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Original Article

Values of lipid and DNA damage in dependence to smoking and plasma antioxidant concentrations

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Antioxidant vitamins; DNA oxidation; Lipid peroxidation; Smoking

Abstract
Smoking is one of the major lifestyle factors influencing the health. Cigarette smoke contains many oxidants, pro-oxidants, free radicals, reducing agents and tobacco-specific carcinogens which can be implicated in the pathogenesis of ischemic heart disease, obstructive lung disease and cancer. Oxidants in cigarette smoke generate oxidative stress which causes oxidative damage to lipids, proteins and DNA. The values of lipid and DNA damage (total peroxides, malondialdehyde, oxidized LDL in plasma, lymphocyte oxidized purines and oxidized pyrimidines) as well as the plasma vitamin concentrations (vitamin C, vitamin E, beta-carotene) were measured in two groups of apparently healthy adults of general population (65 non-smokers and 61 light smokers). Each group was divided into two subgroups with optimal *vs* suboptimal plasma vitamin concentrations (vitamin C > 50 *vs* < 50 μmol/l, vitamin E/cholesterol > 5.2 *vs* < 5.2 μmol/mmol, beta-carotene > 0.4 *vs* < 0.4 μmol/l). The smokers consumed manufactured filter-cigarettes (less than 20 per day) in average number of 9.1 and 8.4 cigarettes per day with duration of smoking for 18 and 16 years for optimal vitamin and suboptimal vitamin groups, respectively. All parameters of lipid and DNA oxidation were found significantly higher in groups of suboptimal *vs* optimal plasma vitamin concentrations, but in smoking group the increase was more expressed (by 31.6-35.2% *vs* 8.9-20.9%). Significantly increased values of all oxidative damage values were seen in smokers *vs* non-smokers with suboptimal vitamin values, while in groups with optimal vitamin concentrations no changes were observed. In conclusion, oxidative stress induced by cigarette smoking increases the lipid and DNA damage at condition of suboptimal, insufficient plasma vitamin values. Optimal vitamin plasma concentrations seem to be protective against harmful effects of free radical in smoke.

INTRODUCTION

Cigarette smoking has been implicated in the pathogenesis of ischemic heart disease, emphysema, obstructive lung disease and neoplastic disorders [1-3]. More than 4000 constituents of smoke, including many oxidants, pro-oxidants, free radicals, reducing agents and tobacco-specific carcinogens have been identified. Cigarette smoke is subdivided into two phases: the hydrophobic tar (cigarette smoke condensate) or particulate fraction, and hydrophilic or gas phase (cigarette smoke extract) [1]. The hydrophilic phase contains nicotine, metals, a large number of oxidants and over 10^{15} short-lived free radicals/puff, whereas the hydrophobic fraction comprises mainly carcinogenic chemicals and more than 10^{17} long-lived free radicals/g. Long-term smoke exposure can result in systemic oxidants-antioxidants imbalance as reflected by increased oxidative damage to biomolecules and depleted concentrations of antioxidants in plasma of smokers [2].

The relationship between smoking and the development and evolution of atherosclerosis and the cardiovascular crises is unquestioned. More than 140,000 smoking-caused premature deaths are calculated to occur annually in the United States, and it has been calculated that 52% of ischemic heart disease deaths in North America are attributable to smoking; the burden is similar elsewhere in the developed world [4, 5].

The risk of myocardial infarction (MI) is dramatically increased by cigarette smoking; the relationship is dose-related and linear. There is an eightfold elevation in the odds ratio for an MI in smokers consuming more than 40 cigarettes a day [6]. The case-control study of acute MI in 52 countries (15152 cases, 14820 controls) showed that smoking (odds ratio 2.87 for current *vs* never; population attributable risk 35.7% for current and former *vs* never) is significantly related to acute MI [6].

