Eur J Nutr (2006) 45 : 97–104
DOI 10.1007/s00394-005-0569-4

ORIGINAL CONTRIBUTION

Karl E. Herbert
Sue Fletcher
Dina Chauhan
Akin Ladapo
Jagdeep Nirwan
Sarah Munson
Pratibha Mistry

Dietary supplementation with different vitamin C doses: no effect on oxidative DNA damage in healthy people

Received: 24 January 2005
Accepted: 24 May 2005
Published online: 18 July 2005

K. E. Herbert (✉) · S. Fletcher
Dept. of Cardiovascular Sciences
University of Leicester
Robert Kilpatrick Building
Leicester Royal Infirmary
Leicester LE2 7LX, UK

D. Chauhan · A. Ladapo · J. Nirwan ·
S. Munson · P. Mistry
Dept. of Pathology
University of Leicester
Leicester Royal Infirmary
Leicester, UK

Summary *Background* Antioxidants are believed to prevent many types of disease. Some previous studies suggest that dietary supplementation with vitamin C results in a decrease in the level of one of the markers of oxidative damage – 8-oxoguanine in the DNA of peripheral blood mononuclear cells (PBMC). *Aim of trial* To investigate the effect of different dose levels of dietary supplementation with vitamin C on oxidative DNA damage. *Methods* A randomised double-blind placebo-controlled trial was carried out using three different levels (80, 200 and 400 mg) of dietary vitamin C supplementation in a healthy population of 160 volunteers; supplementation was for a period of 15 weeks followed by a 10 week washout period. Peripheral blood samples were obtained every 5 weeks from baseline to 25 weeks. *Results* An increase in PBMC vitamin C levels was not observed following supplementation in healthy volunteers. There was no effect found on 8-oxoguanine measured using HPLC with electrochemical detection for any of the three supplemented groups compared to placebo. 8-oxoadenine levels were below the limit of detection of the HPLC system used here. *Conclusions* Supplementation with vitamin C had little effect on cellular levels in this group of healthy individuals, suggesting their diets were replete in vitamin C. The dose range of vitamin C used did not affect oxidative damage in PBMC DNA.

Key words vitamin C – antioxidant – double-blind placebo-controlled trial – DNA damage

Introduction

Background

Reactive oxygen species (ROS) are believed to account for much of the damage to biomolecules, such as DNA, proteins and lipids during oxidative stress in human cells and tissues. Excessive formation of ROS, therefore, may be responsible for the pathology of chronic disorders, e. g. neurodegenerative diseases, connective tissue diseases, atherosclerosis and cancer [1], these being associated with an ageing population where oxidative stress is hypothesised to be a factor in pathogenesis. Protection against oxidative stress and importantly against the deleterious effects of ROS on biomolecules may be afforded by supplementing diets with nutrient antioxidants such as vitamins C and E [2]. However, the precise amounts of dietary antioxidant vitamins are largely a matter of conjecture.

Previous studies [3, 4] on vitamin C supplementation of diets have yielded conflicting results with respect to the antioxidant properties towards DNA. In 1991 Ames and colleagues suggested that vitamin C had a strong protective effect on oxidative DNA damage in sperm [5]. However, a later study reported that although pyrimi-

EJN 569

European Journal of Nutrition (2006) Vol. 45, Number 2

dine damage was reduced following supplementation with a mixture of vitamins, vitamin C was not thought to be responsible for this potentially beneficial effect [6]. Recently, using the comet assay Moller et al. [7] showed vitamin C in slow-release formulation was associated with lower levels of oxidative DNA damage, whereas plain-release formulation had no effect.

8-oxoG is a pre-mutagenic lesion in DNA and the most widely accepted and measured marker of oxidative DNA damage in human cells and tissues, as measured in peripheral blood mononuclear cells (PBMC). Over the last four years in particular, the European Standards Committee on Oxidative DNA Damage (ESCODD) has worked towards a consensus on the absolute or 'background' level of 8-oxoG in human cells [8–11].

Aims and objectives

In order to provide evidence for the merits of taking vitamin C supplements, this study investigated the potential for three different doses of vitamin C supplement to reduce oxidative DNA damage in PBMC from healthy subjects on their usual diet. By measuring vitamin C within the PBMC fraction following supplementation, any changes in oxidative DNA damage could then be related to actual changes in cellular concentration of this antioxidant.

