)	1.83±0.14	1.23±0.05 ***

Results are expressed as means ± S.E.M. * P<0.05, ** P<0.01, * P<0.001.

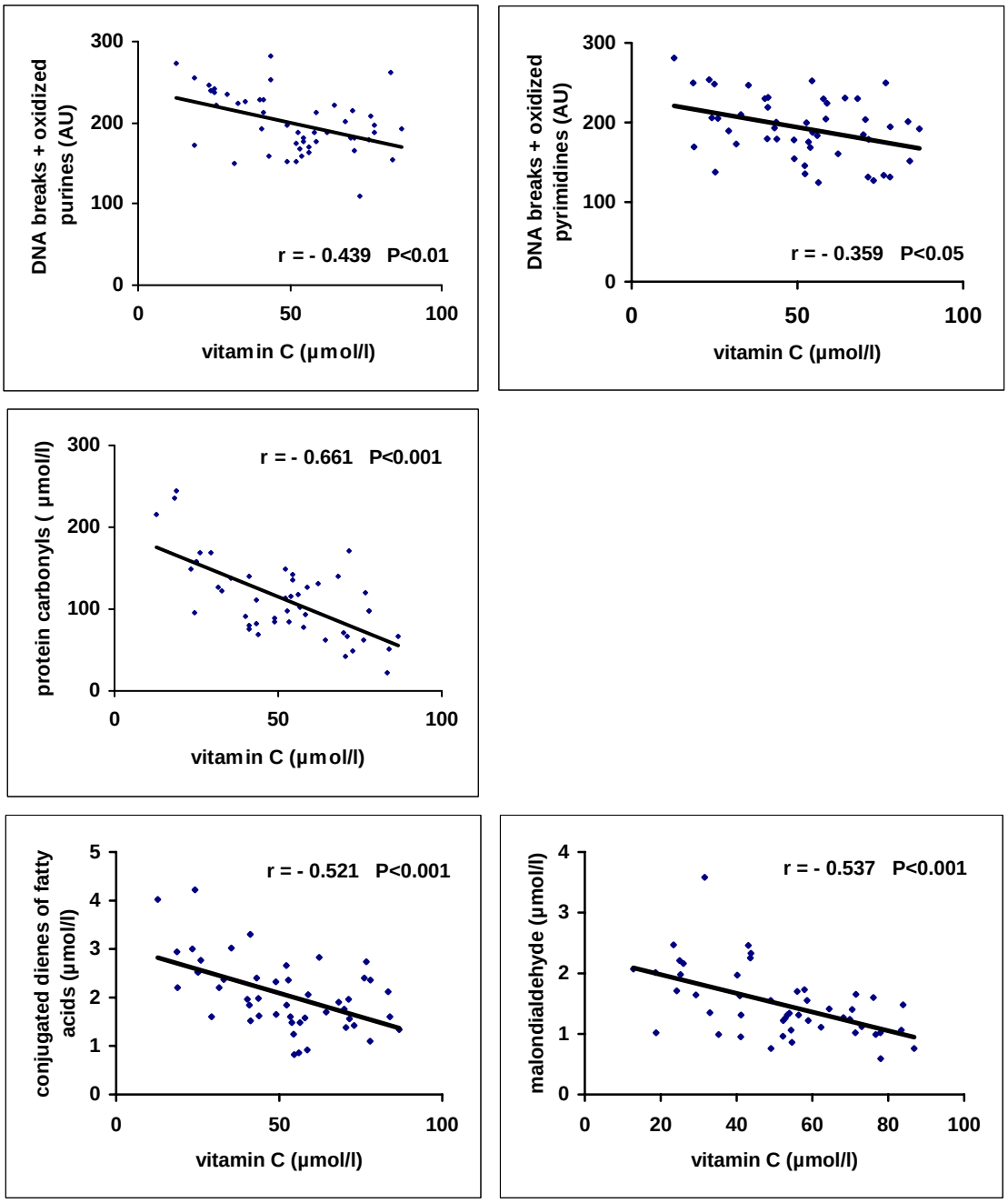

Fig. 1. Relationship of plasma vitamin C concentrations and products of oxidative damage of DNA (DNA breaks + oxidized purines, DNA breaks + oxidized pyrimidines), proteins (protein carbonyls) and lipids (conjugated dienes of fatty acids, malondialdehyde).

