Profiling oxidative DNA damage: Effects of antioxidants

Harold C. Box,¹ Helen B. Patrzyc, Edwin E. Budzinski, Jean B. Dawidzik, Harold G. Freund, Nathalie C. Zeitouni and Martin C. Mahoney

Departments of Cell Stress Biology, Dermatology and Cancer Prevention, Roswell Park Cancer Institute, Buffalo, NY, USA

(Received April 26, 2012/Revised July 2, 2012/Accepted July 16, 2012/Accepted manuscript online July 26, 2012/Article first published online September 18, 2012)

The goal of this research was to determine whether antioxidant usage could be correlated with changes in DNA damage levels. Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) was used to simultaneously measure five different oxidatively-induced base modifications in the DNA of WBC. Measurements of the five modifications were made before and after an 8-week trial during which participants took the SU.VI.MAX supplement. Levels of the five DNA modifications were compared among different groupings: users versus non-users of antioxidant supplements, before versus after the supplement intervention and men versus women. The statistical significance of differences between groups was most significant for pyrimidine base modifications and the observed trends reflect trends reported in epidemiological studies of antioxidant usage. A combination of modifications derived from pyrimidine bases is suggested as a superior indicator of oxidative stress. (*Cancer Sci* 2012; 103: 2002–2006)

The goal of this study was to determine the efficacy of antioxidant supplements in protecting the genome from oxidative damage. Efforts to correlate oxidative stress with oxidative DNA damage have been confounded by controversy over the measurement of 8-oxo-7,8-dihydro-2'-deoxyguanosine, dG^h, the biomarker most often used as an indicator of oxidative stress, and by contradictory reports of the effects of antioxidant usage. The benefits of antioxidants may be genuine but subtle of discernment. In addition to the type of supplement, factors that seem to play a role are dose and gender. An assessment at the molecular level may provide a more immediate evaluation of the value of antioxidant usage. Oxidatively-induced DNA damage is a reasonable molecular basis for such an assessment.⁽¹⁾ This is a particularly attractive approach because modern mass spectrometry, combined with an appropriate methodology, has the capability for assaying multiple DNA modifications simultaneously with excellent sensitivity and high selectivity. A recently developed technology that measures DNA damage at the dimer level is particularly effective, in conjunction with tandem mass spectrometry, for measuring oxidative DNA damage. Most DNA lesions inhibit the action of the endonuclease nuclelease P1. The phosphoester bond 3' to the modified nucleoside resists hydrolysis. The consequence is a tendency for modified nucleosides to accumulate in a nuclease P1 hydrolysate of DNA in the form of modified dimers. An exception is 8-oxo-7,8-dihydrodeoxyguanosine which only minimally inhibits nuclelease P1.⁽²⁾ An advantage of assessing DNA damage at the dimer level is that the sensitivity of triple quadrupole detection operating in the negative ion mode is particularly good due to the presence of the phosphodiester bond. The sensitivity for detection of dinucleotides and dinucleoside monophosphates is much better than for nucleosides or bases.⁽³⁾ In this study we used Liquid Chromatogra-

phy-tandem Mass Spectrometry, (LC-MS/MS), to measure, at the dimer level, five oxidatively-induced base modifications in the DNA of WBC and to correlate damage levels with antioxidant usage.

The status of antioxidants as cancer preventatives may be judged from recent published reports. The well known SU.VI.MAX study involving over 13 000 participants found that a modest supplement reduced cancer incidence in men, but not in women, by 31%.⁽⁴⁾ Another large study found that a 200 µg selenium supplement reduced the incidence of cancer by 25%, whereas a larger supplement, 400 µg, was not efficacious.⁽⁵⁾ On the other hand, a recent meta-analysis that excluded the aforementioned reports concluded that antioxidants were ineffective in preventing cancer.⁽⁶⁾ An evaluation of antioxidants at the molecular level may prove less ambiguous.