Subjects and methods

Trial population

A randomised, double-blind-placebo-controlled trial was conducted on the effect of three different doses of vitamin C on oxidative DNA damage in PBMC in healthy subjects. Local Ethical Committee approval was obtained. Subjects were recruited following poster advertisement throughout the Leicester Royal Infirmary and the University of Leicester in order to have a broad based trial group from both staff and general public. The characteristics of the trial group showed 127/160 (79%) of those recruited were female, which is typical of such volunteer studies. Overall the age range was 18 to 64 years. The mean ages ranged from 34 in the 400 mg group to 38 in both the placebo and 80 mg groups. The overall mean age was 40 for females and 31 for males. The groups had similar female to male ratios (see Table 1). The subjects' General Practitioners were informed of their patients' recruitment and the background to the trial.

Inclusion criteria

Inclusion criteria were: healthy males and females on a normal diet, ages 18–65, written consent which followed GCP guidelines, having been given 48 hours to read the information sheet and consent forms.

Exclusion criteria

Pregnancy, smoking, already taking vitamin supplements, in another trial within 6 months, relatives of, students of and researchers dependent upon Principal Investigator for funding.

Randomisation

Subjects (n = 160) were randomly allocated to one of three vitamin C groups (80, 200 or 400 mg/day) or placebo; there were 40 subjects in each of the four groups (Table 1). The placebo contained calcium carbonate and was manufactured to look and taste like a vitamin C tablet (with citrus fruit flavouring). The vitamin C and placebo capsules were prepared by Nova Laboratories Ltd. Leicestershire; they also prepared the randomisation codes. The four preparations were provided to us in number coded bottles. Allocation of the numbered bottles was sequential at the first/baseline trial visit. The randomisation codes were kept by Nova until after processing of the last final visit 'washout' blood sample. Recruitment took place over a period of 3 months from the end of August to November 2000.

Table 1 Characteristics of the trial groups

	80 mg	200 mg	400 mg	placebo	Total
Numbers	40	40	40	40	160
Females	32	31	30	34	127 (79%)
Males	8	9	10	6	33 (21%)
Mean age (range)	38 (21–62)	38 (21–64)	36 (18–55)	37 (18–64)	40 (18–64)
Weeks between visits*	5	5	5	5	5
Capsules returned*	7	7	7	7	7
Trial withdrawals	1	2	0	3	6 (0.4%)

* medians

Tablets, one per day, were to be taken first thing in the morning. Normal dietary habits were to be maintained. The doses used were chosen since previous work [3] had shown changes to DNA oxidation markers at 500 mg/day; use of doses above 500 mg would involve additional consideration of ethics. Furthermore these relatively low doses of vitamin C would not involve possible gastrointestinal disturbance as a side effect.

Blood sampling

Peripheral blood (40 ml) was taken from subjects at weeks 0, 5, 10, 15, 20 and 25 weeks of the trial, weeks 15, 20 and 25 being the so-called 'washout' period. Blood samples were taken between 08.00 and 10.30 h, a minimum of 0.5 h and maximum of 3 h following ingestion of the tablet. Volunteers were not asked to fast. The blood samples were obtained by venepuncture of an antecubital vein. The 40 ml of blood was collected at each visit in lithium heparin vacutainer syringes, and used to prepare a mononuclear cell fraction by density centrifugation. Processing and analysis of the blood samples were undertaken in batches as the trial progressed; thus from week 5 onwards a mixture of timeframes was collected, processed and analysed at the same time. Samples from weeks 20 and 25 were combined for the DNA damage analysis.

Compliance

Subjects were provided at weeks 0, 5 and 10 with sufficient capsules for 6 weeks' supplementation. They were instructed to take one capsule each morning. Counting of returned capsules at 5, 10 and 15 weeks was used to check compliance. A diary was kept of visit dates and the number of capsules returned at each time period.

Statistical analysis

In most cases data were determined to be non-normally distributed using the Kolmogorov-Smirnov test. Therefore data were $\log_{10}$ transformed prior to analysis by ANOVA. Post-test analysis was performed using the Tukey test.

Analytical methods

Isolation of PBMC

Forty ml of heparinised blood was diluted 1:1 with PBS and layered carefully over Histopaque 1077 (Sigma). Following centrifugation at 400 g for 30 minutes at room temperature PBMC were collected. The cells were then washed twice in PBS (10 ml) by centrifugation at 250 g for 15min at 4°C and finally resuspended in 2 ml PBS on ice. One ml was used for 8-oxodG analysis and 0.5 ml for ascorbic acid determination.

Measurement of ascorbic acid

Ascorbic acid was extracted from approximately 10×10^6 PBMC using 6 % (w/v) metaphosphoric acid containing 100 µM isoascorbic acid internal standard (MPA-IAA). Cells were homogenized on ice and the supernatants collected following centrifugation at 8000 g for 10 min at 4 °C. Samples were stored at –80 °C for no more than two weeks pending analysis. The protein concentration of the extracts was measured by the Bradford assay [12].

