Abstract

Free radicals and reactive oxygen species (ROS) which are generated continuously cause mutagenic alterations resulting in cancer, aging and abnormalities in the nervous system. Accumulating evidence indicates that Vitamin E, the most potent lipid peroxyl radical scavenger, may reduce free radical induced chromosomal damages through inhibition of free radical formation, and activation of endonuclease that can be triggered by intracellular oxidative stress, and by increasing the rate of removal of damaged DNA. Although some studies suggest a potential usefulness of Vitamin E in the prevention of mutagenic effects caused by genotoxic free radicals, other studies report no effects. Thus the data are not conclusive enough to be used as a basis to change the current recommended dietary allowances (RDA). Future research should address molecular mechanisms underlying the protective effects of Vitamin E and develop appropriate biologically relevant biomarkers of DNA damage to further help in determining the dietary levels of Vitamin E needed to protect the genetic pool from internally and externally induced DNA damages. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Free radicals and reactive oxygen species (ROS) are continuously generated from daily background exposure to ionizing radiation [1], cigarette smoke [2], food additives [3], and cellular metabolism damaging a wide range of cell types. In the absence of an adequate repair system, accumulated DNA damages cause mutagenic alterations resulting in cancer, aging and abnormalities in nervous system [4]. Dietary antioxidant supplementation reduces the ROS levels as well as the ROS-induced damages [5]. Accumulating evidences demonstrated that Vitamin E, the most potent lipid peroxyl radical scavenger, significantly reduces free radical induced chromosomal damages [6–8]. As illustrated in Fig. 1, this protective effect of Vitamin E on oxidative stress induced DNA damage may be mediated through inhibition of free radical formation.

In addition to inhibiting free radical formation, Vitamin E has been suggested to inhibit activation of endonuclease that can be triggered by intracellular oxidative stress [10,11] as well as to enhance the repair of DNA damage by increasing the rate of removal of damaged DNA [12].

Although some studies suggest a potential usefulness of Vitamin E in the prevention and treatment of mutagenic activity caused by genotoxic free radicals, other studies reported no effect from either...
in vitro or in vivo studies. This review will summarize the current literature findings on the effects of Vitamin E supplementation in maintaining genome/genetic stability.

2. Role of Vitamin E in reactive oxygen species-induced DNA damage

One of the most damaging intracellular ROS is hydroxyl radical (HO•), which is produced from H2O2 in the presence of metal ions (Fig. 1). Exposure to ionizing radiation gives rise to in vivo production of HO•, which induces chemical changes in the DNA bases by adding itself to guanine residue at C-4, C-5 or C-8 positions to form 8-hydroxy-7,8-dihydroguanine, which is then further oxidized into 8-hydroxyguanine [11,13]. Vitamin E has been shown to reduce H2O2-induced HO• generation and subsequent DNA base pair modification in human oral epithelial cells [14], and H2O2 induced DNA strand breaks in human skin cell line VH10 [15].

High levels of ROS have been shown to be generated by both gas phase [16,17] and tar phase (hydroxy quinone-semiquinone-quinone/hydroxyl radicals) [18] during cigarette smoke. ROS generated from cigarette smoke have been shown to induce DNA single strand break (DNA SSB) [19] and modified DNA base pairs such as 8-hydroxy deoxy guanosine [13]. Vitamin E has been shown to inhibit cigarette smoke induced DNA SSB [17].

Peroxynitrite produced in the presence of O2•− when NO• is released by different cells such as immune cells during inflammation [20], neurons [21], and endothelial cells [22,23], is a potent oxidizing agent. Peroxynitrite has been shown to damage DNA primarily by deamination of cytosine to uracil leading to GC → AT transversion and adenine to hydroxanthine leading to AT → GC mutation [24]. NO•-induced peroxynitrite production also induces DNA cross-link as well as oxidation of DNA base pairs [25,26]. Vitamin E has been shown to inhibit NO•-induced damages in rat pancreatic islet cell nuclei by inhibiting DNA strand breaks [27].

