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Review

Urinary 8-Oxo-2′-Deoxyguanosine – Source, Significance and Supplements

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Oxidative damage to cellular biomolecules, in particular DNA, has been proposed to play an important role in a number of pathological conditions, including carcinogenesis. A much studied consequence of oxygen-centred radical damage to DNA is 8-oxo-2′-deoxyguanosine (8-oxodG). Using numerous techniques, this lesion has been quantified in various biological matrices, most notably DNA and urine. Until recently, it was understood that urinary 8-oxodG derives solely from DNA repair, although the processes which may yield the modified deoxynucleoside have never been thoroughly discussed. This review suggests that nucleotide excision repair and the action of a specific endonuclease may, in addition to the nucleotide pool, contribute significantly to levels of 8-oxodG in the urine. On this basis, urinary 8-oxodG represents an important biomarker of generalised, cellular oxidative stress. Current data from antioxidant supplementation trials are examined and the potential for such compounds to modulate DNA repair is considered. It is stressed that further work is required to link DNA, serum and urinary levels of 8-oxodG such that the kinetics of formation and clearance may be elucidated, facilitating greater understanding of the role played by oxidative stress in disease.

Keywords: Reactive oxygen species, 8-oxo-2′-deoxyguanosine, antioxidants, HPLC-EC, ELISA, urine

Abbreviations: 8-oxodG, 8-oxo-2′-deoxyguanosine; ROS, reactive oxygen species; 8-oxoG, 8-oxoguanine; GC-MS, gas chromatography-mass spectrometry; HPLC-EC, high performance liquid chromatography with electrochemical detection; AP, apurinic–apyrimidinic; Fapy, formamidopyrimidine; Ogg, 8-oxoguanine-glycosylase; NER, nucleotide excision repair; HPLC-MS/MS, high performance liquid chromatography with tandem mass spectrometry; TG, thymine glycol; 8-oxoGuo, 8-oxoguanosine; ELISA, enzyme-linked immunosorbant assay; 8-oxoA, 8-oxoadenine

INTRODUCTION

Reactive oxygen species (ROS) possess an important role in living systems through their beneficial and detrimental effects.[1] The ability of ROS to structurally modify cellular components, activate cytoplasmic/nuclear signal transduction pathways, alter DNA polymerase activity, modulate

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gene expression and protein production[2] has led to the implication of their involvement in a variety of pathological conditions[3,4] including inflammation,[5] carcinogenesis,[6,7] ageing[8,9] and autoimmunity.[10,11] Specific and non-specific defences limit the extent to which ROS, either generated physiologically from cellular metabolic processes or pathologically via toxic insult, may affect the cellular environment.[11] Nevertheless, a proportion of the ROS-generated evade the antioxidant defences and subsequently interact with biomolecules, resulting in a background level of damage. More serious for the cell however, is the condition of oxidative stress which occurs when this prooxidant–antioxidant balance is disturbed, in favour of the former.[12] An inevitable consequence of this is the overwhelming of the antioxidant defences, by ROS, giving rise to elevated levels of damage.

An important target for ROS within the cell is DNA, resulting in a broad range of products including base and sugar modifications, covalent crosslinks with proteins and single- and double-strand breaks.[13] Most attention has focused upon ROS-modification of DNA bases, yet with over twenty products identified,[14] only a few have been investigated in detail. The modified base, 8-oxoguanine or its deoxynucleoside derivative 8-oxodG, has been adopted as the target for intense investigation (comprehensively reviewed in Ref. [15]). The rationale for this has, in part, been due to the sensitivity with which it may be measured.[16] Reflective of this, the methodologies for the measurement of this lesion in DNA are numerous and include gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography with electrochemical detection (HPLC-EC) (both critically reviewed in Ref. [17]), ^{32}P-postlabelling,[18] immunodetection[19,20] and alkaline elution techniques.[21] Additionally, as will be illustrated in this review, 8-oxo-2′-deoxyguananosine (8-oxodG) possesses a documented mutagenic potential and has been shown to be a relevant marker of oxidative stress. Both the base and deoxynucleoside are studied since either may be released from DNA depending upon the method of hydrolysis, acid for the former and enzymic for the latter.[17] Furthermore, both 8-oxoguanine (8-oxoG) and 8-oxodG are present in the urine,[22] the measurement of which has markedly different implications, which is an aim of this review to illustrate.

