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Background level of 8-oxo-2′-deoxyguanosine in lymphocyte DNA does not correlate with the concentration of antioxidant vitamins in blood plasma

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Antioxidantvitamins, being effective free radical scavengers, can protect cellular DNA from ox i da tive dam age. Therefore, in the pres ent study we re port on the re la tion ship be tween basal level of 8-oxo-2′-deoxyguanosine in hu man lym pho cyte DNA and the concentration of antioxidant vitamins (A, C and E). The average level of 8-oxo-2′-deoxyguanosine in lymphocytes of the studied group (15 males and 20 females) was 9.57 per 10^6 dG mol e cules. The en dog e nous level of ascor bic acid (vi ta min C) in the plasma was, on av er age, 56.78 μM, while the mean con cen tra tions of retinol (vi ta min A) and α-tocopherol (vi ta min E) were 1.24 μM and 25.74 μM, re spec tively. No correlations were found between individual 8-oxo-2′-deoxyguanosine levels in lymphocyte DNA and en dog e nous con cen tra tion of the vi ta mins.

8-Oxo-2′-deoxyguanosine, one of the oxidatively modified DNA bases, is a typical biomarker of ox i da tive stress. The pres ence of 8-oxoGua res i dues in DNA leads to GC to TA transversion unless repaired prior to DNA replication [1]. Therefore, the presence of 8-oxoGua may lead to mutagenesis. Furthermore, many observations indicate a direct correlation between *in vivo* 8-oxoGua formation and carcinogenesis [2, 3].

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Abbreviations: 8-oxodGuo, 8-oxo-2′-deoxyguanosine; 8-oxoGua, 8-oxoguanine; dG, 2′-deoxyguanosine; ROS, reactive oxygen spe cies; $OH^{\bullet}$, hydroxyl radical.

Many epidemiological studies have reported an inverse association between vegetable and fruit consumption and the occurrence of cancer and other degenerative diseases [4]. One of the possible mechanisms of this apparent protective effect could be due to the antioxidative activities of such plant food constituents as vitamins A, C and E. These antioxidant vitamins are effective free radical scavengers, therefore they should protect biomolecules such as proteins, lipids and nucleic acids from oxidative damage.

Although vitamin C, E and A are effective antioxidants *in vitro*, there is only a limited number of studies in humans that have addressed the efficacy of these compounds as antioxidants *in vivo*. Most of these studies were focused on the measurement of oxidative DNA damage after supplementation with antioxidant vitamins [5]. Moreover, it has been demonstrated that the extent of oxidative DNA damage measured in human lymphocytes showed large differences between populations of various countries [6]. Also dietary habits may differ between countries. Therefore, in the present study we report on the relationship between the basal level of 8-oxodGuo in lymphocytes and endogenous concentration of antioxidant vitamins in the blood serum of the Polish population.

MATERIALS AND METHODS

Patients. The study was conducted in a group of 35 healthy volunteers (15 males and 20 females) with an age range of 26 to 87 years. None had a history of smoking, diabetes or other diseases connencted with oxidative stress. The patients were asked to abstain from vitamin supplementation for at least a month before the blood samples were collected.

Plasma preparation for vitamins analyses. Blood samples were collected in heparinized Vacuette® tubes and centrifuged for 10 min, at 1800 × ***g***, at 4°C to obtain plasma. The heparin-plasma samples were stored at –85°C for a maximum of 3 months.

Determination of plasma vitamin E (α-tocopherol) and vitamin A (retinol) the vitamins were analyzed by HPLC as described by Shuep *et al.* [7] with some modifications:

Sample preparation. To precipitate proteins aliquots (200 μl) of freshly prepared or freshly thawed plasma samples were mixed with 200μl of HPLC-grade water and 400μl of ethanol. For the vitamins extraction, 800μl of hexane was added, and mixed for 30 min. Then, 600 μl of the upper layer (hexane) was collected, dried in a Speed-Vac system and dissolved in 150 μl of mobile phase with 0.5% butylated hydroxytoluene (BHT) for stabilization of the vitamins. Twenty μl of this solution was injected into the HPLC system.

Standard serum samples, with known vitamin E (α-tocopherol) and vitamin A (retinol) concentrations, were purchased from BIO-RAD and prepared as plasma samples.

Chromatography. A HPLC system with a fluorimetric detector was used. Samples were separated in an isocratic system normal phase column (LC-NH2-NP, 5 μm, 250 mm × 4.6 mm) with an LC-NH2-NP guard column (40 μm, 20 mm × 4.6 mm), both from Supelco [7]. The mobile phase, containing hexane with 4% of ethyl acetate and 5% of 1,4-dioxane was used, at a flow rate of 1.5 ml/min. The effluent was monitored with fluorimetric detection (λ_{ex} = 340 nm, λ_{em} = 472 nm for retinol and λ_{ex} = 290 nm, λ_{em} = 330 nm for α-tocopherol) and analyzed by Dionex Peak Net 4.3 software.

