Free Radical Research, Vol. 35, pp. 917–924
Reprints available directly from the publisher
Photocopying permitted by license only

© 2001 OPA (Overseas Publishers Association) N.V.
Published by license under
the Harwood Academic Publishers imprint,
part of Gordon and Breach Publishing
a member of the Taylor & Francis Group.
All rights reserved.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

γ-Tocopherol is Less Effective than α-tocopherol in Preventing Oxidant-induced Sister Chromatid Exchanges in Chinese Hamster V79 Cells

K. A. O'LEARY, J. A. WOODS and N. M. O'BRIEN*

Department of Food Science, Food Technology and Nutrition, University College Cork, Cork, Republic of Ireland

Accepted by Professor A. Azzi

(Received 9 March 2001; In revised form 2 April 2001)

Although α-tocopherol (α-TOC) is the most biologically active form of vitamin E and is found at high levels in plasma, γ-tocopherol (γ-TOC) has also been found to be a powerful antioxidant *in vitro* and constitutes up to 70% of the dietary intake of TOC. Low plasma levels of γ-TOC and a high α-TOC:γ-TOC ratio may be associated with coronary heart disease, suggesting that there may be a positive protective role for the γ-form of TOC. In this study the ability of different forms of vitamin E to protect against sister chromatid exchanges (SCE) induced by either hydrogen peroxide or menadione was investigated. Chinese hamster V79 cells were pre-treated with 10 μM TOC for 24 h, and then challenged with a genotoxin. After a 24 h pre-treatment, there was a greater incorporation of γ-TOC ($319.8 \pm 66.2\,ng/10^6$ cells) into V79 cells compared to α-TOC ($66.9 \pm 6.4\,ng/10^6$ cells). γ-TOC did not protect the cells against SCE induced by either hydrogen peroxide or menadione, α-TOC acetate was partially protective against both genotoxins, whereas α-TOC completely abolished the oxidant induced SCE. These results demonstrate that, despite a greater incorporation of γ-TOC into V79 cells, α-TOC but not γ-TOC was more effective at inhibiting oxidatively-induced SCE in V79 cells.

Keywords: Sister chromatid exchange; Tocopherol; Menadione; Hydrogen peroxide

Abbreviations: TOC, tocopherol; TOCA, tocopherol acetate; SCE, sister chromatid exchange(s); LDL, low density lipoprotein; NO_2, nitrogen dioxide

INTRODUCTION

Reactive oxygen species (ROS) are by-products of normal, essential cellular metabolism.[1] ROS that escape the numerous antioxidant defence mechanisms within the cell can cause oxidative damage to cellular macromolecules. Oxidative damage resulting from ROS attack has been

*Corresponding author. Tel.: +353-21-4902884. Fax: +353-21-4270244. E-mail: nob@ucc.ie

RIGHTSLINK

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

linked to the development of a variety of human diseases including cardiovascular disease and certain cancers. ROS attack on DNA produces a number of different lesions, including strand breaks and damage to the bases and sugar residues. This damage may result in the formation of chromosomal aberrations,[2,3] and sister chromatid exchanges (SCE).[4–7]

In the present study we examined the ability of vitamin E to protect against oxidatively induced SCE in Chinese hamster V79 cells. Vitamin E is a generic term describing a family of naturally occurring compounds, found mainly in vegetable oils,[8] which exhibit the biological activity of α-tocopherol (α-TOC). Vitamin E is the major lipid-soluble antioxidant in the cell membrane and has membrane stabilizing properties. These effects together with non-antioxidant properties, act to protect the cell against damage cause by ROS.[9] Although α-TOC is the most biologically active form of vitamin E and is the major form of vitamin E detected in plasma, γ-tocopherol (γ-TOC) has also been found to be a powerful antioxidant *in vitro* and constitutes up to 70% of the dietary intake of TOCs in the United States.[10–12]

To date, the chemoprotective and anti-atherogenic properties of α-TOC have been most extensively studied. However it has been reported that low plasma levels of γ-TOC and a high α-TOC:γ-TOC ratio may be associated with increased risk of coronary heart disease.[13] Elmfada and Park[14] compared a diet rich in sunflower/olive oils, which contain higher levels of α-TOC, to a diet rich in corn oil, containing high levels of γ-TOC. They found that the corn oil-rich diet was much more effective at reducing baseline levels of DNA damage, as measured by the SCE assay, in the lymphocytes of young men. Furthermore, recent reports have found that the concentration of both α-TOC and γ-TOC may be important for the subsequent development of prostate cancer.[15] These studies suggest there may be a protective role for the γ-form of vitamin E in the body.