Persistence of base modifications may have potentially deleterious consequences for the cell, for example, mutation. The mutagenic potential of 8-oxodG has been demonstrated to be due to a loss of base pairing specificity, misreading of adjacent pyrimidines,[23] or insertion of cytosine or adenine opposite the lesion.[24] Such mutations arising from 8-oxoG include GC to AT transversions.[25,26] Mispairing of 8-oxoG with adenine appears to be possible due to the predominance of the energetically favoured *syn* conformation of 8-oxodG, whereas in the *anti*-form pairing with dC is possible.[27] It may be that these structural alterations are involved in the recognition and subsequent repair of this lesion and yet 8-oxoadenine (8-oxoA), a structurally similar lesion, is reported to be at least one order of magnitude less mutagenic than 8-oxoG, in *Escherichia coli*.[28] It is therefore imperative that 8-oxoG does not persist in the DNA; to achieve this there exist, broadly speaking, four repair systems (Figure 1).

BASE EXCISION REPAIR

Base excision repair is largely responsible for the removal of non-bulky base adducts, involving specialised enzymes which recognise a specific repertoire of lesions. In this process, a DNA glycosylase removes the modified base leaving an apurinic–apyrimidinic (AP) site (AP-deoxyribose), which is subsequently removed by two AP endonucleases which incise 3′ (AP lyase) and 5′ (AP hydrolase) to the AP site.[29] However, some repair enzymes have been shown to possess both glycosylase and AP lyase activities. The resultant gap is then filled by a DNA polymerase.

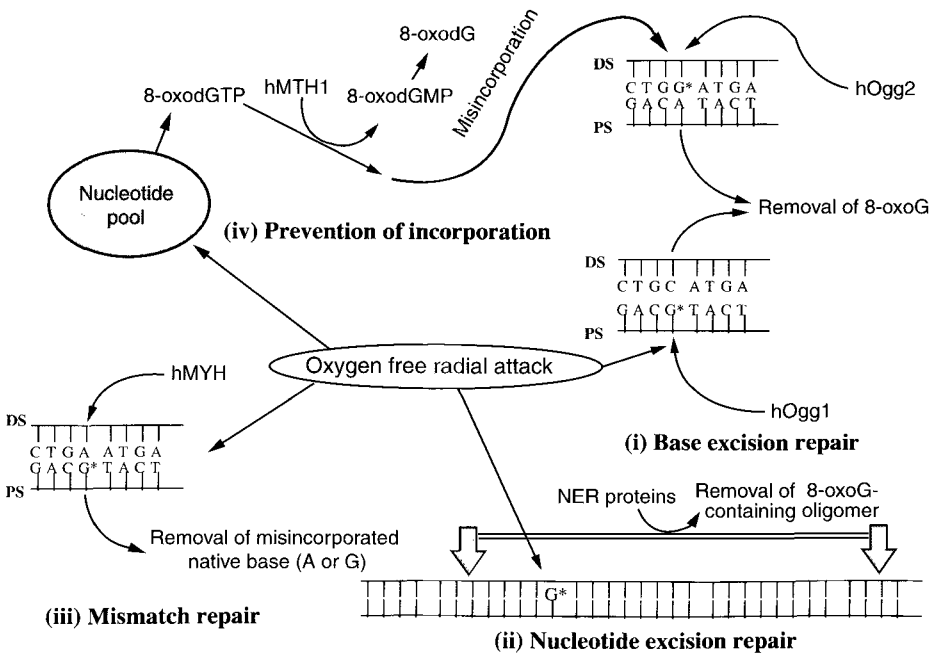

FIGURE 1 Representation of the four most established repair systems which maintain the integrity of the human genome, with respect to 8-oxodG: base excision repair (human homologue of the 8-oxoguanine-glycosylase enzyme: hOgg1 and hOgg2), nucleotide excision repair, mismatch repair (human MutY homologue: hMYH) and prevention of incorporation (human MutT homologue: hMTH1). DS and PS refer to daughter and parent strands of DNA respectively. G* indicates 8-oxodG.