Ascorbic acid and isoascorbic acid were separated on a fused silica capillary of 50 µm internal diameter and 47 cm length. The capillary was conditioned prior to use with 10 min washes with 0.1 M NaOH and then water followed by 0.1 M tricine (pH 8.5). Samples were introduced into the capillary by applying positive pressure over 10 s. Ascorbic acid and isoascorbic acid were resolved under a constant voltage of +30 kV over 8 min at a constant temperature of 25 °C and detected by absorbance at 254 nm UV. Between each sample analysis the capillary was rinsed for 20 s in 0.1 M NaOH and 20 s water followed by 30 s 0.1 M tricine (pH 8.5). Each sample was analysed in duplicate. Verification of the ascorbic acid peak involved migration time and treatment of some samples with ascorbate oxidase. This enzyme oxidises ascorbic acid leading to a reduction of the ascorbic acid peak on the electrophorogram.

Peak areas of ascorbic acid measured in cell lysate samples were integrated using System Gold Software (Beckman), and the values were normalised to the internal standard, isoascorbic acid. Concentrations of ascorbic acid in cell extracts were calculated from an ascorbic acid standard curve ranging 0–1000 µM prepared in the MPA-IAA stock solutions specific to each daily run of samples. The final concentration of ascorbic acid in each cellular sample was expressed as µmol ascorbic acid per g of protein.

Sodium iodide DNA extraction method

DNA for 8-oxodG analyses was extracted using a method modified from that described for whole blood by Wang et al. [13]. For each sample, approximately 20×10^6 PBMC were washed twice in PBS and transferred into an Eppendorf tube. The cell pellets were dispersed prior to the addition of 0.5 mL lysis solution [10 mM Trizma®-HCL, 5 mM magnesium chloride, 320 mM sucrose, pH 7.5, 1 % (w/v) Triton-X-100 with

0.1 mM desferrioxamine (DFO) added on the day of use]. The cells were vortex mixed for 30 s and pelleted at 8000 g for 20 s. The supernatant was discarded and the process repeated with 1 mL of lysis solution. To each pellet 0.2 mL of enzyme reaction solution (20 mM Trizma®-HCl, 5 mM DTPA, 10 mM NaCl, pH 6.0 with 0.1 mM DFO added on the day of use) was added and mixed, followed by heat inactivated RNase A (0.1 mL equal to 16 Kunitz units ribonuclease type III-A from bovine pancreas in enzyme reaction solution). The samples were incubated at 37 °C for 15 min, then 0.1 mL (equal to 50 Kunitz units) Proteinase K (in enzyme reaction solution) was added and the samples further incubated at 37 °C for 60 min.

Following incubation, 0.3 mL sodium iodide solution (7.6 M sodium iodide, 5 mM DTPA and 40 mM Trizma®-HCL, pH 8.0) and 0.5 mL ice-cold isopropanol were added and the sample inverted several times. Once the DNA precipitate was visible the samples were centrifuged at 8000 g for 20 s and the supernatants removed. The DNA was subsequently washed with 40 % (v/v) ice-cold isopropanol and then 70 % (v/v) ice-cold ethanol. The DNA pellets were allowed to dry before being reconstituted in 300–400 µL 20 mM sodium acetate (pH 4.8) and dissolved overnight at room temperature on a rotating mixer. DNA purity and quantity was assessed by measuring absorbance at 260 nm and at 280 nm.

Digestion of DNA to deoxynucleosides

DNA extracted by the sodium iodide method was digested prior to analysis of 8-oxodG by HPLC with electrochemical detection. To each minimum 50 µg aliquot of DNA in 190 µL, 20 mM sodium acetate was added 10 µL 2 mM DFO, 5 µL 1 M sodium acetate containing 45 mM zinc chloride (pH 4.8) and 0.1 Kunitz units/µg DNA Nuclease P1 in 20 mM sodium acetate. The samples were incubated at 37 °C for 60 min, then 20 µL 1.5 M Trizma®-HCl, pH 8.0 and 0.07 Kunitz units/µg DNA alkaline phosphatase in 100 mM Trizma®-HCl, pH 8.0 were added and the samples further incubated at 37 °C for 30 min and stored on ice pending analysis.

HPLC measurement of 8-oxodG

In order to determine the concentration of 8-oxodG in DNA samples, standards were prepared using stock solutions of 1 mM dG and 100 µM 8-oxodG in water. To determine the exact concentration of these stocks, spectrophotometric absorbance measurements at 253 nm for dG and 245 nm for 8-oxodG were taken. The concentrations were then determined using their extinction coefficients, where $E_{253} = 13.0\ mM^{-1}cm^{-1}$ and $E_{245} = 12.3\ mM^{-1}cm^{-1}$ for dG and 8-oxodG respectively. The stock solutions of standards were further diluted in 20 mM sodium acetate containing 100 µM DFO to provide a range of standards from 0–100 µM dG and from 0–10 nM 8-oxodG. A 190 µL aliquot of each standard was processed through the DNA digestion procedure to provide the estimation of 8-oxodG in DNA samples.