Materials and Methods

Study design. Oxidative DNA damage was profiled before and after an 8-week intervention trial, which involved taking a daily oral supplement containing a mixture of antioxidants and minerals. The supplement used the same combination of antioxidants and minerals as in the SU.VI.MAX study, namely 120 mg vitamin C, 30 mg vitamin E, 6 mg beta carotene, 100 µg selenium and 20 mg zinc (Pacific Biologic, Concord, CA, USA). The SU.VI.MAX study is a large prospective epidemiologic assessment of oxidative supplements.⁽⁴⁾ Our study entailed measurements of five oxidatively-induced DNA modifications in the WBC of healthy donors. The main objective of the trial was to determine whether changes in DNA damage levels could be detected and correlated with the antioxidant regimen. A new approach to assaying DNA damage was used that measures multiple lesions at the dimer level.

Measuring DNA damage at the dimer level. The structures of the five oxidatively-induced DNA modifications observed in this study are shown in Figure 1. The modifications were measured in the dimer forms shown in Figure 1. Some comment concerning the modified deoxynucleoside constituent, N', of these modified 2'-deoxynucleoside (3'-5')-2'-deoxyadenosines and their generation in labeled oligomers is appropriate:

d(N^apA), 2'-deoxyribose-(3'-5')-2'-deoxyadenosine. The internal standard for this lesion, d(TN^aA*T), was synthesized from d(TGA*T) where A* denotes universally ¹⁵N-labeled deoxyadenosine. The tetramer was subjected to partial acid hydrolysis, followed by isolation of the product of interest by HPLC. The mutagenic potential of abasic sites has been ascertained from studies on yeast. Deoxycytosine is the preferred nucleoside inserted opposite an abasic site.⁽⁷⁾ d(P^fpA), 1'-(formamido)-2'-deoxyribose-(3'-5')-2'-deoxyadenosine. Synthesis of an internal standard for measuring the

¹To whom correspondence should be addressed.
E-mail: harold.box@roswellpark.org

Fig. 1. Structures of the five base modifications measured in this work. The modifications are shown in the dimer form in which the modifications were actually measured.

formamido lesion was accomplished by treating labeled oligomer with potassium permanganate to obtain the glycol followed by treatment with sodium periodate to obtain the formamido modification as described previously.⁽⁸⁾ The formamido lesion promotes the misincorporation of guanine.⁽⁹⁾ d(C^fpA), 1'-(1-carbamoyl-2-oxo-4,5-dihydroxy-3-imidazolidinyl)-2'-deoxyribose-(3'-5')-2'-deoxyadenosine. The modification of an oligomer to contain 1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine was accomplished using the Fenton reaction.⁽¹⁰⁾ The mutagenic consequences of this lesion have not been determined.

d(G^fpA), 1'-(2-amino-4-oxo-5-formamido-6-amimopyrimidinyl)-2'-deoxyribose-(3'-5')-2'-deoxyadenosine. This lesion, more familiarly known as Fapy-dG, is mutagenic causing transversions.⁽¹¹⁾ Generation of the lesion by radiation synthesis is favored.^(12,13)

d(T^gpA), 5,6-dihydroxy-5,6-dihydrothymidyl-(3'-5')-2'-deoxyadenosine. The thymine glycol lesion was produced in labeled oligomer by treatment with potassium permanganate.⁽³⁾ The cis isomers were isolated by HPLC. It is generally agreed that the thymine glycol lesion does not have significant mutagenic effects.⁽¹⁴⁾

Another advantage of measuring DNA damage at the dimer level relates to the isotopically labeled internal standards that are necessary for accurate quantitation of species by mass spectrometry. The mass of the isotopically labeled internal standard should be displaced from the mass of the product of interest by two or more mass units. Our measurements employ as internal standards DNA oligomers bearing the modified nucleoside with an isotopically labeled nucleoside 3' to the modified nucleoside. The internal standards are added to the DNA sample before digest (the oligomer is hydrolyzed to an isotopically labeled modified dinucleoside monophosphate concomitant with the hydrolysis of the DNA). It is generally easier to incorporate the isotopic label as an additional unmodified nucleoside than to synthesize a suitable labeled modified nucleoside as a standard. Another consideration relates to quantitation of the isotopically labeled internal standard itself. The absorption maximum of nucleotides at 254 nm, commonly the basis for quantitation of nucleic acids,

typically is shifted or eliminated in a damaged nucleoside, thus complicating quantitative determinations by spectrophotometric methods. In contrast, the extinction coefficients of the unmodified nucleosides in an oligomer internal standard serve nicely for determining the molar content of the standard.