Cigarette smokers are chronically exposed to oxidants including reactive oxygen species (ROS), peroxynitrite as well as other toxic and potentially toxic elements. Oxidative stress, a state of excessive ROS activity, enhances oxidative modification of LDL, endothelial dysfunction, platelet aggregation ability and activation of matrix-degrading enzymes, all of which are key early events in atherosclerosis. It has been reported that ROS can cause oxidative damage to cells of lipids, proteins and DNA [7, 8]. The antioxidative vitamins play a significant protective role. In long-term epidemiological and clinical studies the protective optimal plasma values of antioxidative vitamins on the base of correlations between occurrence of disease or risk markers for its genesis on one hand and plasma vitamin values on the other were determined [9]. Nutrition is a key environmental factor implicated in health and disease. Improved antioxidant status helps to minimize oxidative damage, thus can delay and prevent pathological changes [10, 11]. Food naturally containing antioxidants but not super-rich in calories, namely fruit, vegetables, nuts, seeds and cereal grains help maintain human health and delay disease onset.

The aim of this study was to assess the plasma values of lipid peroxidation and lymphocyte DNA oxidation in smokers and non-smokers of general population in relation to optimal and suboptimal (insufficient) plasma antioxidative vitamin concentrations.

SUBJECTS AND METHODS

Randomly selected apparently healthy adult non-obese 126 subjects without diagnosed cardiovascular, oncological, gastrointestinal, renal, diabetes and thyroid gland disorders (79 men, 47 women) aged 21-60 years were divided into two groups depending on smoking as the volunteers recorded in questionnaires:

Group I: 65 non-smokers of general population on traditional western or central European diet;

-Group Ia: 31 subjects with optimal plasma concentrations of vitamin C (> 50 μmol/l), vitamin E/cholesterol (> 5.2 μmol/mmol), beta-carotene (> 0.4 μmol/l),

-Group Ib: 34 subjects with suboptimal plasma concentrations of vitamin C (< 50 μmol/l), vitamin E/cholesterol (< 5.2 μmol/mmol), beta-carotene (< 0.4 μmol/l).

Group II: 61 light smokers (general population) consuming manufactured filter-cigarettes less than 20 per day;

-Group IIa: 29 smokers with optimal plasma concentrations of vitamins (see group Ia) on the average of 9.1 cigarettes per day and duration of smoking for 18 years,

-Group IIb: 32 smokers with suboptimal plasma vitamin concentrations (see group Ib), who smoked average amount of 8.4 cigarettes per day and duration of smoking for 16 years.

All subject lived in the same region (Bratislava and surroundings). The volunteers have an approximately similar physical activity (no sports). They were selected from database of addresses of university projects about health benefits and risks of alternative forms of nutrition *vs* general population as the control group. The Regional Ethic Committee approved this study and all participants gave their written informed consent. The grouping characteristic is shown in Table 1.

Blood was sampled after an overnight fasting by a standard procedure. Total peroxides in plasma (direct correlation between free radicals and circulating biological peroxides) were detected by OxyStat colorimetric assay for the quantitative determination of peroxides in EDTA plasma, serum and other biological fluids (reaction of biological peroxides with peroxidase and photometric measurement of produced coloured liquid) (Biomedica, Vienna, Austria). Malondialdehyde (MDA) in plasma was assessed by HPLC (high-performance liquid chromatography; HP 1200, Hewlett-Packard, Germany) using the fluorescence detection [12]. Oxidized low density lipoprotein (LDL) concentration in serum was measured by a commercial oxidized LDL ELISA kit (Mercodia, Uppsala, Sweden) on instrument Elisa Reader (Biotek, Winooski, VT, USA).

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 (using density gradient). For detection of oxidized purines slides were incubated with formamidopyrimidine glycosylase, oxidized pyrimidines were detected after incubation with endonuclease III. Comets were analyzed by visual scoring of 100 randomly selected images per gel, classifying them into five categories representing relative tail intensity and thus increasing degrees of damage. This method was calibrated by reference to computer image analysis based on fluorometric measurement of DNA intensities in head and tail (software: Comet Assay IV; Perceptive Instruments, Suffolk, UK) [13, 14].