A number of glycosylases for the removal of oxidative base damage have been identified, which include hydroxymethyluracil DNA glycosylase, thymine glycol (TG) DNA glycosylase and 8-oxoG DNA glycosylase.[30] It has been the glycosylases associated with the removal of 8-oxoG which have received most attention. Early studies of 8-oxoG DNA glycosylase were in *E. coli*, where it was identified as a formamidopyrimidine (Fapy) glycosylase (or the Fpg protein from the *fpg* or *MutM* gene) for removing Fapys.[31] The substrate specificity of Fapy glycosylase has been further extended to include singlet oxygen damaged DNA[32] (predominantly 8-oxoG) and also an appreciable amount of 8-oxoA.[33] The repair mechanism of this enzyme has been shown to involve two stages, hydrolysis of the N-glycosyl bond, resulting in removal of the damaged base, followed by an active lyase activity towards the apurinic site leaving a strand break.[34] Demonstration that this enzyme can be induced in *E. coli* by conditions of stress, in particular molecular oxygen, emphasises the profound protective nature of such repair processes.[35]

van der Kemp *et al.*[36] describe the cloning of a *Saccharomyces cerevisiae* gene named *OGG1*. Expression of this *OGG1* gene produced a glycosylase/lyase enzyme (8-oxoguanine-glycosylase 1, Ogg1) which acts on 8-oxoG when paired opposite either cytosine or thymine, but not when paired with adenine.[36] The substrate repertoire for the Ogg1 protein was later found to include AP sites opposite cytosine.[37] 8-oxoA was also found to be removed by Ogg1,[38] but only when inserted opposite cytosine. Girard *et al.*[37] concluded that whilst being functional analogues, differences in substrate specificity and catalytic mechanism suggested MutM and Ogg1 proteins are not closely related. The genes thought to be the human and mouse homologues of *OGG1* (*hOGG1* and *mOGG1*), were recently cloned by several groups.[39–43] Expression of *hOGG1* suppressed endogenous mutation in an *E. coli* mutant, deficient in both 8-oxoG glycosylase repair (MutM)

and 8-oxoG : A mis-match repair (MutY)[44] and was shown to be ubiquitously expressed in a variety of human organs.[39] Similarity of substrate specificity and mechanism between Ogg1 and hOgg1 suggested hOgg1 to be indeed the mammalian homologue of Ogg1.[44,45] Nash *et al.*[46] furthered work in this field of repair by reporting a second 8-oxoG glycosylase/lyase in yeast (tentatively designated yOgg2) which possesses a substrate preference for 8-oxoG paired opposite guanine or adenine.[47] Indeed, Hazra *et al.*[48] define hOgg2 in human cells as removing 8-oxoG when derived from the nucleotide pool and misincorporated opposite G or A.

The importance of the hOgg pathway for removal of 8-oxoG from DNA is exemplified in a study which reported that inactivation of hOgg1 may occur in some lung and kidney tumours.[49] Given the nature of inactivation, the authors speculate that *OGG1* may represent a new tumour suppressor gene.[49] Induction of *OGG1* mRNA by toxic insult[50] further supports this importance, suggesting the involvement of hOgg1, like the *E. coli* MutM protein,[51] in an adaptive response to cellular oxidative stress.

Bessho *et al.*[52] reported the existence of two human 8-oxoG repair enzymes, one being 8-oxoG glycosylase (which in contrast to MutM does not have a lyase activity) and an 8-oxoG endonuclease (which does not possess a glycosylase activity), although the activity of the former enzyme may be identical to mammalian N-methylpurine-DNA-glycosylase.[46] In addition to its main substrate of N-alkylpurine, mammalian N-methylpurine-DNA-glycosylase removes 8-oxoG by glycosylase action.[53] Glycosylases, by virtue of their mechanism, result in release of the modified base. The action of the endonuclease reported by Bessho *et al.*[52] gives rise to 3′,5′,8-oxodGDP as the putative product from modified DNA, which may subsequently be hydrolysed to 8-oxodG by nucleotidase(s). Such a mechanism would provide one means by which DNA-derived 8-oxodG may appear in urine.