Internal standards. Measurements of DNA damage were carried out at the dimer level using labeled modified tetramers as internal standards. Labeled modified tetramers have been synthesized containing the base modifications shown in Figure 1. The syntheses are described elsewhere^(3,8,10) and above. The internal standard for d(G^fpA) was unstable over the duration of the trial and had to be recalibrated.

Sample preparation. Human blood samples were obtained under an approved research protocol (RPCI # I 155409). The buffy coat was isolated from a sample of whole blood, typically 10 mL, by centrifugation. Lysing solution, 10 mL, was added to the sample and centrifuged. The pellet was washed in PBS and stored at -20°C. DNA was extracted from the cells using a kit designed to minimize spurious oxidation reactions (ZeptoMetrix, Buffalo, NY, USA). The kit uses chaotropic precipitation of the DNA together with desferol in the extraction procedure. Samples were prepared for LC-MS/MS analysis as follows: 100 µg DNA was lyophilized, denatured by addition of 25 µL H₂O and heated to 95°C for 5 m, then cooled in dry ice for 5 min. Internal standards were added to the DNA sample before enzymatic digest. Enzymatic hydrolysis was accomplished by adding 25 µL of 3.0 mM ZnCl₂, 7.5 µL 0.25 M Na acetate, pH 5.2, 1.0 µL of nuclease P1 equivalent to 1.0 unit, followed by incubation at 37°C for 2 h. Next, 12.5 µL of Tris HCl, 1.0 M, pH 9.0, and 75 units of alkaline phosphatase from bovine intestinal mucosa were added and the solution incubated an additional 2 h at 37°C. The sample was filtered using a Spin-X HPLC micro centrifuge 0.2 µm nylon filter by centrifuging at 2000g for 2 min. The filtrate was lyophilized to dryness and transferred to a MS/MS vial in 25 µL H₂O.

LC-MS/MS measurements. Liquid Chromatography-tandem Mass Spectrometry is especially suited to the sensitive and simultaneous detection of multiple DNA modifications. LC-MS/MS measurements were made on an AB Sciex Qtrap 5500 instrument operating as a triple quadrupole in the nega-

tive ion mode. The LC function used a Phenomenex Kinetex column (2 × 150 mm). Mobile phase A was 5.0 mM ammonium formate. Mobile phase B was acetonitrile. The gradient was 1.5–2.5% B over 0.0 to 0.5 min, 2.5% B from 0.5 to 5.0 min, 2.5–4.0% B from 5 to 10 min, then a gradient to 100% B. The total run time was 30 min. The flow rate was 0.25 mL/min.

Study population. Studies were carried out in accordance with accepted ethical practices. Potential participants were recruited from adult patients (>18 years old) seen at a dermatology practice. Volunteers had to report themselves to be in generally good health; those noting a history of kidney or liver disease were required to provide medical clearance from their primary care physician prior to enrollment. Smokers and patients with a prior history of malignant melanoma or kidney stones were not eligible. Females had to be post-menopausal. All participants were provided with an overview of the study and completed an informed consent prior to enrollment.

Subjects ($n = 38$) had a mean age of 59 years (median = 59 years, range 27–85 years). Participants already taking antioxidants, mainly multivitamins and vitamin C, were not excluded from the trial. This protocol was adopted because it was useful to know how DNA damage levels were affected by our supplement; whether or not the participant had been taking vitamin C or multivitamins. A baseline phlebotomy specimen prior to starting the supplement was provided by 38 persons, while an end of study specimen was provided by 35 persons (three participants declined to complete this draw).

Use of the antioxidant supplement was monitored via a mid-trial telephone call and by a residual capsule count. The overall adherence rate was 92% based upon a count of residual capsules at the end of the study.

Among the 38 participants who were started on the supplement, two participants withdrew due to the development of adverse events (two episodes of upper respiratory infection) from which both fully recovered. Both occurrences were judged not to be associated with the use of the supplement. No other clinically significant adverse events were noted.