In addition, Vitamin E plays a protective role against oxidative stress induced by other environmental mutagens such as food additives and insect repellants. For example, potassium bromate (KBrO3), which has been used as a food additive, is an oxidizing agent capable of generating modified guanine base pairs (reviewed in [28]). Vitamin E inhibits KBrO3-induced guanine base pair modification in rat kidney cells [28]. Other environmental toxins such as naphthalene, used as components of moth ball, have been shown to generate lipid peroxidation, and Vitamin E treatment has been shown to inhibit naphthalene-induced lipid peroxidation as well as DNA SSB in rat liver and brain cells [29].

3. Role of Vitamin E in chromosomal aberration, DNA adducts and micronucleus formation

Vitamin E supplementation has been shown to inhibit chromosomal alterations that are measured by chromosome gaps and breaks in c-myc proto-oncogene and transforming growth factor (TGF) co-over expressing transgenic mice [7]. It is interesting to note that over expression of c-myc and TGF increases intracellular peroxide production [30]. Further, these authors demonstrated that oxidative stress-induced chromosomal aberration is inhibited by Vitamin E supplementation. Inhibition in chromosomal aberration is also observed in bone marrow cells from rats that are fed Vitamin E (300 mg/kg) for 6 months [31].
Vitamin E inhibited metal ion (chromium)-induced DNA strand breaks [32] and chromosomal aberration in Chinese hamster lung V79 cells [33]. It is possible that Vitamin E inhibits chromosomal alteration by reducing metal ion adducts to DNA. However, Blankenship and colleagues [34] showed that the inhibitory effect of Vitamin E on sodium chromate-induced chromosomal aberration was not due to reduction in chromium adducts to DNA in Chinese hamster ovary cells [34]. However, Vitamin E (Trolox, water soluble Vitamin E) decreased H$_2$O$_2$-induced DNA adducts in the presence of Fe$^{3+}$ ion [35].

Micronuclei which arise from chromosomal breaks and from chromosomes that are not incorporated into daughter cells during mitosis is used as a biomarker of chromosomal alterations. It has been shown that Vitamin E pretreatment (200 mg/kg per day for 5 days) inhibits irradiation-induced micronucleus formation in bone marrow polychromatic erythrocytes of C57Bl/6 mice [12].

4. Role of Vitamin E in apoptosis

A variety of stimuli induces programmed cell death or apoptosis in different cell types. Removal of serum can induce increased lipid peroxidation as well as apoptosis in HL-60 cells, and Vitamin E inhibited both lipid peroxidation and apoptosis [36]. Oxidative stress also has been shown to induce apoptosis [37], and Vitamin E has been shown to inhibit Prostaglandin F2 ($\text{PGF}_2$)-induced apoptosis in ovine corpora luteal cells [38]. In addition, proto-oncogenes such as bcl-2 (inhibitor of apoptosis) have been shown to exert their anti-apoptotic effect through their ability to protect cells against oxidative stress [39]. Indeed, bcl-2 was shown to inhibit oxidation of phospholipid oxidation [40]. Furthermore, Tyurina et al. [40] showed that Vitamin E analogue (2,2,5,7,8-pentamethyl-6-hydrochromane) inhibits phospholipid oxidation. In addition, lipopolysaccharide (LPS)-induced apoptosis in human endothelial cells was also inhibited by Vitamin E [41]. Taken together, results from these studies suggest that Vitamin E inhibition of apoptosis occur mainly through its antioxidant function. Consequently, antioxidants may prevent development of cancer through their ability to induce apoptosis.

5. Role of Vitamin E in DNA repair

The addition of Vitamin E, immediately after radiation treatment to bone marrow polychromatic erythrocytes, reduced radiation-induced micronucleus formation [12]. It has been suggested that this inhibitory effect of Vitamin E on micronucleus formation may be due to modulation of the DNA repair system [12]. Indeed, measurement of DNA repair ability tested in lymphocytes indicated that Vitamin E increases the removal rate of damaged DNA compared to cells that are not treated with Vitamin E [12].

Zearlenone, a non-steroidal oestrogenic toxin, is found in human foods such as cereals and has been shown to cause DNA damage [42]. Zearlenone was also reported to induce DNA damage-induced SOS response (detection of genotoxic activity), and Vitamin E has been shown to prevent Zearlenone-induced induction of SOS repair response [42].