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Table 1. Characteristic of groups

	Non-smokers (antioxidant concentration)		Smokers (antioxidant concentration)	
	Optimal	**Suboptimal**	**Optimal**	**Suboptimal**
n (men + women)	31 (19 + 12)	34 (23 + 11)	29 (18 + 11)	32 (19 + 13)
Average age (years)	40.8	38.9	39.7	37.8
BMI (kg/m^2)	24.1	23.8	24	23.7
Cigarettes/day	-	-	9.1	8.4
Duration of smoking (years)	-	-	18	16

Suboptimal antioxidant concentration: vitamin C < 50 μmol/l, vitamin E/cholesterol < 5.2 μmol/mmol, beta-carotene < 0.4 μmol/l.
Optimal antioxidant concentration: vitamin C > 50 μmol/l, vitamin E/cholesterol > 5.2 μmol/mmol, beta-carotene > 0.4 μmol/l.

Plasma concentrations of vitamins C, E and β-carotene were measured by HPLC (HP 1200 UV detector connected with a programmable fluorescence detector) [15, 16]. Ethylenediaminetetraacetate (EDTA) was used as an anticoagulant. Serum concentration of total cholesterol was measured by standard laboratory method on a Vitros 250 autoanalyzer (Johnson & Johnson, New York, NY, USA).

The intake of vitamins, mineral and trace elements in natural form only was allowed (no supplementation). The study was carried out during spring. The quantitative data are presented as means ± SEM. The significance of differences in measured values between groups was determined by unpaired Student´s t-test. P values less than 0.05 were considered statistically significance.

RESULTS

In Table 2 values of lipid and DNA damage in non-smokers and smokers in dependence to optimal or suboptimal plasma antioxidative vitamin concentrations are introduced. In the group of non-smokers with suboptimal *vs* optimal plasma concentrations of vitamin C, lipid-standardized vitamin E and β-carotene all parameters of oxidative damage (total peroxides, MDA, oxidized LDL, oxidized purines and oxidized pyrimidines) were measured significantly higher. In the group of active long-term smokers (average 18 years for optimal vitamin group, and 16 years for suboptimal vitamin group) who smoked in average 9.1 and 8.4 cigarettes per day the values of total peroxides, MDA, oxidized LDL, oxidized purines and oxidized pyrimidines were also found significantly increased in suboptimal *vs* optimal group, at which the increase is more expressive than in non-smokers (by 31.6-35.2% *vs* 8.9-20.9%). Furthermore, all oxidative parameters were recorded significantly higher in smokers *vs* non-smokers with suboptimal vitamin values, while remained unchanged in case of optimal vitamin concentrations.

DISCUSSION

The Nurses' Health Study found a positive association between the number of cigarettes smoked per day and risk of fatal coronary heart disease (relative risk, RR = 5.5 for more than 25 cigarettes per day) and non-fatal MI (RR = 5.8) [17]. Intake of antioxidant micronutrients from foods represent a key for the prevention of atherosclerotic disease [18, 19]. Nutritional compounds which display anti-inflammatory and antioxidant effects have specific applications in preventing oxidative stress induced injury. It is relevant that purified micronutrients isolated from natural products may be less effective than a combination seen in the natural products due to synergistic effects of interacting agents. The dietary intakes of ascorbic acid and alpha-tocopherol were negatively associated linearly with F2-isoprostanes (non-cyclooxygenase-derived prostanoids) [19].