The oxidants chosen for this study were hydrogen peroxide (H_2O_2) and menadione (K_3). H_2O_2 can easily penetrate cell membranes and is diffusible both inter- and intra-cellularly. H_2O_2 can generate the hydroxyl radical ($^{\cdot}OH$), which can attack DNA and is thought to be responsible for the ability of H_2O_2 to induce SCE *in vitro*. Menadione, a synthetic vitamin K derivative, is a redox cycling compound that causes an increase in intracellular superoxide (O_2^{-}) by monovalent reduction of molecular oxygen. The action of menadione results in DNA strand breakage and eventual cell death.[7,16–20]

In the present study we report a greater incorporation of γ-TOC into V79 cells, however α-TOC but not γ-TOC was more effective at inhibiting oxidatively induced SCE in V79 cells.

MATERIALS AND METHODS

Materials

All chemical and cell culture materials were obtained from Sigma-Aldrich Ireland Ltd (Dublin, ROI) unless otherwise indicted. DPX mountant, giemsa and colcemid were supplied by Boehringer Mannheim UK (East Sussex, England). Gurr's buffer tablets were purchased from Life Technologies (Paisley, Scotland). Cell culture plastics were obtained from Costar (Cambridge, MA). All solvents used were high performance liquid chromatography grade. H_2O_2 supplied as a 30% (w/w) solution was prepared in ice-cold phosphate buffered saline (PBS) immediately prior to use. A stock solution of menadione was prepared in dimethyl sulfoxide (DMSO) and stored at −20°C. TOCs were dissolved in ethanol for delivery to the cells. The final concentration of solvents in the cultures did not exceed 0.5% (v/v) for DMSO or 0.1% (v/v) for ethanol. These solvent concentrations were found to have no effect on either cell viability or SCE induction.

RIGHTSLINK

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

Cell Line Maintenance

Chinese hamster lung V79 cells were obtained from the European Collection of Animal Cell Cultures (Wiltshire, UK). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) foetal calf serum (FCS), and 2 mM L-glutamine. Cells were grown in a humidified incubator in an atmosphere of 5% CO_2, 95% air at 37°C and passaged every 8–10 days. The cells were grown in the absence of antibiotics and were screened for mycoplasma using a Hoechst staining method.[21] Exponentially growing cells were used throughout this study.

Cell Treatments

Cells were seeded at a density of 2×10^4 cells/cm^2 in either six well plates or 10 cm petri dishes and incubated for 18 h prior to treatment (37°C/5% CO_2). After this time, the media was removed and replaced with DMEM supplemented with 2.5% FCS containing either α-TOC, α-tocopherol acetate (α-TOCA), γ-TOC or no form of TOC. The final concentration in growth media of these compounds was 10 μM. The samples were incubated for a further 24 h. After washing twice with PBS, cells were then incubated for 1 h (37°C/5% CO_2) in serum-free DMEM containing either H_2O_2 (100 μM), menadione (10 μM) or no added genotoxin. Following incubation, the genotoxin was removed by washing the cells twice with PBS. The cells were then cultured for a further 30 h in DMEM supplemented with 5% FCS. Control samples not treated with either genotoxin or antioxidant were incubated with the equivalent volume of carrier vehicle.

Neutral Red Assay

Cytotoxicity was assessed by the neutral red uptake assay as previously described[22] and cell viability was expressed as a percentage of the control samples. Preliminary experiments were carried out to determine the concentrations of H_2O_2, menadione and TOCs to be used. Concentrations were chosen such that cell viability did not fall below 90% for any of the treatments.