NUCLEOTIDE EXCISION REPAIR (NER)

It has been suggested that NER acts simply as a "back-up" system for base excision in the repair of oxidative lesions,[54,55] yet in yeast, NER is reported to play a major role in processing oxidative DNA damage.[56] Whilst removal of these non-bulky lesions would appear to occur due to the non-specific binding of the NER recognition subunits to DNA,[54] the differing capacities of XP complementation groups to process 8-oxodG would suggest that more specificity is present than previously thought.[57,58] Once bound, the recognition subunits cause conformational changes in the DNA at the site which, if already conformationally altered due to a lesion, produces a higher affinity interaction and a more long-lived complex,[59] the result of which becomes an excinuclease target. Thus a process exists in mammalian cells[60] whereby oxidative lesions, which induce conformational changes and base mismatches, may be recognised and removed by NER.[54] Indeed, Reardon *et al.*[61] demonstrated the removal of 8-oxoG by NER to occur 1.5-fold faster than *cis*, *syn* cyclobutane thymine dimers (T<>T), the reference bulky lesion, concluding this to be physiologically significant. Such a finding adds, in mammalian cells, to the bacterial GO system described by Michaels and Miller[62] for preventing 8-oxoG-derived, spontaneous mutagenesis. Furthermore, NER would result in a lesion-containing oligomer, classically some 24–29 nucleotides in length.[63] Although again relying on data derived from studies of T<>T, there exists some evidence to suggest that such excised oligomers rapidly become subject to 5′ → 3′ exonucleolytic attack, which the authors speculate may continue until the lesion is encountered, resulting in a 6- or 7-mer,[64] or perhaps, ultimately, the isolated lesion itself. We suggest that such a process would represent another means by which DNA-derived 8-oxodG, or 8-oxodG-containing oligomers, may appear in the urine.

MISMATCH REPAIR

First demonstrated by Holmes *et al.*[65] and later characterised by McGoldrick *et al.*[66] MYH, the mammalian homologue of MutY in *E. coli*, removes the mismatched, but undamaged, adenine opposite to an 8-oxoG by a glycosylase action. Failure of this action would induce a G : C → T : A transversion. More recently, the repertoire of this enzyme has been extended to include the removal of guanine, when mismatched opposite 8-oxoG, thus preventing G : C → C : G transversions.[67] Removal of 8-oxoG from the parent strand is then performed by hOgg2. The human homologue of the *MutY* gene (*hMYH*) has been cloned and sequenced by Slupska *et al.*[68] establishing, along with *hOgg1* and *hMTH*, the human equivalent of the bacterial GO system.

PREVENTION OF INCORPORATION

The A → C mutation was shown to occur following misincorporation of 8-oxodGTP, from the nucleotide pool, opposite dA in DNA.[69] The existence of a protein, 8-oxodeoxyguanosine triphosphatase or 8-oxo-2′-deoxyguanosine 5′-triphosphate pyrophosphohydrolase (8-oxodGTPase[70]), detected in human tissue, (a homologue of *E. coli* MutT protein, known as human MutT homologue, hMTH[71]) which hydrolyses the damaged triphosphate to the monophosphate, 8-oxodGMP[72] (Figure 2), assigns considerable importance to this mechanism for base lesion appearance in DNA. This is further supported by the finding in *E. coli* of another enzyme, GTP cyclohydrolase II, with the ability to hydrolyse 8-oxodGTP (and 8-oxoGTP, although favouring the former) to their corresponding (deoxy)nucleoside monophosphates.[73] Whilst 8-oxodGTP can arise from oxidation of dGTP and the phosphorylation of 8-oxodGDP, it cannot be generated via the repeated phosphorylation of 8-oxodGMP[74] (Figure 2). Subsequent digestion of 8-oxodGMP by a 5′(3′)-nucleotidase (8-oxodGMPase) generates 8-oxodG which can be transported across the cell membrane and excreted in the urine.[74]