Statistical considerations. The test statistic used for evaluating the difference between means of two distributions, presumed normal, was the following equation.

$$t = \frac{(\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$$

The subscripts 1 and 2 refer to the two groups being compared. The quantity μ refers to the mean value of a group and n is the number of participants in the group. The standard deviation, σ , for each of the five DNA modifications was derived from the totality of measurements of that modification. The statistical computations were facilitated using EXCEL.

Results

The mean values obtained for the five DNA modifications are listed in Table 1. Participants in the study are distinguished according to whether they were, or were not, antioxidant users before the start of the trial and according to gender.

Several observations are pertinent:

- 1 The first comparison to be made is between the two groups of participants who either did, or did not take antioxidants before the trial began. Comparing levels of oxidative damage, the levels are considerably higher for d(P^fpA), d(N^apA), d(T^epA) and d(CⁱpA) in non-users compared with

users. The level of the modification, d(G^fpA), is not significantly altered.

The difference between means of the different groups and the statistical significance of the measured difference between groups is given in Table 2 for each type of damage. Compared with non-users antioxidant users have significantly lower levels of DNA damage in all types of damage, except d(NapA) and d(G^fpA) (first row, Table 2). Thus, antioxidants are effective in limiting oxidative DNA damage.

- 2 Comparing the levels of the group of non-users before and after the trial, Tables 1 and 2, show that the effect of taking antioxidants produced a damage profile remarkably similar to that of the group that took antioxidants before the start of the trial. In other words, nearly the same lowered profile of oxidative damage was achieved by those who took antioxidant only during the trial.
- 3 Another insight comes from the group that took the supplement in addition to their customary supplement. Unlike the non-user group, the user group damage levels changed little over the course of the trial. No clear trend is indicated since two of the damage levels actually increase, whereas the other three decrease (see Table 1). We conclude that the additional supplement taken by prior users had no additional benefit and perhaps had a negative effect. These results bring to mind the negative results obtained in intervention trials where the dose was probably too large.⁽⁵⁾
- 4 Men, but not women, are reportedly benefitted by antioxidant supplements.⁽⁴⁾ It was important, therefore to examine our data with respect to gender. At the start of the trial all five DNA modifications are lower in women than in men (Table 1) with the modification, d(P^fpA), being the most significantly lower. The consistently lower levels of oxidative DNA damage in women suggests a possible explanation why women are reportedly not benefitted by antioxidant supplements. They may already be at some basal level such that supplements are not helpful as previously suggested.⁽⁴⁾ On the other hand, at the end of the trial no significant difference could be discerned between DNA damage levels in men versus women (Table 1).
- 5 Another goal of this research was to obtain better biomarkers of oxidative DNA damage than the familiar 8-oxo-7,8-dihydroguanine marker, dG^h.^(15,16) Consider the three pyrimidine base modifications, d(P^fpA), d(T^epA) and d(CⁱpA). Table 2 shows that at the start of the trial all three pyrimidine markers are consistent indicators of lowered oxidative DNA damage in users compared with non-users. Similarly, Table 2 shows that the pyrimidine base modifications are better indicators of the effects to prior non-users of taking antioxidants. Consistent with these observations the correlation coefficients, shown in Table 3, are generally high between pyrimidine modifications. The formamide marker, dP^fpA, which can be derived from either of the pyrimidine bases by oxidative stress, distinguishes most definitively between DNA damage levels in women versus men. These results suggest that a profile of DNA damages assessment might serve as a useful clinical guide in the use of antioxidants. Interestingly, the abasic lesion, although formed in high yield, is less responsive to varying conditions and shows little correlation with the other lesions.

Table 1. Levels of oxidative DNA damage are given in femtomoles per microgram of DNA $\pm$ SEM