Sister Chromatid Exchange (SCE) Assay

The SCE assay was carried out as previously described.[23] Briefly, after removal of genotoxin, cells were washed twice in PBS and incubated with DMEM supplemented with 5% FCS and bromodeoxyuridine (8 μg/ml). Samples were wrapped in foil and incubated for 30 h (37°C/5% CO_2). For the final 3 h of this incubation colcemid (30 ng/ml) was added to the cultures to arrest the cells in metaphase. Cells were collected by gentle scraping, harvested by centrifugation (200 g) and hypotonically shocked with 0.075 M potassium chloride (10 min, 37°C). The cells were fixed three times in freshly prepared ice-cold Carnoy's fixative (Methanol:AceticAcid, 3:1). Chromosome spreads were prepared by the air drying method.

Visualisation of SCE

Chromosome spreads were treated with (5 μg/ml) Hoechst 33258 in PBS for 30 min in the dark. Slides were rinsed and mounted in dilute PBS before being exposed to UV light (366 nm, 2.5 h, 10 cm distance from light source). Following light exposure, slides were rinsed and stained with 3% (v/v) giemsa in Gurr's buffer (30 min). Mitotic index was determined by counting 1000 cells and expressing the number of mitoses as a percentage of the total number of nuclei. Replicative index was evaluated by determining the number of first (M1), second (M2) and third (M3) mitotic divisions in 100 randomly

RIGHTSLINK

selected mitoses. The replicative index was calculated according to the formula:

$$\frac{M1 + 2(M2) + 3(M3)}{100}$$

The frequency of SCE was determined from 25 well spread second cycle metaphases and expressed as the number of SCE/chromosome.

HPLC Analysis

The TOC content of the cells was measured according to the method of Burton *et al.*[24] Approximately 7–8 million cells/sample were required for HPLC analysis. Briefly, cells were incubated with either α-TOC or γ-TOC (10 μM, 24 h). Samples were thoroughly washed with PBS, and harvested by trypsinisation. α-TOCA was used as an internal standard for measuring TOC content.[25] Methanol, containing 100 μM internal standard and 0.05% butylated hydroxytoluene (BHT), was added to the cell pellets which were vortexed vigorously. The samples were then extracted three times in hexane, and the hexane fractions pooled and dried under a gentle stream of nitrogen. The extracts were reconstituted in dichloromethane: acetronitrile: methanol (1:7:2) and analysed by HPLC.[26] Samples (50 μl) were injected onto a temperature controlled (25°C) reverse phase HPLC system (Shimadzu, model SCL-10A). The column system included Spherisorb ODS-2 guard cartridges in line with two Spherisorb ODS-2 150×4.5 mm C18 columns (Alltech, Lancashire, UK). Samples were eluted using a mobile phase of acetonitrile: methanol: dichloromethane (15:4:1) containing 10 mM ammonium acetate, 4.5 mM BHT and 3.6 mM triethylamine at 1.5 ml/min. Peaks were detected using a Shimadzu SPD-10AV UV-visible detector. Retinol was detected at 292 nm and the TOCs were detected at 325 nm. Data were collected and analysed using Millenium Chromatography Manager data collection software (Waters Corporation, Milford, MA).

Statistical Analysis

Where appropriate, data were analysed using one way analysis of variance (ANOVA) followed by Dunnett's test or students t-test.

RESULTS

Tocopherol Content of V79 Cells

The TOC content of V79 cells following 24 h supplementation with either α-TOC or γ-TOC was determined by HPLC. There was a significant enrichment of cell-associated TOC prior to treatment with the genotoxins. The α-TOC content increased by approximately 11 fold, and γ-TOC content increased by approxiamately 30 fold following supplementation, indicating a preferential uptake of γ-TOC by V79 cells. Supplementation of the growth media with α-TOC did not affect the baseline level of γ-TOC detected in the cells, and vice-versa (Table I). There was no effect on cell retinal content following supplementation with TOCs (data not shown).

