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Review

Vitamin C for DNA damage prevention

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ABSTRACT

The ability of vitamin C to affect genetic damage was reviewed in human studies that used molecular epidemiology methods, including analysis of DNA adducts, DNA strand breakage (using the Comet assay), oxidative damage measured as levels of 8-oxo-7,8-dihydroxy-2′-deoxyguanosine (8-oxodG), cytogenetic analysis of chromosomal aberrations and micronuclei, and the induction of DNA repair proteins. The protective effect of vitamin C was observed at plasma levels > 50 μmol/l. Vitamin C supplementation decreased the frequency of chromosomal aberrations in groups with insufficient dietary intake who were occupationally exposed to mutagens, and also decreased the sensitivity to mutagens as assessed using the bleomycin assay. High vitamin C levels in plasma decreased the frequency of genomic translocations in groups exposed to ionizing radiation or c-PAHs in polluted air. The frequency of micronuclei was decreased by vitamin C supplementation in smokers challenged with γ-irradiation, and higher vitamin C levels in plasma counteracted the damage induced by air pollution. The prevalence of DNA adducts inversely correlated with vitamin C levels in groups environmentally exposed to high concentrations of c-PAHs. Increased vitamin C levels decreased DNA strand breakage induced by air pollution. Oxidative damage (8-oxodG levels) was decreased by vitamin C supplementation in groups with plasma levels > 50 μmol/l exposed to PM2.5 and c-PAHs. Modulation of DNA repair by vitamin C supplementation was observed both in poorly nourished subjects and in groups with vitamin C plasma levels > 50 μmol/l exposed to higher concentrations of c-PAHs. It is possible that the impact of vitamin C on DNA damage depends both on background values of vitamin C in the individual as well as on the level of exposure to xenobiotics or oxidative stress.

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Abbreviations: AA, ascorbic acid; AB.C., aberrant cells – cells carrying chromosomal aberrations; B[a]P, benzo[a]pyrene; BCME, bis(chloromethyl)ether; CMME, chloromethyl methyl ether; c-PAHs, carcinogenic polycyclic aromatic hydrocarbons; F_G/100, genomic frequency of translocations/100 cells; FISH, fluorescence in situ hybridization; *hMTH1*, human MutH homologue; *hOGG1*, 8-oxoguanine DNA glycosylase 1; H_2O_2, hydrogen peroxide; MN, micronuclei; NS, nonsmokers; 8-oxodG, 8-oxo-7,8-dihydroxy-2′-deoxyguanosine; PBL, peripheral blood lymphocytes; PBMC, peripheral blood mononuclear cells; PM2.5, particulate matter of aerodynamic diameter < 2.5 μm; RDA, Recommended Dietary Allowances; SCE, sister chromatid exchanges; *XRCC1*, X-ray repair complementing defective repair in Chinese hamster cells 1.

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1. Introduction

Vitamin C (ascorbic acid, AA) is one of the most natural antioxidants in living tissues. Unlike other mammals, man is incapable of synthesizing vitamin C from D-glucose and may thus be considered a mutant whose inborn genetic defect must be compensated by food-borne vitamin C, or in the case of insufficient intake, especially under conditions of stress, by its direct supply [1]. This idea was repeatedly proposed by Ginter [1–3]. The Recommended Dietary Allowance (RDA) in many countries ranges from 40 to 90 mg/day (UK 40 mg/day, WHO 45 mg/day, Canada and USA females 75 mg/day, males 90 mg/day). However, a comparison of the kinetic parameters of AA in animals and man shows that the officially recommended doses of vitamin C do not ensure maximum steady-state levels in plasma and tissues [2]. Therefore, Ginter [3] suggested that an intake of AA that ensures a maximum body pool and maximum steady-state level of vitamin C in tissues is optimal. It is probable that in healthy adults, such a dose ranges from 100 to 200 mg/day and that under stress it exceeds 200 mg/day [3]. 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 [4]. This corresponds to an average vitamin C intake of 124.2 mg/day (in the range of 92–181 mg) [5]. Consistent with this, Carr and Frei [6] recommended a daily intake of 120 mg.

The significance of vitamin C for man lies in its biochemical functions. It affects xenobiotic biotransformation, apparently by influencing microsomal hydroxylation enzymes and reacting with free radicals – particularly those derived from oxygen. Ginter et al. [7] proposed that vitamin C increases mixed function oxidase (cytochrome P450) activity acting as the electron donor for cytochrome b_5 oxidoreductase or for the formation of hydroxyradicals. By speeding up the microsomal hydroxylation process, vitamin C enhances the solubility of xenobiotics and consequently their elimination in urine. This ability to affect the activity of cytochrome P450 explains the significance of vitamin C for xenobiotic biotransformation.