	d(P ^f pA)		d(N ^a pA)		d(G ^f pA)		d(T ⁹ pA)		d(C ⁱ pA)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Non-users, start of trial ⁽¹¹⁾	12	4.7	190	57	68	6	8.1	3.6	17	0.7
Users, start of trial ⁽²⁶⁾	5.4	0.6	121	24	84	13	4.4	0.7	7.01	1.1
Non-users, end of trial ⁽¹¹⁾	6.6	1.3	153	57	94	24	2.8	0.4	8.7	3.4
Users, end of trial ⁽²⁶⁾	6.4	1.2	87	12	98	14	3.0	0.4	5.2	0.6
Men, start of trial ⁽¹⁴⁾	10	3.7	170	42	89	23	6.5	3.0	12	6.3
Women, start of trial ⁽²³⁾	5.5	0.7	124	30	74	8.1	5.0	1.1	9.5	2.2
Men, end of trial ⁽¹⁴⁾	6.2	0.9	124	44	89	14	3.5	0.7	5.7	0.9
Women, end of trial ⁽²³⁾	6.7	1.4	96	15	102	17	2.6	0.3	6.6	1.8

The DNA was obtained from WBC of users and non-users of antioxidants before and after an intervention trial during which all participants took our supplement for 8 weeks. See Figure 1 for structures of base modifications.

Table 2. Differences in DNA damage levels between groups of users and non-users of antioxidants at the start of the trial and at the end of the trial and between men and women

	d(P ^f pA)		d(N ^a pA)		d(G ^f pA)		d(T ⁹ pA)		d(C ⁱ pA)	
	Mean	P	Mean	P	Mean	P	Mean	P	Mean	P
Non-users, start–users, start	+6.6	0.01	+69	0.09	–16	0.26	+3.7	0.04	+10	0.02
Non-users, start–Non-users, end	+5.4	0.04	+37	0.26	–26	0.17	+5.3	0.02	+8.3	0.05
User, start–users, end	–1.0	0.42	+34	0.18	–14	0.23	+1.4	0.20	+1.8	0.31
Men, start–women, start	+4.5	0.03	+46	0.16	+15	0.27	+1.5	0.21	+2.5	0.40
Men, end–women, end	–0.5	0.42	+28	0.27	–13	0.28	+0.9	0.43	–0.9	0.40

Table shows differences between group means and *P*-values for differences corresponding to calculated *t*-values. The most significant differences are in bold type.

Table 3. Correlation coefficients between DNA modifications

	d(P ^f pA)	d(N ^a pA)	d(G ^f pA)	d(T ⁹ pA)	d(C ⁱ pA)
d(P ^f pA)	1	0.10	–0.13	0.64	0.61
d(N ^a pA)		1	–0.19	0.07	0.11
d(G ^f pA)			1	–0.14	–0.18
d(T ⁹ pA)				1	0.88
d(C ⁱ pA)					1

Discussion

This research used an assay for measuring multiple oxidative DNA damages and explored its usefulness in relation to antioxidant usage. The assay includes three pyrimidine base modifications that are known products of ROS activity and a modification derived from guanine, also a product of ROS activity. Pyrimidine bases have a higher oxidizing potential than guanine and are less likely than guanine to be oxidized artifactually during sample preparation. Nevertheless, our sample preparations were carried out taking precautions against artifactual oxidation (see Materials and Methods). The fifth DNA modification consisted of nucleotides that lost the base moiety and probably derived mainly from purine nucleosides. The level of the abasic modification was the highest of the five DNA modifications measured. The level of d(N^apA), measured in non-users of antioxidants, is 190 fmol/μg, corresponding to 52 modified dinucleoside monophosphates per 10⁶ nucleotides. Assuming the modification is distributed randomly among all sequences, this value corresponds to 172 abasic lesions per 10⁶ nucleotides. However, it is not altogether appropriate to classify abasic sites as ROS-induced since they can also be generated by other processes, such as enzymatic.

We compared differences in levels of DNA damage between groups and observed the following trends: (i) Taking antioxidant supplements reduces levels of oxidative DNA damage; (ii) excessive use of antioxidants provides no additional benefit and may be deleterious; and (iii) women have lower levels of oxidative DNA damage than men. These same trends are observed in epidemiological studies of cancer prevention by antioxidants.^(4,5) Additionally, with respect to the main goal of the research, we found that measurements of oxidatively-induced pyrimidine base damage distinguished most effectively between various groups.