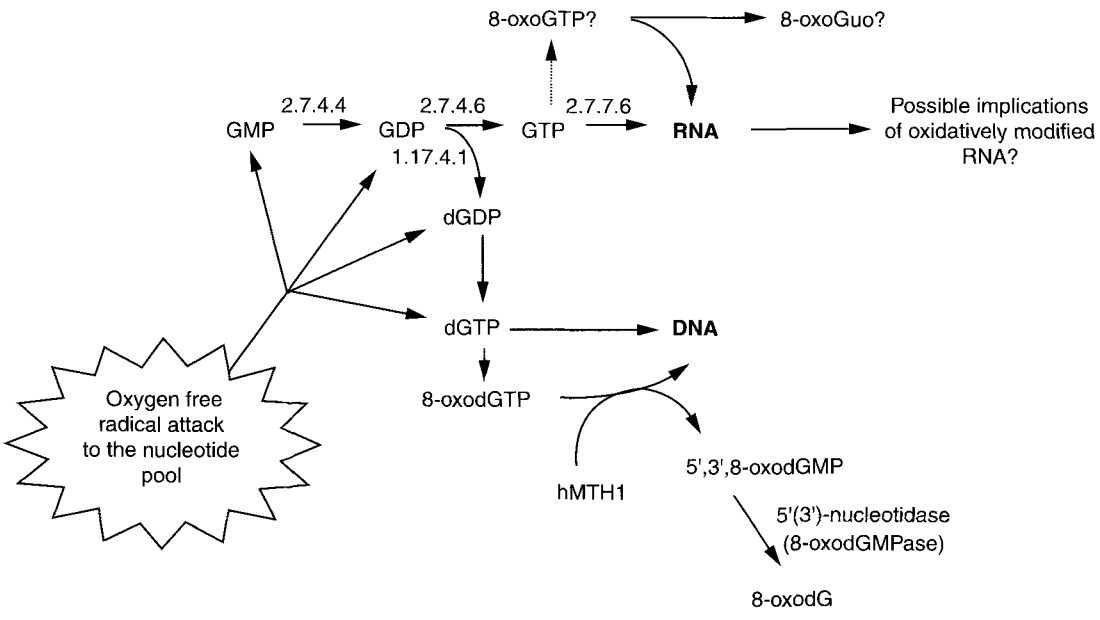

FIGURE 2 Illustration of how oxidative insult to the nucleotide pool may give rise to urinary 8-oxodG via the action of hMTH1 (human MutT homologue) (EC2.7.4.4: Nucleoside-phosphate kinase; EC2.7.4.6: Nucleoside 5′-diphosphatephosphotransferase; EC2.7.7.6: DNA-directed RNA polymerase; EC1.17.4.1: Ribonucleotide-diphosphate reductase).

Given that oxidation of the free deoxynucleotide occurs more rapidly than as a paired polynucleotide,[69] it might be implied that the deoxynucleotide pool represents a greater source of 8-oxodG than DNA. Also, mitochondrial sources of deoxynucleotides are disproportionally large compared to the nuclear/cytosolic pool when corrected for corresponding DNA content (*cellular pool*: 8.0 pmol dGTP/μg cellular DNA versus *mitochondrial pool*: 18.0 pmol dGTP/μg cellular DNA)[75] which, given the potential for elevated oxygen radical production in mitochondria, could result in significant oxidation of deoxynucleotides. In keeping with this, mitochondrial levels of hMTH1 are reported to be high.[76] Equally, it may be argued that the nucleotide pool is so large that any possible modulation of 8-oxodG levels would not be seen, unless the technique was extremely sensitive. However, whilst the total nucleotide pool is indeed likely to be large (800.8 pmol/cell[75]), the deoxynucleotide pool would represent only a fraction of this (10.8 pmol/cell[75]), easily influenced by prooxidant/antioxidant factors. It may be that these factors combined contribute significantly to urinary 8-oxodG levels, but the questions of their quantitative and qualitative importance compared to DNA, remains to be elucidated.