6. Role of Vitamin E in DNA damage-induced gene expression

Although the effects of Vitamin E on ROS-induced gene expression has been studied extensively, limited data are available regarding the role of Vitamin E in DNA damage-induced gene expression. Although not gene specific, it has been shown that the total and non-specific liver transcriptional activities (measured by the amount of different lengths of RNA) of rats that were fed 30 IU alpha-tocopherol acetate/kg body weight were higher than those of control rats (the levels of Vitamin E in the control diet is not specified and could be zero mg/kg diet) [43].

Glucose-regulated protein 78 (GRP 78) is a heat shock 70-related protein that resides in the lumen of the endoplasmic reticulum (ER) which is involved in multimeric protein assembly, the degradation of proteins, and the storage and regulation of ER luminal calcium. Sodium chromate treatment inhibited GRP78 gene expression up to 80–90% [44]. Chromium has been shown to induce DNA SSB and DNA adducts, and Vitamin E has been shown to inhibit chromium-induced DNA damage [34]. Thus, it is plausible that chromium-induced suppression of GRP78 may be upregulated by the addition of Vitamin E. Vitamin E has been shown to induce heat shock protein (HSP 70)
expression in keratinocytes [45]; this implies a protective role of Vitamin E in chromium-induced genotoxicity.

The c-fos expression is induced when cells are exposed to H$_2$O$_2$ [46] and UV, and both H$_2$O$_2$ and UV induce DNA damage [47]. The c-fos encodes a nuclear protein that forms heterodimer with Jun, Fos or ATF (activating transcription factor) transcription factors, and binds a common DNA site (AP-1) to regulate various transcriptional activity. Vitamin E treatment has been shown to induce c-jun expression as well as AP-1 binding activity in human breast cancer cells [48]. Furthermore, addition of Trolox (a water-soluble Vitamin E derivative) increased c-fos expression in human fetal lung fibroblasts [49]. Collectively, these findings suggest that the protective role of Vitamin E against DNA damage induced by H$_2$O$_2$ and UV is, in part, mediated by upregulation of c-fos expression and AP-1 binding activity.

7. Interaction of Vitamin E with other vitamins

The interaction of Vitamin E with other vitamins such as Vitamin C is perplexing. Vitamin C has been shown to inhibit formation of guanosine base modification in both in vivo [50,51] and in vitro studies [52]. Furthermore, when Vitamin E (30 μM) or ascorbic acid (600 μM) are added separately, each vitamin has a protective effect against DNA damages in human sperms [53]. However, in the same study, the addition of Vitamins C and E together produced damaging effects. Interestingly, addition of Vitamin C (60 μM) together with Vitamin E (30 μM) showed neither a harmful nor a beneficial effect on H$_2$O$_2$-induced DNA damage in human lymphoblastoid cells. However, combined treatment with the two vitamins increased radiation-induced DNA damage [54]. Whether different types of genotoxic stress such as irradiation and H$_2$O$_2$ trigger different modes of protective action by these antioxidants resulting in either beneficial or harmful effects need to be examined further.

The addition of Vitamins E and C separately inhibited formation of micronucleus levels in bone marrow polychromatic erythrocytes in C57Bl/6 mice [12]. However, when Vitamins E and C were added together levels of micronucleus were higher in bone marrow polychromatic erythrocytes than in those of control cells [12].

It has been suggested that the addition of Vitamin E hinders the protective effect of Vitamin C because Vitamin C is utilized during the recycling process of Vitamin E (from oxidized form to reduced form) which results in decreased protective effects of Vitamin C against oxidative stress-induced DNA damage [54]. The exact mechanism as to why these two antioxidants, which traditionally work beneficially together, exert harmful effects on DNA damage needs to be further studied.

8. In vivo cross-sectional and intervention studies in humans

Vitamin E supplementation (80 mg per day for 4 weeks) in healthy male non-smokers, who were given increased dietary polyunsaturated fatty acid (PUFA, 15% higher PUFA compared to the control diet), significantly increased plasma Vitamin E concentration (from 3.85 ± 0.16 μmol/mmol cholesterol to 4.67 ± 0.26 μmol/mmol cholesterol, P < 0.001) compared to baseline, and reduced PUFA-induced DNA SSB in peripheral blood lymphocytes [55].

Normal healthy males (between ages of 50 to 59 years) supplemented with Vitamin E (280 mg per day) showed increased Vitamin E levels in plasma after 20 weeks (from baseline of 5.30 ± 0.38 μM/mM cholesterol to 7.93 ± 0.77 μM/mM cholesterol after supplementation, P < 0.002) which was associated with reduced oxidized pyrimidines in peripheral blood lymphocytes collected from them [56].