Duplicate 50 µL injections of deoxynucleoside standards or samples were separated by reversed-phase HPLC using a 3 µm Hypersil® ODS column [octadecyl silane (C18) bonded to silica, 15 cm × 4.6 mm internal diameter] and mobile phase consisting of 75 mM sodium acetate (pH 5.5) with 8 % (v/v) methanol, pumped at a flow rate of 1 mL/min. 2-deoxyguanosine was detected by its UV absorbance at 254 nm and 8-oxodG by coulometric detection at 375 mV (Guard cell output 700 mV). Peaks were integrated using a Beckman 32 karat software package and the levels of 8-oxodG expressed per 10^6 dG.

Results

General

Initially, 160 healthy volunteers were recruited into the trial; of these, 154 completed the full trial consisting of a single daily dose of vitamin C for 15 weeks, followed by a 'washout' period of ten weeks. The 6 subjects who withdrew took no further part and samples were not analysed. No adverse effects of vitamin supplements were reported during this study. Characteristics of the trial are shown in Table 1.

Compliance was excellent; the median number of capsules returned for any one period was 7 and the median time between visits was 5 weeks (Table 1). Six subjects (0.4 %) withdrew from the trial. Four withdrew by week 5, one by week 10 and one by week 15. Three withdrawals were from group 4 (although one did complete 15 weeks), two from group 2 and one from group 1 (who completed 10 weeks).

Vitamin C levels in PBMC

No statistically significant differences were observed in the level of vitamin C in PBMC between the four groups of volunteers, at any stage of the trial (Fig. 1). The variation in 'n' value, for cellular vitamin C levels, was due to loss of samples to analysis.

Each individual trial group was then assessed for any statistically significant changes in PBMC vitamin C before and following supplementation and during the washout period (Fig. 1). Analysis of the placebo group by ANOVA showed that the differences between transformed means were not statistically significant.

Analysis of the transformed data from the 80 mg/day vitamin C group revealed a significantly lower level of