Support for the hypothesis that oxidative damage is an important cause of mutations leading to cancer seems to be growing. All cells are subject to oxidative stress stemming from their necessity to synthesize ATP.⁽¹⁷⁾ It has been demonstrated experimentally that the level of oxidative damage is higher in the DNA of WBC of cancer patients compared with healthy controls. Thus, the well known indicator of oxidative stress, 8-oxo-7,8-dihydroguanine, has been reported to be significantly higher in the DNA from WBC of patients with cancer of the lung,^(18,19) lymphocytic system,⁽²⁰⁾ colorectum,⁽²¹⁾ bladder,⁽²²⁾ breast⁽²³⁾ and oesophagus⁽²⁴⁾ compared with controls. Unfortunately, control levels reported by these various laboratories varied by almost three orders of magnitude. Also in all of these studies the control levels were higher than the maximum considered acceptable by the European Standards Committee on Oxidative DNA Damage (ESCODD).⁽¹⁵⁾ However, also in every case, patient levels were significantly higher than controls.

Confidence in 8-oxo-7,8-dihydro-2'-deoxyguanosine lesion as a biomarker for oxidative stress has been guarded for two reasons: (i) deoxyguanosine has a low redox potential and may be oxidized inadvertently during preparation of the DNA sample; (ii) The redox potential of the biomarker,

8-oxo-7,8-dihydro-2'-deoxyguanosine, is even lower than that of deoxyguanosine; thus the biomarker may also be lost by inadvertent oxidation.⁽²⁵⁾ Thus, the assessment of oxidative stress has a controversial history. Alternative methods should be considered.⁽²⁶⁾ Here we assayed for five DNA modifications of the perhaps 50 or so that *in vitro* studies suggest may be present in human DNA.

Some additional observations regarding further work in this area of research are in order. Our preliminary study was structured as interventional and longitudinal. The information needed to follow up on the implications of the work could better be garnered from a retrospective observational study. Data associated with consistent long term usage of specific individual or specific combinations of antioxidants and minerals could best be obtained from a survey of potential participants followed by recruitment of participants whose histories conform to the needs of the study. The SU.VI.MAX supplement,

deservedly established as a benchmark for antioxidant intervention, could be approximated by the multivitamins taken by many people in Western countries.

Acknowledgments

The work was supported by grant RO3 CA139513 from the National Cancer Institute. The original Principal Investigator was Dr Alan Oseroff, now deceased, whose unfailing encouragement is gratefully recalled. The assistance of Ms Anne Paquette and Ms Donna Wagner in carrying the clinical aspects of the work is gratefully acknowledged. Mass spectrometer facilities are shared resources supported by NCI grant CA 016056.

Disclosure Statement

The authors have no conflict of interest.