Oxidative stress is believed to be a significant factor for speeding up the process of aging. There is evidence that a higher intake of vitamin C is associated with a reduced risk of cancer and cardiovascular disease, probably due to its antioxidant effects [8]. It is therefore believed that elevated vitamin C levels in man may decrease oxidative damage and thereby prevent pathological changes. Experiments on animals (especially detecting increased synthesis and then increased excretion of vitamin C in the urine of rats) proved the relationship between the level of vitamin C in an organism and the biotransformation of drugs and other xenobiotics. These include analgesics (aminopyrine and antipyrine), antihistamines (chlorcyclizine), antirheumatics (phenylbutazone), hypnotics (barbital), myorelaxants (orphenadine) [9], antibiotics (tetracycline) [10], pesticides (organophosphates [11], chlorinated hydrocarbons [12]) and some metals. Studies in humans investigated the effect of vitamin C in speeding up the metabolism of alcohol [13].

The increased requirement for vitamin C was also proven for the metabolism of chemicals with known mutagenic and carcinogenic activity as benzene [14], benzidine and 3-naphtylamine [15], chloroform and tetrachloromethane [16], nitrates (affecting the formation of nitrosamines) [17], nitrosamines [18], polycyclic aromatic hydrocarbons [19], polychlorinated biphenyls [12,20] and tobacco smoke [21,22]. Studies in guinea-pigs and rats indicated the increased need for vitamin C during chronic consumption of moderate doses of alcohol. This is due to the participation of ascorbate in redox processes connected with ethanol metabolism [23].

The relationship between vitamin C levels and the efficiency of humans to metabolize xenobiotics was studied using the antipyrine test (this test determines the rate of antipyrine biotransformation by the liver and its elimination from the organism, and was understood to be an indicator of mixed-function oxidase activity [24]). Ginter and Vejmolova [25] studied the effect of a daily intake of 500 mg vitamin C for 12 months in volunteers. A significantly higher elimination constant and shorter half-life of antipyrine were found in the AA-treated group (17.0 h vs. 10.0 h, $p < 0.001$). It was concluded from these results that vitamin C stimulated the microsomal cytochrome P450-containing hydroxylase system in the liver.

Most in vitro studies with chemical mutagens indicate that vitamin C has an antimutagenic activity. AA decreased the frequency of mutations induced by mutagens in studies with *Salmonella typhimurium* strains [26–28], the mutagenicity of human feces in tests on microorganisms [29,30], the frequency of chromosomal aberrations [31], and the frequency of SCE (sister chromatid exchanges) in cell cultures [26,32]. However, Stich et al. [33,34] found that ascorbate induced chromosomal aberrations in the Chinese hamster ovary cells, clastogenicity was significantly increased by Cu^{2+}, Mn^{2+} and Fe^{2+} ions (10^{-4} to 10^{-5} M), which probably induce hydrogen peroxide and free radical formation. The mutagenic activity of AA was not proven in in vivo experiments that studied the frequency of SCE in the bone marrow of Chinese hamsters [35] and dominant lethal in rats [36] with a dose of 10 mg/kg b.w. It is believed that mammalian organisms are protected against the damage caused by hydrogen peroxide (H_2O_2) and free radicals by their reaction with enzymes such as glutathione peroxidase, catalase and superoxide dismutase [35,37].

2. Effect of ascorbic acid prophylaxis on groups occupationally exposed to carcinogens

Cytogenetic analysis of peripheral blood lymphocytes (PBL) has been accepted as a suitable technique for the biological monitoring of genetic damage in somatic cells since the early 70s. In the Czech Republic, cytogenetic analysis has proven its utility, particularly in the area of occupational health. To prevent genetic injury to workers and its expected consequences, such as an increase in cancer rates or in the rates of malformations in their children, cytogenetic analysis has been implemented as a component of preventive medical check-ups since 1976/1977. It quickly became a particularly useful tool to test the safety of industrial hygiene standards. In many instances, cytogenetic analysis provided the chief argument used to enforce improved working conditions. Cytogenetic analysis was often the only method available to evaluate the clastogenic effects of mixtures of chemicals present in the working environment and their carcinogenic potency in real time. Today, chromosomal aberrations in human PBL are recognized as a valuable biomarker. It is generally accepted that a high frequency of chromosomal aberrations in peripheral lymphocytes is predictive of an increased risk of cancer [38–42].

Some workers have been occupationally exposed to human carcinogens, such as bis(chloromethyl)ether (BCME), chloromethyl methyl ether (CMME) and carcinogenic polycyclic aromatic hydrocarbons (c-PAHs). The ion exchanger unit, exposed to both halogenated ethers, was in operation from 1973 to 1987. In 1974 it became the very first workplace (Usti n. L., Czech Republic) in which chromosomal aberrations were used as a biomarker of exposure to evaluate the biological significance of occupational exposure. Since 1976 cytogenetic analysis became part of their periodic check-ups, and 80–110 workers were examined each year. The frequency of AB.C. was 4.5–7.5% for exposed workers compared to 1.5% in controls. Wattenberg's recommendation (1978) to use chemical carcinogen inhibitors in high-risk categories of workers in order to prevent possible health damage resulting from chronic occupational overexposure proposed using AA for this purpose [43].