A randomly selected group consisted of 48 apparently healthy non-smoking adult subjects of the general population from the Bratislava region. The subjects were divided into two subgroups. In the first subgroup the vitamin C plasma concentrations were deficient from the point of view of antioxidative defense (< 50 µmol/l). The persons in the second subgroup had an optimal vitamin C concentration with a protective effect > 50 µmol/l, that means a reduced risk of free radical disease. The subgroup characteristics are presented in Table 1. Vitamin C concentrations in the plasma were detected by HPLC (Cerhata *et al.* 1994). EDTA was used as an anticoagulant and as an inhibitor of free radical reactions. The alkaline comet assay modified with lesion specific enzymes was used for detection of DNA strand breaks, oxidized purines and oxidized pyrimidines in isolated lymphocytes (Collins *et al.* 1996). Carbonyl group content in plasma was evaluated by the 2,4-

dinitrophenyl-hydrazine assay (Levine *et al.* 1990). Conjugated dienes of fatty acids in plasma were estimated by the spectrophotometric method (Recknagel and Glende 1984). The plasma malondialdehyde concentration was determined according to the method of Wong *et al.* (1987). The survey was carried out in the spring. The intake of vitamins, mineral and trace elements only in natural form was considered (no supplementation).

The protective effect of vitamin C in the plasma at a concentration > 50 μmol/l is documented in Table 1. Under these conditions we found significantly lower products of oxidative damage to DNA (DNA breaks with oxidized purines or pyrimidines), proteins (carbonyls) and lipids (conjugated dienes of fatty acids, malondialdehyde) in comparison with the vitamin C-deficient group (< 50 μmol/l). The plasma values of lipid and protein oxidative damage products were decreased by 28-33 % and a reduction of DNA damage in lymphocytes was by 12-16 %. Greater intake of fruit and vegetables was associated with a lower risk of cancer and cardiovascular disease death (Genkinger et al. 2004). A daily intake of 700-1000 g of fruit and vegetables produced an improvement in redox status parameters of diabetic patients (Giammarioli *et al.* 2004). Observational studies including mainly healthy individuals have shown a favorable relationship between the intake of vitamin C and E and subsequent cardiovascular events. However, clinical intervention studies including patients with manifest atherosclerotic disease do not support a beneficial effect of antioxidant supplements (Blomhoff 2005). In a group of apparently healthy subjects without vitamin supplementation we found an inhibitory effect of higher consumption of fruit and vegetables (higher intake of vitamin C and higher vitamin C plasma concentration) on the formation of products of oxidative damage to DNA, proteins and lipids. Figure 1 shows an inverse significant correlation between plasma vitamin C concentration and products of oxidative damage and indicates that sufficient plasma vitamin C concentrations together with other antioxidants present in fruit and vegetables may inhibit the formation of DNA, protein, lipid products induced by reactive oxygen species.

In our repeated epidemiological studies we showed that more than 90 % of subjects with predominant or exclusive plant consumption has a protective plasma concentrations of vitamin C (Krajčovičová-Kudláčková *et al*. 2000, 2004b). This fact is a consequence of frequent and sufficient consumption of fruit and vegetables in the vegetarian population. Carr and Frei (1999) suggested that the recommended dietary allowance for vitamin C must be 120 mg/day. Fain (2004) concluded that a protective daily intake of vitamin C is 110 mg. Our epidemiological-nutritional research has shown that vegetarians consume 122 mg of vitamin C on the average vs. 83 mg in the general population (Krajčovičová-Kudláčková *et al.* 2000).

Conclusion

A significantly lower incidence of oxidative damage to DNA, proteins and lipids was recorded in the population group with protective plasma concentrations of vitamin C over 50 μmol/l in comparison to the group with vitamin C concentration < 50 μmol/l. Higher concentrations of diet-derived antioxidative vitamins eliminate the harmful effect of free radicals, decrease the formation of products of oxidative damage of molecules and protect against the free radical disease.

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Reprint requests

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