References

- Walters JL, Satia JA, Kupper LL, Swenberg JA, Schroeder JC, Switzer BR. Associations of antioxidant nutrients and oxidative DNA damage in healthy African-American and white adults. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 1428–38.
- Falcone JM, Box HC. Selective hydrolysis of damaged DNA by nuclease P1. *Biochim Biophys Acta* 1997; **1337**: 267–75.
- Bailey DT, DeFedericis H-C, Greene KF *et al*. A novel approach to DNA damage assessments: measurement of the thymine glycol lesion. *Radiat Res* 2006; **165**: 438–44.
- Hercberg S, Czernichow S, Galan P. Antioxidant vitamins and minerals in prevention of cancers: lessons from the SU.VI.MAX study. *Br J Nutr* 2006; **96**: S28–30.
- Reid ME, Duffield-Lillico AJ, Slate E *et al*. The nutritional prevention of cancer: 400 Mcg per day selenium treatment. *Nutr Cancer* 2008; **60**: 155–63.
- Myung SK, Kim Y, Ju W, Choi HJ, Bae WK. Effects of antioxidant supplements on cancer prevention: meta-analysis of randomized controlled trials. *Ann Oncol* 2010; **21**: 160–79.
- Kow YW, Bao G, Minesinger B *et al*. Mutagenic effects of abasic and oxidized abasic lesions in *Saccharomyces cerevisiae*. *Nucleic Acids Res* 2000; **33**: 6196–202.
- Greene KF, Budzinski EE, Iijima H *et al*. Assessment of DNA damage at the dimer level: measurement of the formamide lesion. *Radiat Res* 2007; **167**: 146–51.
- Maufrais C, Fazakerley GV, Guy A, Cadet J, Boulard Y. Structure of an oligonucleotide containing a N-(2-deoxy-β-D-erythro-pentofuranosyl) formamide residue facing a guanine. *Biochimie* 2000; **82**: 65–7.
- Iijima H, Patrzyc HB, Budzinski EE, Dawidzik JB, Freund HG, Box HC. The 1-carbamoyl-2-oxo-4,5-dihydroimidazolidine component of ROS-induced DNA damage in white blood cells. *Radiat Res* 2010; **173**: 101–6.
- Kalam MA, Haraguchi K, Chandani S *et al*. Genetic effects of oxidative DNA damage: comparative mutagenesis of the imidazole ring-opened formamidopyrimidines (Fapy lesions) and 8-oxo-purines in simian kidney cells. *Nucleic Acids Res* 2006; **34**: 2305–15.
- Cadet J, Berger M. Radiation-induced decomposition of the purine bases within DNA and related model compounds. *Int J Radiat Biol Relat Stud Phys Chem Med* 1985; **47**: 127–43.
- Dizdaroglu M, Kirkali G, Januga P. Formamidopyrimidines in DNA: mechanisms of formation, repair and biological effects. *Free Radic Biol Med* 2008; **43**: 1610–21.
- Cooke MS, Evans MD, Dizdaroglu M *et al*. Oxidative DNA damage: mechanisms, mutations and disease. *FASEB* 2003; **17**: 1195–214.
- Collins AR, Cadet J, Moller L, Poulsen HE, Vina J. Are we sure we know how to measure 8-oxo-7,8-dihydroguanine in DNA from human cells? *Arch Biochem Biophys* 2004; **423**: 57–65.
- Dizdaroglu M. Facts about the artifacts in the measurements of oxidative DNA base damage by gas chromatography-mass spectrometry. *Free Radic Res* 1998; **29**: 551–63.
- Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. *Proc Natl Acad Sci USA* 2006; **90**: 7915–22.
- Vulimiri SV, Wu X, Baer-Dubowska W *et al*. Analysis of aromatic DNA adducts and 7,8-dihydro-8-oxo-2'-deoxyguanosine in lymphocyte DNA from a case-control study of lung cancer involving minority populations. *Mol Carcinog* 2000; **27**: 34–46.
- Gackowski D, Speina E, Zielinska M *et al*. Products of oxidative DNA damage and repair as possible biomarkers of susceptibility to lung cancer. *Cancer Res* 2003; **63**: 4899–902.
- Oltra AM, Carbonell F, Tormos C, Iradi A, Saez GT. Antioxidant enzyme activities and the production of MDA and 8-oxo-DG in chronic lymphocytic leukemia. *Free Radic Biol Med* 2001; **30**: 1286–92.
- Gackowski D, Banaszkiwicz Z, Rozalski R, Jawien A, Olinski R. Persistent oxidative stress in colorectal carcinoma patients. *Int J Cancer* 2002; **101**: 395–7.
- Akay T, Saygili I, Andican G, Yalcin V. Increased formation of 8-hydroxy-2'-deoxyguanosine in peripheral blood leukocytes in bladder cancer. *Urol Int* 2003; **71**: 271–4.
- Soliman AS, Vulimiri SV, Kleiner HE *et al*. High levels of oxidative DNA damage in lymphocyte DNA of premenopausal breast cancer patients in Egypt. *Int J Environ Health Res* 2004; **14**: 121–34.
- Breton J, Sichel F, Pottier D, Prevost V. Measurement of 8-oxo-7,8-dihydro-2'-deoxyguanosine in peripheral blood mononuclear cells: optimization and application to samples from a case-control study on cancers of the oesophagus and cardia. *Free Radic Res* 2005; **39**: 21–30.
- Leipold MD, Muller JG, Burrows CJ, David SS. Removal of hydantoin products of 8-oxoguanine oxidation by the *Escherichia coli* DNA repair enzyme FPG. *Biochemistry* 2000; **39**: 14984–92.
- Iijima H, Patrzyc HB, Budzinski EE *et al*. A study of pyrimidine base damage in relation to oxidative stress and cancer. *Br J Cancer* 2009; **101**: 452–4.