Effect of Treatments on Mitotic Index and Replicative Index

The mitotic index was not affected by any of the treatment used in this study (Table IIa). Addition of α-TOC, but not α-TOCA or γ-TOC to the growth medium significantly increased the replicative index of V79 cells (Table IIb, $P>0.01$). In contrast, both H_2O_2 (100 μM) and menadione

TABLE I TOC Content of V79 Cells. (Experiments were carried out as previously described in the materials and methods section. Results are the mean values ±SD for four monolayers obtained from two independent experiments)

Treatment	Content in 10^6 V79 cells (ng)	
	α-TOC	γ-TOC
Control	6.86 ± 0.56	9.07 ± 2.75
α-TOC	66.94 ± 6.39	11.33 ± 3.33
γ-TOC	5.18 ± 1.16	319.84 ± 66.15

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

RIGHTSLINK

TABLE II Effect of TOCs on Mitotic and replicative index. (Experiments were carried out as previously described in the materials and methods section. Results are the mean value ±SE from 8/10 monolayers obtained from three independent experiments)

Treatment	α-TOC	α-TOCA	γ-TOC
	(a) Mitoses/1000 Nuclei (%)		
Control	3.96 ± 0.03	4.12 ± 0.06	4.14 ± 0.07
Antioxidant	4.08 ± 0.05	4.03 ± 0.06	4.06 ± 0.09
H_2O_2	4.12 ± 0.08	4.21 ± 0.08	4.35 ± 0.07
Menadione	4.11 ± 0.07	4.09 ± 0.05	3.98 ± 0.08
Antioxidant and H_2O_2	4.08 ± 0.04	4.08 ± 0.07	4.25 ± 0.09
Antioxidant and Menadione	4.16 ± 0.06	4.19 ± 0.08	4.09 ± 0.09
	(b) Replicative Index/100 Nuclei (%)		
Control	1.94 ± 0.02	1.98 ± 0.01	1.97 ± 0.02
Antioxidant	2.00 ± 0.01*	2.01 ± 0.01	1.99 ± 0.02
H_2O_2	1.78 ± 0.01*,†	1.83 ± 0.01*,†	1.77 ± 0.02*,†
Menadione	1.87 ± 0.01*,‡	1.89 ± 0.02*	1.86 ± 0.03*,‡
Antioxidant and H_2O_2	1.97 ± 0.01†	1.93 ± 0.01†	1.98 ± 0.01†
Antioxidant and Menadione	1.91 ± 0.01‡	1.94 ± 0.01	1.96 ± 0.02‡

*Significantly different from control ($P < 0.01$).
†Significantly different from each other ($P < 0.01$).
‡Significantly different from each other ($P < 0.05$).

(10 μM) significantly decreased the replicative index of the cells ($P < 0.01$). This genotoxin-induced decrease in proliferation was prevented by the addition of the TOCs in all cases (Table IIb). None of the vitamins or genotoxins were cytotoxic at the concentrations used in the experiments as measured by the neutral red uptake assay (Table III).

Effect of Treatments of SCE Frequency

The addition of either TOCs or solvents to the growth medium did not affect the background frequency of SCE. In both cases, treatment with either menadione (10 μM) or H_2O_2 (100 μM) significantly increased the number of SCE by approximately 2 fold with respect to the control samples. Treatment with α-TOC (10 μM) for 24 h prior to addition of H_2O_2 completely abolished the increase in SCE induced by this oxidant. However, although menadione-induced SCE were substantially reduced by the addition of TOC, there was still a significantly greater frequency of SCE present compared to the control samples (Table IV).

Addition of α-TOCA (10 μM) to the growth medium 24 h prior to the addition of the oxidants

TABLE III Cytotoxicity as determined by the neutral red uptake assay. (Experiments were carried out as previously described in the materials and methods section. Results were expressed as a percentage of the solvent control and are the mean values ±SD from four monolayers obtained from two independent experiments)

Treatment	Viable Cells (%)		
	α-TOC	α-TOCA	γ-TOC
Antioxidant	99.2 ± 1.3	100.4 ± 0.2	103.9 ± 0.6
H_2O_2	97.7 ± 0.2	98.8 ± 0.9	103.5 ± 0.4
Menadione	97.7 ± 0.1	99.2 ± 0.5	103.7 ± 0.3
Antioxidant and H_2O_2	99.6 ± 2.3	97.7 ± 3.1	103.7 ± 0.1
Antioxidant and Menadione	98.8 ± 1.3	100.4 ± 0.3	103.5 ± 0.2

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

RIGHTSLINK

TABLE IV Effect of TOCs on SCE. (Experiments were carried out as previously described in the materials and methods section. Results are the mean values ±SE from 8/10 monolayers obtained from three independent experiments.)