Supplementation of male smokers (approximately 24 years of age) with Vitamin E 200 IU per day (for 4 weeks) increased plasma levels of Vitamin E approximately two-fold, and reduced the carbonyl content and 8-hydroxydeoxyguanosine levels in leukocytes [57].

Contrary to results from the above studies, others have reported no beneficial effect of Vitamin E supplementation against cellular DNA damage. For example, despite the fact that Vitamin E supplementation (800 IU per day for 6 weeks) increased plasma level of Vitamin E by 2.5-fold, no correlation between increased Vitamin E levels and chromatid breaks was observed [58]. No beneficial effects of Vitamin E on the prevention of chromosomal damage were
observed in another study in which male subjects were supplemented with 50 mg per day for 8 weeks (provided in cereal), followed by 500 IU for 8 weeks (provided as capsules), as measured by micronuclei originated from damaged chromosomes in peripheral blood lymphocytes [59]. In addition, no significant reduction in DNA SSB has been observed in blood lymphocytes with 400 IU per day treatment of Vitamin E for 8 weeks for both type 1 diabetic patients and in healthy control subjects [60].

Furthermore, no effects of Vitamin E on oxidative DNA damage, as measured by urinary 8-hydroxydeoxyguanine levels, was reported following 2 months of supplementation with 200 mg Vitamin E compared to the control group [61]. In addition, higher levels of 8-hydroxydeoxyguanine levels (biomarkers of DNA oxidative damage) were reported in people with higher plasma Vitamin E levels [62]. These findings suggest that the plasma levels of Vitamin E do not necessarily correlate with the protective effects of Vitamin E on cellular DNA damage.

Results from in vitro human studies provide mostly beneficial effects. Vitamin E treatment (200 μM) to in vitro cultured human peripheral blood lymphocytes has been shown to decrease unscheduled DNA synthesis, a measure of DNA repair synthesis [63]. As low as 10 μM Vitamin E treatment significantly reduced chromosomal breakage in blood leukocytes [64]. A moderate decrease in glucose oxidase-induced DNA SSB has been observed in human peripheral blood lymphocytes with 100 μM Vitamin E treatment [65]. Furthermore, 30 μM Vitamin E treatment to cultured human lymphoblastoid cells has been shown to decrease mean comet tail moment [54]. Comet assay is based on the micro-electrophoresis of cells that are lysed and embedded in agarose gel, followed by DNA-binding fluorescent staining. DNA strands that are broken migrate farther in the electric field, creating a “comet” like image with a fluorescent head and a tail region that increases as DNA damage level increases.

9. Concluding remarks

The current recommended dietary allowances (RDA) of Vitamin E is 10 mg for the adult male and 8 mg for the adult female. The literature findings discussed in this review and summarized in Table 1, indicate that there is an inconsistency in the current literature regarding the preventive effect of Vitamin E
on DNA damage; some studies reported beneficial effects of Vitamin E while others reported no beneficial effects of higher than the RDA intake of Vitamin E. Thus, it is not possible at this time, to recommend a level of dietary Vitamin E above the RDA for optimal prevention of DNA damage. Some of the inconsistencies in the literature are due to variations in the age and health status of the subjects used as well as the “biomarkers” utilized to access DNA damage. Standardization of “relevant biomarkers” as well as the study population should help in answering the question of the adequacy of the current RDA of Vitamin E for optimal protection against genetic damage. Furthermore, based on inconclusive correlations between plasma levels of Vitamin E and protective effects of Vitamin E on cellular DNA damage reduction, it is difficult to suggest the level of Vitamin E in blood that corresponds to minimal DNA damage. Although adverse effects of Vitamin E treatment (when added together with Vitamin C) are reported, the highest intake levels of Vitamin E alone that might produce adverse effects has not been defined. Future research should address molecular mechanisms underlying the protective effects of Vitamin E on DNA damage and DNA damage-induced changes in gene expression regulation as well as the appropriate clinically/biologically relevant biomarkers of DNA damage. Such information will further help in determining the dietary levels of Vitamin E needed to protect the genetic pool from internally and externally induced DNA damage.

References