Determination of plasma vitamin C (ascorbic acid) by HPLC. Vitamin C was analyzed as described by Hultqvist *et al.* [8] with some modifications:

Standard solutions. A standard stock solution (1 mM ascorbic acid) was made by dissolving ascorbic acid in 5% metaphosphoric acid. Aliquots of this solution were immediately frozen at –85°C and stored no longer than 1 month. Working standards (in the range 1–60 μM) were freshly prepared for each analysis. All solutions were carefully pro

tected from light during preparation and analysis.

Sample preparation. Aliquots (200 μl) of freshly prepared or freshly thawed plasma samples were mixed with 200 μl of 10% metaphosphoric acid for protein precipitation and ascorbic acid stabilization. After centrifugation (10 min, 3000 × ***g***, 4°C), supernatants were collected and filtered through a Millipore microcentrifuge filter (NMWL 5000). Twenty μl of these filtrates was injected into the HPLC system.

Chromatography. A HPLC system with a diode-array detector was used. Samples were separated in an isocratic system C18 reversed phase column (5 μm, 250 mm × 4.6 mm) with a C18 guard column (40 μm, 20 mm × 4.6 mm), both from Supelco, at a flow rate of 1 ml/min. The mobile phase containing 5 mM KH_2PO_4, 1 mM Na_2EDTA was adjusted to pH 3.0 with phosphoric acid. The effluent was monitored with a UV detector at 245 nm and analysed by Class software.

Isolation of lymphocytes from venous blood. Lymphocytes were isolated on Histopaque 1077 solution (Sigma), according to the procedure laid down by the manufacturer. Lymphocytes were stored at –85°C.

DNA isolation and determination of 8-oxo-2′-deoxyguanosine. DNA isolation from lymphocytes and quantification of 8-oxodGuo by the use of HPLC with electrochemical detection were as described previously [9].

RESULTS AND DISCUSSION

The 8-oxodGuo levels in lymphocytes of the studied group ranged from 2.86 to 20.46 per 10^6 dG molecules (mean value ± S.D.: 9.57 ± 3.95), in the range of values reported by others [10, 11]. The mean endogenous level of ascorbic acid in the plasma was 56.78 μM (range 9.88–139.03 μM), while the mean concentrations of retinol and α-tocopherol were 1.24 ± 0.63 and 25.74 ± 16.02 μM, respectively (range 0.19–2.69 μM and 2.47–65.70 μM, respectively). No correlations were found between 8-oxodGuo level in lymphocyte DNA and any of the investigated vitamins (Fig. 1).

Most of the studies concerning the association of antioxidant vitamins with the background level of oxidative DNA damage were supplementation studies [12–15]. Duthie *et al.* [15] using single cell gel electrophoresis (comet assay) found that supplementation of healthy volunteers with vitamin C (100 mg/day), vitamin E (280 mg/day) and β-carotene (25 mg/day) significantly reduced base damage in lymphocyte DNA. The study of Rehman *et al.* [12] suggested that supplementation of volunteers with an initial high endogenous level of vitamin C (71.7 μM) (co-supplemented with iron) may result in an increase of total oxidative DNA base damage. However, when volunteers with lower endogenous level of vitamin C (50.4 μM) were studied, vitamin C and iron co-supplementation suppressed total oxidative DNA base damage.

Collins *et al.* [16] demonstrated a significant negative correlation between basal concentration of serum carotenoids and oxidatively modified pyrimidines. Supplementation of patients with carotenoids did not influence oxidative DNA damage. The authors did not find any correlation between the damage and the concentration of vitamins E and C, either.

Oxidative DNA damage, as measured by urinary excretion of 8-oxodGuo, is not significantly influenced by plasma concentration of antioxidants (ascorbic acid, α-tocopherol and β-carotene) [17].

As mentioned above, our results suggest that there is no correlation between the endogenous concentration of the investigated vitamins and the modified guanine level in DNA.