It is therefore clear that, due to the potential for 8-oxodG to be derived from the nucleotide pool, urinary measurements of this lesion do not reflect solely excision repair of DNA, but also the processes which prevent incorporation of damaged deoxynucleotides during DNA synthesis (Figure 2). Indeed, this argument is unlikely to be unique for 8-oxodG, as DNA polymerase incorporation of other oxidatively-modified deoxynucleotide triphosphates and their potential for mutation, have both been shown.[77,78] Although recent work has extended the substrate repertoire of hMTH to include oxidised dATP,[79] it is likely that enzymes, functionally analogous to hMTH, will be identified for other deoxynucleotides.

A further possible source of urinary 8-oxodG may be that derived from dead cells. Lindahl[80] asserted that, as 8-oxodG cannot be produced from repair via a glycosylase action, its source in the urine is presumably due to the action of non-specific nucleases and phosphatases upon DNA released from dead cells. Such processing would yield free deoxynucleosides, oxidation of which would give rise to urinary 8-oxodG. Whilst this may indeed represent a contributory pathway for urinary 8-oxodG generated intra-cellularly prior to or during cell death, there is evidence to suggest that free deoxynucleosides are not subject to oxidative modification in the systemic circulation.[81] In this thorough study, Shigenaga *et al.*[81] demonstrated that intra-venous immunisation with 2′-deoxyguanosine leads to rapid incorporation into the cellular nucleotide pools, with no 8-oxodG production. Furthermore, oxidation of the trace dG quantities present in the urine was also shown to be insignificant.[81] The same workers showed that 8-oxodG is not generated from free 2′-deoxyguanosine when incubated with liver cytosolic enzymes or microsomal enzymes (cytochrome P-450), nor is 8-oxodG degraded in the circulation or through prolonged incubation in urine.[81] Therefore, whilst the potential exists for 8-oxodG derived from dead cells to contribute to urinary levels, its artefactual production or loss through metabolism or exposure to the circulation is insignificant, establishing its stability for use as a biomarker. Repair-based mechanisms for the generation of 8-oxodG are illustrated in Figure 3.

METHODOLOGY FOR THE MEASUREMENT OF URINARY 8-oxodG

It is generally accepted that a consequence of repair is the appearance of lesions, or their derivatives, in the urine. On this basis, the development of assays which measure urinary levels of damage products would provide a non-invasive approach to the monitoring of *in vivo* repair. The technique of HPLC-EC, has been applied to the analysis of

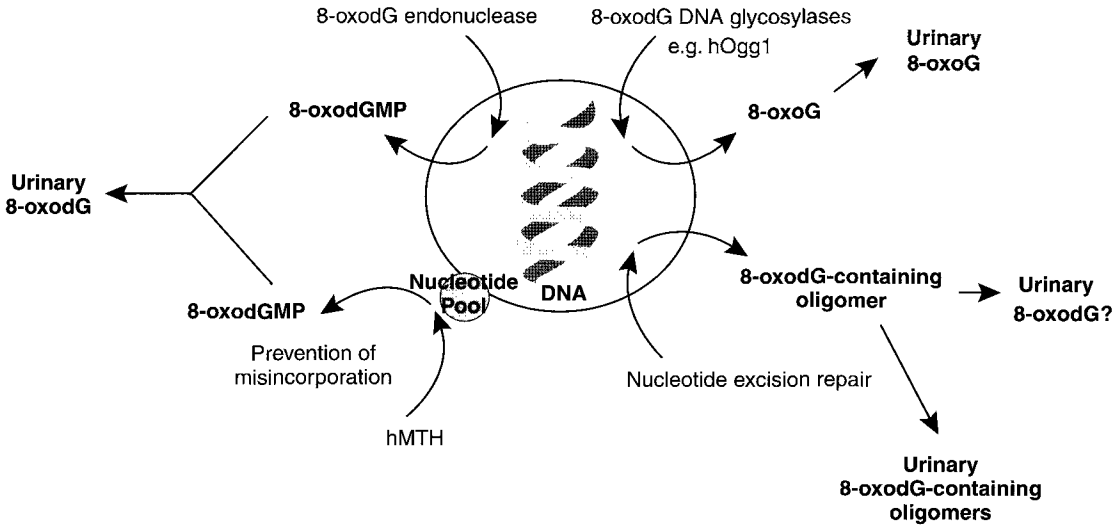

FIGURE 3 Schematic for possible sources of urinary 8-oxodG (hOgg1: human homologue of the Ogg enzyme; hMTH human MutT homologue).