Treatment	SCE/chromosome		
	α-TOC	α-TOCA	γ-TOC
Control	0.244 ± 0.003†,‡	0.249 ± 0.004†,‡	0.227 ± 0.005†,‡
Antioxidant	0.228 ± 0.003†,‡	0.241 ± 0.003†,‡	0.233 ± 0.006†,‡
H_2O_2	0.491 ± 0.011*	0.505 ± 0.009*	0.481 ± 0.006*
Menadione	0.487 ± 0.004*	0.485 ± 0.006*	0.495 ± 0.007*
Antioxidant and H_2O_2	0.252 ± 0.003†	0.362 ± 0.010*,†	0.435 ± 0.006*,†
Antioxidant and Menadione	0.279 ± 0.007*,‡	0.387 ± 0.006*,‡	0.481 ± 0.006*

*Significantly different from control ($p > 0.01$).
†Significantly different from H_2O_2 treatment ($p < 0.01$).
‡Significantly different from menadione treatment ($p < 0.01$).

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

reduced the extent of H_2O_2-induced SCE by approximately 30%, and menadione-induced SCE by approximately 20% (Table IV). Despite the fact that γ-TOC (10 μM) was apparently taken up efficiently by the V79 cells, pretreatment of cultures with this compound did not result in any decrease in oxidant-induced SCE frequency (Table IV).

DISCUSSION

The SCE assay is a sensitive indicator of genotoxicity[23] and involves the exchange of DNA between two homologous sister chromatids. α-TOC has been shown to reduce the frequency of SCEs induced by various genotoxins in a number of different *in vitro* cell models. α-TOC reduced cyclophosphidamide and Trp-2-P induces SCEs in human lymphocytes, and was also effective at reducing oxygen radical induced SCEs in Chinese hamster ovary cells.[6,27,28]

Treatment of V79 cells with α-TOC prevented H_2O_2- and menadione-induced SCE. α-TOCA also protected the cells against oxidant-induced SCE, but not as effectively as α-TOC. Despite the increased uptake of γ-TOC into the cells, this compound was the least protective of the three compounds, having no effect against menadione-induced SCE and only a very slight effect against H_2O_2-induced SCE. Both α-TOCA and γ-TOC are known to have less antioxidant activity than α-TOC under certain conditions; the former due to esterification of the –OH group at position 6, and the latter as a result of the smaller number of methyl groups on the chromanol ring.[16] As α-TOCA caused some reduction of oxidant-induced SCE, it is possible that this protection resulted from a non-antioxidant mechanism.[9]

In iron loaded rats, γ-TOC was shown to have 30–40% of the antioxidant activity of α-TOC[29] indicating that it was a more effective antioxidant *in vivo* than previously thought. More recently, a study has shown that a diet rich in corn oil resulting in increased plasma levels of γ-TOC reduced the baseline levels of SCE in healthy young men to a greater extent than a diet that increased levels of plasma α-TOC.[14] γ-TOC has been shown to be efficient at removing reactive nitrogen species[10,11], and preventing NO_2-induced DNA strand breaks in V79 cells.[30] However, Takahashi *et al.*[31] have demonstrated that γ-TOC was less effective than α-TOC in preventing hyperoxia-induced cell death. γ-TOC was also less effective at preventing oxidized LDL-induced apoptosis in human smooth muscle cells.[32] γ-TOC incorporates in cultured cells to a greater extent than α-TOC, however it also disappears more quickly than α-TOC.[12] Our results demonstrate that despite its preferential uptake, γ-TOC is much less effective than

RIGHTSLINK

either α-TOC or α-TOCA at preventing ROS-induced SCE in cultured cells.

In conclusion, γ-TOC may be a more effective antioxidant under physiological conditions than in cultured cells, and may be more protective against RNS than ROS. The behaviour of γ-TOC and α-TOC in cell membranes is influenced by their orientation within the membrane, their relative ratios and their interaction with co-antioxidant molecules. In addition, conditions *in vitro* are very different from those found in the body with regard to, for example, oxygen concentration. Clearly more research is required to define the actions of TOCs at the molecular level before their *in vivo* actions can be fully explained.