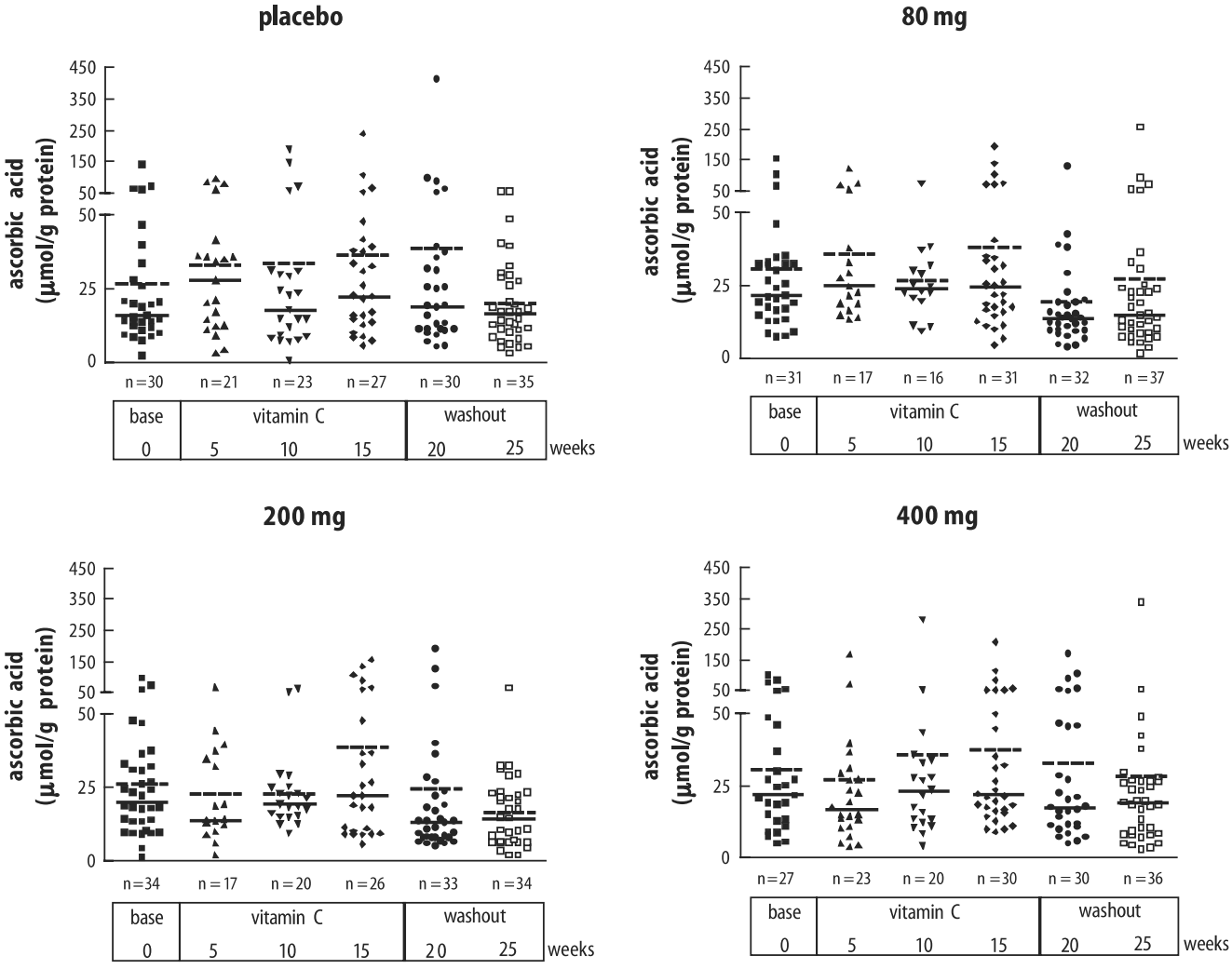

Fig. 1 Effect of vitamin C supplementation on PBMC ascorbate levels. Bars indicate the median (——) and mean (- - - - -) values

vitamin C in PBMC at week 20 (washout week) compared to the weeks representing the supplementation period (weeks 5 and 15; $p < 0.05$ for both). A significant washout effect was noted in the individuals provided with 200 mg/day vitamin C ($p < 0.05$) with lower values at week 25 compared with week 15.

No statistically significant trends were observed in the data from the group receiving the highest level of vitamin C supplement (400 mg/day) (Fig. 1).

Oxidative DNA damage in PBMC

Analytical work performed as part of this study showed that 8-oxodA could be detected by coulometric detection (at 650 mV) at a limit of detection of approximately 1 nmol/l (50 fmol on column). Importantly however, this limit of detection was not sufficient to detect levels present in DNA extracted, using our protocol optimised for detection of 8-oxodG, from the PBMC of human volunteers.

It was possible, however, to detect and quantify 8-oxodG by HPLC with electrochemical detection; values were obtained for each study group at baseline, during and after vitamin C supplementation (Fig. 2). Importantly, there were no statistically significant differences between any of the four subject groups on comparison of values obtained during vitamin C supplementation or during washout. A significant difference between the baseline values of 8-oxodG content of the placebo and 80 mg groups was noted ($p < 0.05$; Fig. 2). There was no correlation between baseline values of 8-oxodG and baseline vitamin C levels ($r^2 < 0.001$).