8-oxodG in urine. However being a complex mixture of constituents, the urine requires considerable prepurification, which include; solid-phase clean-up of the urine,[81] column coupling,[82,83] carbon column capture[84] and immunoaffinity clean-up.[85] The latter principle was extended by Park *et al.*[86] using a monoclonal antibody column to enrich for 8-oxodG in blood plasma and culture medium. Whilst such antibodies may represent an improvement in the HPLC method for urinary 8-oxodG measurement, they also appear to be a respectable alternative to HPLC methodology,[87] with good correlation between the two techniques (Ref. [88], Ochi *et al.**). Urinary 8-oxodG has also been measured by GC-MS and, although this has required extensive sample work-up including both solid-phase extraction and/or HPLC prepurification,[89,90] it does represent a means by which multiple lesions may potentially, be simultaneously measured.[91] Preliminary analysis of five urinary oxidative DNA products, 8-oxodG included, by GC-MS with HPLC prepurification, indicated an approximately constant ratio between the levels of each lesion.[91] Such a result would support suggestions that lesions other than 8-oxodG may also be suitable urinary markers of oxidative stress. Conversely, HPLC with tandem mass spectrometry requires comparatively little sample work-up, whilst offering both measurement and confirmatory identification of 8-oxodG.[92]

URINARY MEASUREMENT OF OXIDATIVE DAMAGE REPAIR PRODUCTS

TG was one of the first ROS-induced DNA lesions to be studied and found to be a significant marker of oxidative stress.[93] However, there is a 1000-fold greater sensitivity associated with the detection of 8-oxodG by HPLC with electrochemical detection, compared to the UV detection of

* Ochi, H., Yoshikawa, T., Cutler, R., Takeuchi, M. and Ramarathnam, N. Development of a monoclonal antibody ELISA for the quantification of 8-hydroxy-2′-deoxyguanosine (submitted for publication).

TG[94] and this has led to urinary measurements of TG being superseded by 8-oxodG. This simple rationale highlights a number of *caveats* which should be noted when measuring a lesion in urine and interpreting the results.

Dietary Contribution

Only a small amount of TG in urine is derived from the diet,[95] whereas the diet represents a major contributor to urinary 8-oxoG.[85] Measurement of the modified deoxynucleoside (thymidine glycol and 8-oxodG) appears to overcome this problem, although it is argued that this prevents the assay from being directly reflective of DNA repair. Such a view assigns considerable input from the deoxynucleotide pool and minimal contribution from the DNA directly. Furthermore, it implies that 8-oxoG-generating, DNA repair processes are of primary quantitative importance.

Levels of Lesion in Urine

8-oxodG predominates over thymidine glycol in human urine.[94] Reasons for this are believed to include the relative instability of the TG, resulting in its decomposition, site-specific hydroxyl radical formation in DNA (largely in guanine-rich regions) and selectivity in the occurrence or repair of damage.[94] Furthermore, the stability of 8-oxodG allows the frozen storage of urine for up to one year prior to analysis.[83]

Artefactual Oxidation

The possibility of 8-oxodG arising from enzymatic or chemical oxidation of dG has been rigorously examined by Shigenaga *et al.*[81] who concluded that neither of these routes, nor exposure of dG to the systemic circulation, give rise to 8-oxodG.