In our previous studies [11, 20, 21] we found the protective effects of optimal plasma concentrations of antioxidants (for vitamin C > 50 μmol/l, for lipid-standardized vitamin E > 5.2 μmol/mmol, for beta-carotene > 0.4 μmol/l) against the oxidative damage. Significantly increased products of lipid peroxidation and DNA damage were measured in subgroups of suboptimal *vs* optimal plasma vitamin concentrations in group of long-term but light smokers (less than 20 cigarettes per day) and also in group of non-smokers, but these changes (increase) are more pronounced in smokers. Long-term smoke exposure causes systemic oxidative stress and suboptimal concentrations of antioxidants are insufficient to protect against harmful effects of free radicals from cigarette smoke. In case of optimal vitamin plasma concentrations the effective defense against free radicals from all sources, also from cigarette smoke is provided.

The cigarette smoke with high number of pro-oxidants and free radicals affects the antioxidant protective action, *i.e.* increases the depletion of antioxidants and oxidation of lipids, proteins and DNA [22-24]. Intervention studies using antioxidants may provide effective protection against smoking-related disorders [25, 26].

Table 2. Values of lipid and DNA oxidative damage in dependence to smoking and antioxidant concentration (mean ± SEM)

	Non-smokers (antioxidant concentration)		Smokers (antioxidant concentration)	
	Optimal	**Suboptimal**	**Optimal**	**Suboptimal**
Vitamin C (µmol/l)	57.1 ± 0.8	36.4 ± 1.4***	59 ± 1.4	34.2 ± 1.3***
Vitamin E/total cholesterol (µmol/mmol)	5.93 ± 0.1	4.94 ± 0.17***	5.76 ± 0.11	4.61 ± 0.11***
Beta-carotene (µmol/l)	0.65 ± 0.02	0.39 ± 0.03***	0.61 ± 0.02	0.37 ± 0.01***
Total peroxides (µmol/l)	354 ± 26	415 ± 16*	375 ± 19	494 ± 26***^{B}
Malondialdehyde (µmol/l)	0.88 ± 0.05	1.02 ± 0.05*	0.83 ± 0.05	1.12 ± 0.03***^{A}
Oxidized LDL (U/l)	25.8 ± 0.6	28.1 ± 0.7**	25.6 ± 0.6	34.6 ± 1.4***^{C}
Oxidized, purines (AU)	47.8 ± 2.2	57.9 ± 2.2**	54.5 ± 2.6	72.4 ± 3.7***^{B}
Oxidized pyrimidines (AU)	48.8 ± 2.3	59 ± 2**	55.4 ± 2.4	73.5 ± 3.3***^{C}

Suboptimal antioxidant concentration: vitamin C < 50 µmol/l, vitamin E/cholesterol < 5.2 µmol/mmol, beta-carotene < 0.4 µmol/l.
Optimal antioxidant concentration: vitamin C > 50 µmol/l, vitamin E/cholesterol > 5.2 µmol/mmol, beta-carotene > 0.4 µmol/l.
*P < 0.05, **P < 0.01 ***P < 0.001 suboptimal *vs* optimal antioxidant concentration; ^{A}P < 0.05, ^{B}P < 0.01 ^{C}P < 0.001 smokers *vs* non-smokers.

A relative lymphocyte protection factor of 17.6 against nitrogen dioxide and 6.3 against singlet oxygen was obtained by supplementation of lycopene rich foods and by 10-fold increased serum lycopene in comparison to typical western European diet [26]. The protective effect of broccoli intake (200 g during 40 days) was detected as significantly decreased strand breaks in smokers and non-smokers and significantly lowered oxidized purines in smokers [27].

In conclusion, oxidative stress, induced by cigarette smoking but also from other sources, increased all the parameters of lipid and DNA damage as it was observed in smokers and in non-smokers at conditions of insufficient suboptimal plasma vitamin concentrations. This increase is more expressed in smokers. In groups of smokers *vs* non-smokers with optimal, sufficient plasma antioxidant concentrations no changes were found in oxidative damage values. The results suggest that long-term maintenance of correct antioxidative status sufficiently protects against potential free radical damage.

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COMPETING INTERESTES

The authors declare that they have no conflict of interest.

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