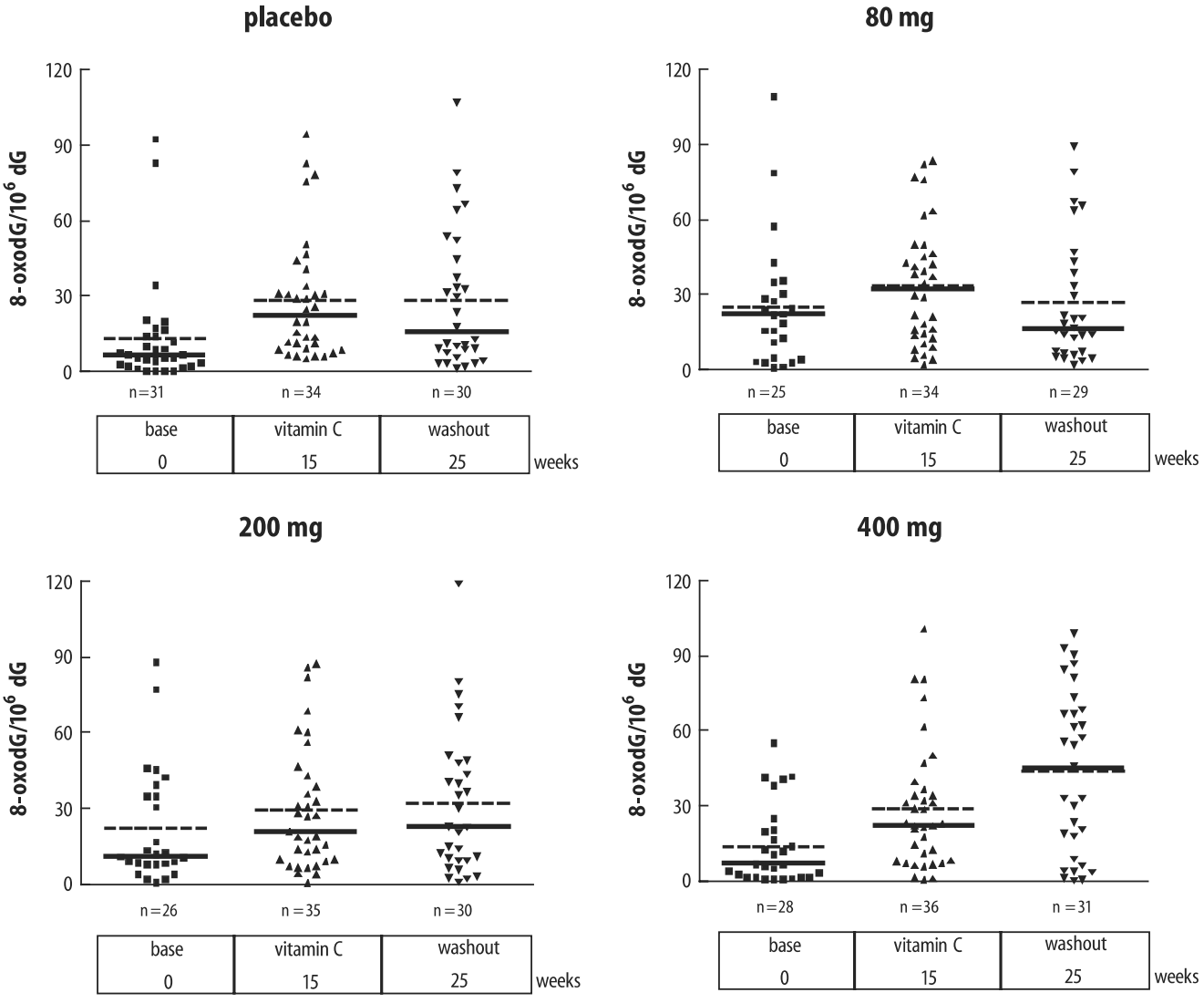

Fig. 2 Effect of vitamin C supplementation on 8-oxodG levels in PBMC. Bars indicate the median (——) and mean (- - - - -) values

Discussion

The main finding of this study is that 8-oxodG levels measured by HPLC in PBMC DNA were not altered at any of the doses of vitamin C used. There has been considerable controversy associated with reports investigating the ability of dietary vitamin C to modulate oxidative stress in humans. Initial data suggested oxidative DNA damage could be attenuated in sperm from human volunteers by such dietary augmentation [5]. Later it was reported that supplementation of diets of healthy volunteers with 500 mg/day ascorbic acid resulted in a reduction in 8-oxoguanine in DNA yet an increase in 8-oxoadenine, as measured by GC-MS [3]. The latter study was not strictly placebo-controlled and analytical measures of the oxidised purines were made by GC-MS, which was heavily criticised at the time and subsequently due to the potential formation of adventitious 8-oxodG in the sample work-up procedure [4]. The present study aimed to address these issues in a double-blind placebo-controlled trial investigating three vitamin C doses (and a placebo) with 8-oxopurines, as markers of oxidative DNA damage, being measured by HPLC with electrochemical detection.

The levels of 8-oxodG reported here are in the order of ten-fold less than those reported by Podmore et al. [3]. Several improvements to analytical procedures, over the intervening years, include optimised and validated DNA extraction [14] with much reduced adventitious oxidative DNA damage and the formation of some degree of consensus between individual laboratories by

collaborative studies, in particular by ESCODD participants [8–11]. Nevertheless it is possible that the HPLC assay still lacks the sensitivity to detect the possibly small changes induced by vitamin C supplementation due to remaining yet unidentified problems with spurious oxidation during analysis [11]. Currently perhaps the most widely accepted markers of oxidative stress are the isoprostanes [15]. Levine et al. [16] used urinary isoprostane levels to demonstrate that vitamin C does not appear to cause a pro-oxidant effect in vivo. A recent report claims a pro-oxidant effect of dietary ascorbate [17]; however the study used different and less widely investigated markers than those used here and moreover a less rigorous trial format.