References

[1] Ames, B.N. and Shigenaga, M.K. (1991) "Oxidants are a major contributor to cancer and aging", In: Halliwell, B. and Arouma, O.I., eds, DNA and Free Radicals (Ellis Horwood, New York), pp 1–15.

[2] Oya, Y., Yamamoto, K. and Tonomura, A. (1986) "The biological activity of hydrogen peroxide. (1) Induction of chromosomal-type aberrations susceptible to inhibition by scavengers of hydroxy radicals in human embryonic fibroblasts", *Mutation Res.* **172**, 245–253.

[3] Ochi, T. (1989) "Effects of iron chelators and glutathione depletion on the induction and repair of chromosomal aberrations by tert-butylhydroperoxide in cultured Chinese hamster cells", *Mutation Res.* **213**, 243–248.

[4] Bradley, M.O., Hus, I.C. and Harris, C.C. (1979) "Relationship between sister chromatid exchange and mutagenicity, toxicity and DNA damage", *Nature* **282**, 318–320.

[5] Speit, G., Vogel, W. and Wolf, M. (1982) "Characterisation of sister chromatid exchange induction by hydrogen peroxide", *Environmental Mutagenesis* **4**, 135–412.

[6] Weitberg, A.B., Weitzman, S.A., Clack, E.P. and Stossel, T.P. (1985) "Effects of antioxidants on oxidant induced SCE formation", *J. Clin. Invest.* **75**, 1835–1841.

[7] Larramendy, M., Mello-Filho, A.C., Martins, E.A. and Meneghini, R. (1987) "Iron mediated induction of sister chromatid exchanges by hydrogen peroxide and superoxide anion", *Mutation Res.* **178**, 57–63.

[8] Sheppard, A.J., Pennington, J.A.T. and Weihrauch, J.L. (1992) "Analysis and distribution of vitamin E in vegetable oils and foods", In: Packer, L. and Fuchs, J., eds, Vitamin E in Health and Disease (Marcel Dekker, New York), pp 235–243.

[9] Azzi, A. and Stocker, A. (2000) "Vitamin E: non-antioxidant roles", *Prog. Lipid Res.* **39**, 231–255.

[10] Wolf, G. (1997) "γ-Tocopherol: an efficient protector of lipids against nitric oxide-initiated peroxidative damage", *Nutr. Rev.* **55**, 376–378.

[11] Christen, S., Woodall, A.A., Shigenaga, M.K., Southwell-Keely, P.T., Duncan, M.W. and Ames, B.N. (1997) "γ-Tocopherol traps mutagenic electrophiles such as NOx and complements α-tocopherol: Physiological implications", *Proc. Natl. Acad. Sci. USA.* **94**, 3217–3222.

[12] Tran, K. and Chan, A.C. (1992) "Comparative uptake of γ- and α-tocopherol by human endothelial cells", *Lipids* **27**, 38–41.

[13] Ohrvall, M., Sundlof, G. and Vessby, B. (1986) "γ-, But not α-tocopherol levels in serum are reduced in coronary heart disease patients", *J. Internal Med.* **239**, 111–117.

[14] Elmadfa, I. and Park, E. (1999) "Impacts of diets with corn oil or olive/sunflower oils on DNA damage in healthy young men", *Eur. J. Nutr.* **38**, 286–292.

[15] Helzlsouer, K.J., Huang, H.-Y., Alberg, A.J., Hoffman, S., Burke, A., Norkus, E.P., Morris, J.S. and Comstock, G.W. (2000) "Association between α-tocopherol, γ-tocopherol, selenium, and subsequent prostate cancer", *J. Natl. Cancer Inst.* **92**, 2018–2023.

[16] Halliwell, B. and Gutteridge, J.M.C. (1989) Free radicals in Biology and Medicine, 2nd ed. (Clarendon Press, Oxford), pp. 105–245.