The results of our study are in good agreement with the hypothesis which assumes that 8-oxodGuo mainly arises due to the reaction of hydroxyl radical ($OH^{\bullet}$) with cellular DNA [18]. Because of its high reactivity hydroxyl radical must be formed in close proximity to

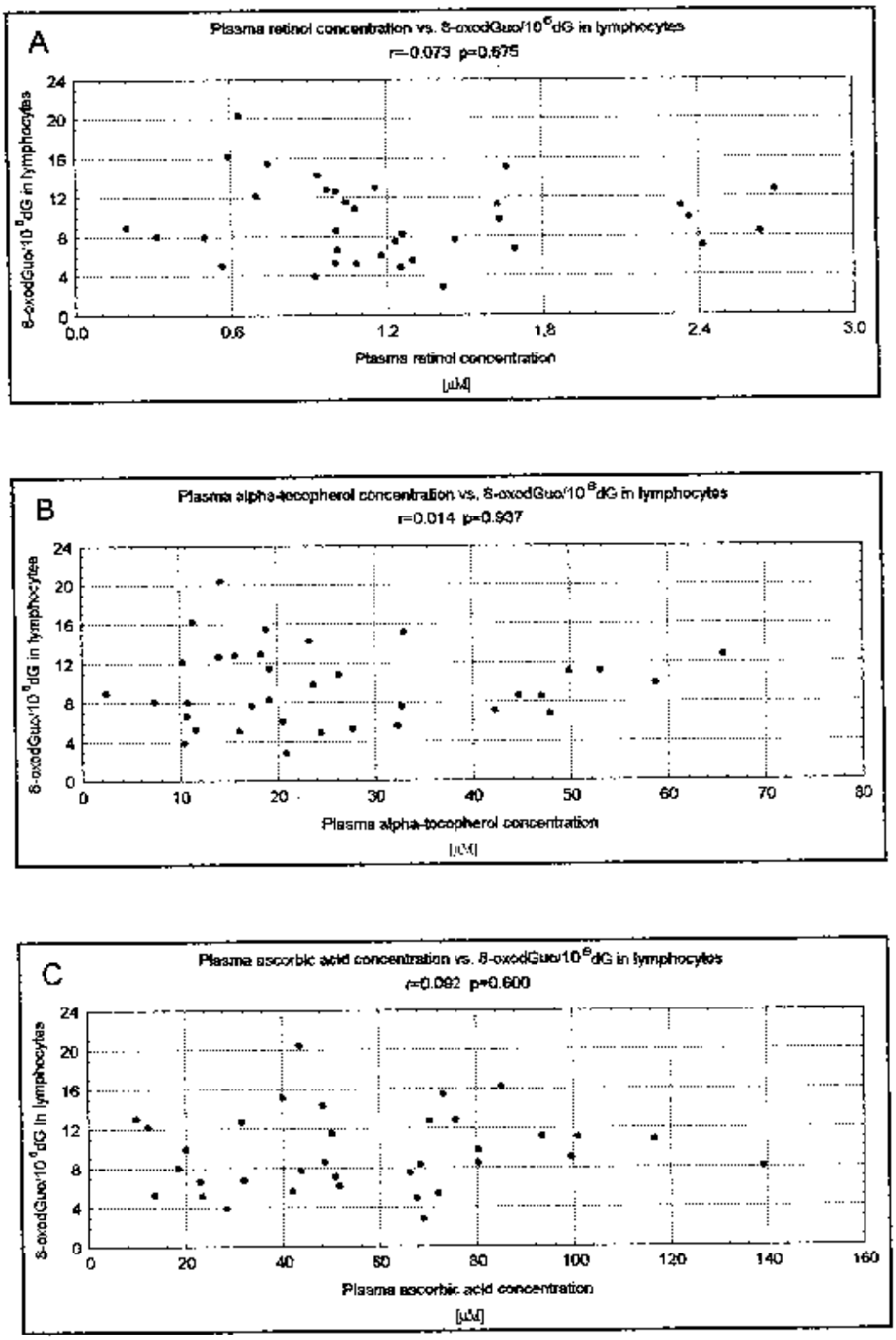

Figure1. Relationship between level of 8-oxodG in lymphocytes DNA and plasma concentrations of retinol (A), α-tocopherol (B) and ascor bic acid (C).

the base to produce the damage (half-life of $OH^{\bullet}$ in cells is 10^{-9} s). Most likely $OH^{\bullet}$ is produced during Fenton reaction between H_2O_2 (which can easily penetrate cell membranes) and transition metal ions (Fe^{2+} and/or Cu^{+}), which may form complexes with cellular DNA [19]. In this case it is rather unlikely that vitamin C (or the other vitamins) can scavenge the radicals and protect DNA. However, 8-oxoguanine may also arise as a product of guanine reaction with several different reactive oxygen species (ROS) (most likely with singlet oxygen with the half-life time about 10^{-5} s [18, 19]). Therefore, we cannot exclude the possibility that under such circumstances antioxidant vitamins can protect DNA from oxidative damage.

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