Our initial aim was to investigate changes in the DNA content of both 8-oxodG and 8-oxodA in PBMC from people supplemented with vitamin C, in order to investigate mechanisms for the changes reported previously [3]. The assay was, indeed, capable of detecting 8-oxodA but importantly only at relatively high levels, for example as present in heavily oxidised DNA. For example, 8-oxodA could be detected when calf thymus DNA was incubated with 2,2'-diazobis(2-amidinopropane) dihydrochloride, a peroxyl radical generator, in a dose-dependent manner (unpublished observations). However, 8-oxodA was not detected in untreated calf thymus DNA, nor calf thymus DNA irradiated with 5 or 20 Gy γ irradiation, iron/peroxide, nor importantly was it observed in untreated PBMC DNA from human volunteers (unpublished observations). Other workers, using LC-MS/MS analysis, have arrived at the same conclusion [18]. The measurement of 8-oxodA in human cellular DNA awaits more sensitive mass spectrometric and/or electrochemical detection systems. However, we can conclude with some certainty that previous reports of levels of 8-oxoA in human PBMC DNA by GC-MS [3, 19] and, indeed, modulations thereof induced by vitamin C, are questionable. Possible explanations for the data in previous reports include adventitious oxidation of DNA during sample work-up, for example, during DNA extraction from cells; or alternatively during derivatisation of bases prior to GC-MS analysis, or both.

This study aimed to relate any changes in PBMC DNA oxidation to actual changes in the level of vitamin C within the cells; previously plasma vitamin C had been used as a surrogate marker of cellular levels. Previous studies have demonstrated that the level of ascorbic acid in plasma may be increased significantly following dietary supplementation with vitamin C in healthy individuals. This is the case for healthy subjects on their normal diet [3, 17, 20] and for subjects depleted of ascorbate [21, 22]. Naturally the daily dose of ascorbate is important in determining the amount and possibly rate of increase in plasma ascorbate. We have previously shown that doses of 400–500 mg ascorbate/day caused an approximate 50 % increase in measured plasma levels in three weeks [17, 20]. In these previous studies the significant rise in plasma ascorbate was believed to account for changes observed in DNA damage in PBMC obtained from the same volunteers. The assumption was that if plasma levels of vitamin C could be increased then so could cellular levels of the vitamin. Although this was demonstrated clearly for seven healthy individuals who were depleted of vitamin C to levels near those which would result in 'scurvy' [21], this is an extreme set of circumstances and there are few data on the effect of increasing plasma ascorbate on cellular levels in individuals with more usual plasma levels at onset of supplementation. However, Levine et al. [21] also imply that when plasma levels saturate (at around 65–75 μmol/l) then so do cellular levels, suggesting that supplementing individuals with vitamin C would have little effect on cellular levels if those individuals entered the study with plasma concentrations approaching the 65 μmol/l plateau. The data obtained in this study represent the first confirmation of this in a cross-section of healthy subjects on a 'normal' diet. If we assume that, as in previous studies, the subjects here possessed plasma vitamin C levels at or around 50–60 μmol/l, then any supplementation would have only a small effect on cellular levels – entirely consistent with our current observations. However, this does not imply that the increased availability/flux of vitamin C would not have some effect(s) on cellular metabolism.

The limited data in the literature on ascorbic acid levels in human cells concur with the data presented here. The overall data for all 4 groups: baseline median, 20.5 (13.5–33.3; 25^{th}–75^{th} percentile) μmol ascorbate/g protein, are in good agreement with other reports on levels in PBMC (20–30 μmol ascorbate/g protein [23–25]; and leukaemia cell lines following in vitro supplementation with ascorbate (~40 μmol ascorbate/g protein [26]).

For the placebo group, over the duration of the trial period, the level of ascorbic acid in PBMC was unchanged. The use of such a placebo group was aimed at accounting for any seasonal (or other) variations in intake of ascorbic acid and hence potentially in cellular levels that could lead to changes in levels of markers of oxidative damage such as 8-oxodG. These data on the placebo group allows us to confidently assert that supplementation of diets with vitamin C did not increase the ascorbate content within PBMC.

In conclusion, supplementation with vitamin C (80–400 mg/day) had little effect on ascorbate levels in PBMC in this group of individuals suggesting their diets were replete with vitamin C. The dose range of vitamin C used did not affect levels of oxidative damage measured by HPLC in DNA extracted from PBMC.

Acknowledgements Funding was from the Food Standards Agency under the MAFF Antioxidant Programme. Thanks also to those members of ESCODD throughout Europe, who have shared facilities, techniques and ideas over the past few years.