[17] Ward, J.F., Evans, J.W., Limoli, C.L. and Calabro-Jones, P.M. (1987) "Radiation and hydrogen peroxide induced free radical damage to DNA", *Br. J. Cancer* **55**(suppl. VIII), 105–112.

[18] Martins, E.A.L. and Meneghini, R. (1990) "DNA damage and lethal effects of hydrogen peroxide and menadione in Chinese hamster cells: Distinct mechanisms are involved", *Free Radical Biol. Med.* **8**, 433–440.

[19] Samali, A., Nordgren, H., Zhivotovsky, B., Peterson, E. and Orrenius, S. (1999) "A comparative study of apoptosis and necrosis in HepG2 cells: oxidant-induced caspase inactivation leads to necrosis", *Biochem. Biophys. Res. Commun.* **255**, 6–11.

[20] Woods, J.A., Young, A.J. and Bilton, R.F. (1997) "Measurement of menadione-mediated DNA damage in human lymphocytes using the comet assay", *Free Radical Res.* **26**, 113–124.

[21] Mowles, J.M. (1990) "Mycoplasma Detection", In: Pollard, J.W. and Walker, J.M., eds, Methods in Molecular Biology Volume V: Animal Cell Culture (Humana Press, New Jersey), pp 65–74.

[22] Hunt, S.M., Chrzannovoska, C., Barrett, C.R., Brand, H.N. and Fawell, J.K. (1987) "A comparison of *in vitro* cytotoxicity assays and their application to water samples", *Alternatives Lab. Anim.* **15**, 20–29.

[23] Perry, P. and Wolff, S. (1974) "New Giemsa method for the differential staining of sister chromatids", *Nature* **251**, 156–158.

[24] Burton, G.W., Webb, A. and Ingold, K.U. (1985) "A mild, rapid and efficient method of lipid extraction for use in determining Vitamin E/lipid ratios", *Lipids* **20**, 29–39.

[25] Carroll, Y.L., Corridan, B.M. and Morrissey, P.A. (1999) "Carotenoids in young and elderly healthy humans: dietary intakes, biochemical status and diet–plasma relationships", *Eur. J. Clin. Nutr.* **53**, 644–653.

[26] Craft, N.E., Wise, S.A. and Soares, J.H. (1992) "Optimization of an isocratic high performance liquid chromatographic separtion of carotenoids", *J. Chromatogr.* **589**, 171–176.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

RIGHTSLINK

[27] Edenharder, E., Kerkoff, G. and Dunkilberg, H. (1998) "Effects of β-carotene, retinal, riboflavin, α-tocopherol and vitamins C and K1 on sister-chromatid exchanges induced by 3-amino-1-methyl-5H-pyrido [4,3-b] indole (Trp-P-2) and cyclophosphamide in human lymphocyte cultures", *Food Chem. Toxicol.* **36**, 897–906.

[28] Weitberg, A.B. (1987) "Effects of inhibitors of arachidonic acid metabolism and vitamin E on oxygen radical-induced sister chromatid exchanges", *Carcinogenesis* **8**, 1619–1620.

[29] Dillard, C.J., Gavino, V.C. and Tappel, A.L. (1983) "Relative antioxidant effectiveness of α-tocopherol and γ-tocopherol in iron-loaded rats", *J. Nutr.* **113**, 2266–2273.

[30] Bittrich, H., Matzig, A.K., Kraker, I. and Appel, K.E. (1993) "NO_2 induced DNA single strand breaks are inhibited by antioxidative vitamins in V79 cells", *Chem.-Biol. Interact.* **86**, 199–211.

[31] Takahashi, H., Kosaka, N. and Nakagawa, S. (1998) "α-Tocopherol protects PC12 cells from hyperoxia-induced apoptosis", *J. Neurosci. Res.* **52**, 184–191.

[32] de Nigris, F., Franconi, F., Maida, I., Palumbo, G., Anania, V. and Napoli, C. (2000) "Modulation by α- and γ-tocopherol and oxidized low-density lipoprotein of apoptotic signaling in human coronary smooth muscle cells", *Biochem. Pharmacol.* **59**, 1477–1487.

Free Radic Res Downloaded from informahealthcare.com by Library of Health Sci-Univ of Il on 11/23/11
For personal use only.

RIGHTSLINK