References

1. Halliwell B, Gutteridge JMC (1999) Free Radicals in Biology and Medicine, Third Edition, Oxford University Press, Oxford UK
2. Sies H (1991) Oxidative Stress: Oxidants and Antioxidants, Academic Press
3. Podmore ID, Griffiths HR, Herbert KE, Mistry N, Mistry P, Lunec J (1998) Vitamin C exhibits pro-oxidant properties. Nature 392:559
4. Poulsen HE, Weimann A, Salonen JT, Nyyssonen K, Loft S, Cadet J, Douki T, Ravanat JL (1998) Does vitamin C have a pro-oxidant effect? Nature 395: 231–232
5. Fraga CG, Motchnik PA, Shigenaga MK, Helbock HJ, Jacob RA, Ames BN (1991) Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. Proc Natl Acad Sci (USA) 88: 11003–11006
6. Duthie SJ, Ma A, Ross MA, Collins AR (1996) Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. Cancer Res 56: 1291–1295
7. Moller P, Viscovich M, Lykkesfeldt J, Loft S, Jensen A, Poulsen HE (2004) Vitamin C supplementation decreases oxidative DNA damage in mononuclear blood cells of smokers. Eur J Nutr 43: 267–274
8. ESCODD (European Standards Committee on Oxidative DNA Damage) (2000) Comparison of different methods of measuring 8-oxoguanine as a marker of oxidative DNA damage. Free Radic Res 32:333–341
9. ESCODD (2002) Inter-laboratory validation of procedures for measuring 8-oxo-7,8-dihydroguanine/8-oxo-7,8-dihydro-2'-deoxyguanosine in DNA. Free Radic Res 36:239–245
10. ESCODD (2002) Comparative analysis of baseline 8-oxo-7,8-dihydroguanine in mammalian cell DNA, by different methods in different laboratories: an approach to consensus. Carcinogenesis 23:2129–2133
11. ESCODD, Gedik CM, Collins A (2005) Establishing the background level of base oxidation in human lymphocyte DNA: results of an interlaboratory validation study. FASEB J 19:82–84
12. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
13. Wang L, Hirayasu K, Ishizawa M, Kobayashi Y (1994) Purification of Genomic DNA from Human Whole-Blood by Isopropanol-Fractionation with Concentrated NaI and SDS. Nucleic Acids Research 22:1774–1775
14. Ravanat JL, Douki T, Duez P, Gremaud E, Herbert K, Hofer T, Lasserre L, Saint-Pierre C, Favier A, Cadet J (2002) Cellular background level of 8-oxo-7,8-dihydro-2'-deoxyguanosine: an isotope based method to evaluate artefactual oxidation of DNA during its extraction and subsequent work-up. Carcinogenesis 23:1911–1918
15. Roberts LJ, Morrow JD (2000) Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. Free Radic Biol Med 28:505–513
16. Levine M, Wang Y, Padayatty SJ, Morrow J (2001) A new recommended dietary allowance of vitamin C for healthy young women. Proc Natl Acad Sci (USA) 98:9842–9846
17. Cooke MS, Mistry N, Ahmad J, Waller H, Langford L, Bevan RJ, Evans MD, Jones GD, Herbert KE, Griffiths HR, Lunec J (2003) Deoxycytidine glyoxal: lesion induction and evidence of repair following vitamin C supplementation in vivo. Free Radic Biol Med 34:218–225
18. Weimann A, Belling D, Poulsen HE (2001) Measurement of 8-oxo-2'-deoxyguanosine and 8-oxo-2'-deoxyadenosine in DNA and human urine by high performance liquid chromatography-electrospray tandem mass spectrometry. Free Radic Biol Med 30: 757–764
19. Rehman A, Collis CS, Yang M, Kelly M, Diplock AT, Halliwell B, Rice-Evans C (1998) The effects of iron and vitamin C co-supplementation on oxidative damage to DNA in healthy volunteers. Biochem Biophys Res Commun 246: 293–298
20. Cooke MS, Evans MD, Podmore ID, Herbert KE, Mistry N, Mistry P, Hickenbotham PT, Hussieni A, Griffiths HR, Lunec J (1998) Novel repair action of vitamin C upon in vivo oxidative DNA damage. FEBS Lett 439:363–367
21. Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, Park JB, Lazarev A, Graumlich JF, King J, Cantilena LR (1996) Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance, Proc Natl Acad Sci USA 93: 3704–3709
22. Polidori MC, Mecocci P, Levine M, Frei B (2004) Short-term and long-term vitamin C supplementation in humans dose-dependently increases the resistance of plasma to ex vivo lipid peroxidation. Arch Biochem Biophys 423: 109–115
23. Lenton KJ, Therriault H, Fulop T, Payette H, Wagner JR (1999) Glutathione and ascorbate are negatively correlated with oxidative DNA damage in human lymphocytes. Carcinogenesis 20:607–613
24. Lenton KJ, Therriault H, Cantin AM, Fulop T, Payette H, Wagner JR (2000) Direct correlation of glutathione and ascorbate and their dependence on age and season in human lymphocytes. Am J Clin Nutr 71:1194–1200
25. Lenton KJ, Sane AT, Therriault H, Cantin AM, Payette H, Wagner JR (2003) Vitamin C augments lymphocyte glutathione in subjects with ascorbate deficiency. Am J Clin Nutr 77:189–195
26. Mistry P, Herbert KE (2003) Modulation of hOGG1 DNA repair enzyme in human cultured cells in response to pro-oxidant and antioxidant challenge. Free Radic Biol Med 35:397–405