Renal Function

Urinary levels of any oxidative lesion rely on renal excretion of damage products[96] and this raises the issue of "in which units should the lesion be expressed?" Whilst some groups favour expression of urinary 8-oxodG in terms of pmol/kg/24 h,[83,97] others express relative to creatinine.[82,98,99] Tagesson *et al.*[100] later suggest that creatinine levels co-vary with 8-oxodG independently of urine concentration. However, creatinine is used routinely to correct for variations in urine concentration and also acts as an indicator of renal function, particularly important when examining urinary 8-oxodG in patient groups with disease. Furthermore a study by Bogdanov *et al.*[84] showed close correlation between total 8-oxodG output per 24 h and "spot" urines corrected for creatinine, demonstrating low intra-individual variability. It was subsequently concluded that 8-oxodG measurements of these "spot" urines were excellent markers in intervention studies.[84] This is supported by Poulsen *et al.*,[101] given the *caveat* that the creatinine concentrations are unchanged in paired experiments and comparable in unpaired experiments.

A major drawback of either unit is that it prevents comparison of data between the "two schools' of thought". Other problems, associated with the 24 h collections, are the stringency with which the 24 h is timed and the logistics of collection (large volumes of urine etc.).

Specificity of the Technique

A frequent criticism of antibody techniques relates to their specificity. In our studies (Refs. [88,98], Evans *et al.*[†]), we employed an antibody which has been thoroughly characterised.[102] No recognition of DNA or RNA bases was shown, whether modified or not. Whilst some recognition of the ribonucleoside, 8-oxoguanosine (8-oxoGuo) was reported, it was at a concentration at least

[†]Evans, M.D., Cooke, M.S., Akil, M. and Lunec, J. Aberrant processing of 8-oxo-2'-deoxyguanosine in Systemic Lupus Erythematosus (Submitted).

two orders higher than for 8-oxodG. The relative amounts of urinary 8-oxodG and 8-oxoGuo have not yet been accurately demonstrated preventing quantitative comparison. Even if this still represents a significant contribution from RNA and/or the nucleotide pool, it does not, as discussed below, preclude this as an assessment of oxidative stress.

The antibody used in the above studies (and others[87,103–108]) has a demonstrated ability to detect "free", monomeric 8-oxodG and that contained within oligomers and indeed isolated DNA itself[‡]. Given that the urinary products of NER may be 8-oxodG-containing oligomers, the potential of the antibody to detect these highlights a limitation of assays such as HPLC-EC and GC-MS which are strictly limited to monomeric 8-oxodG, thereby not detecting 8-oxodG derived from a potentially important repair pathway. Although no literature precedent exists for the presence of 8-oxodG-containing oligomers in the urine, experiments within the authors' laboratory confirm the presence of oligomers[§] with work to identify potential lesions on-going. This may also account, in part, for the difference in basal levels of 8-oxodG noted by enzyme-linked immunosorbant assay (ELISA) and HPLC-EC techniques, the ELISA-derived values being higher (Table I). The HPLC-EC method is not without analytical difficulties, Bogdanov *et al.*[84] recently reported the presence of peaks which co-elute with 8-oxodG, identified by multi-channel coulometric electrochemical detection. Despite these issues, within technique agreement of levels in control subjects between labs appears strong (Table I). Furthermore, in longitudinal or comparative studies, the significance of absolute levels is superseded by the ability to detect variation.

TABLE I Inter-laboratory and inter-technique comparison of baseline levels of urinary 8-oxodG in healthy individuals, as measured by ELISA and HPLC-EC

Technique	
ELISA (ng 8-oxodG/mg creatinine)	HPLC-EC (ng 8-oxodG/mg creatinine)
20.5 ± 7.5[98]	3.8 ± 1.9
19.4 ± 8.5[87]	2.2 ± 0.9
18.61[106]	5.0 ± 1.1
24.3 ± 15.2[107]	2.4 ± 1.3

Values are derived from those tabulated by Loft and Poulsen.[109]

[‡] Unpublished observations.
[§] Manuscript in preparation.

Contribution from RNA

Whilst not an issue for TG, urinary levels of 8-oxoG may include a contribution from RNA, particularly if mechanisms exist to maintain the integrity of RNA molecules. Indeed, damage to RNA may play a significant role in pathology through abnormal protein translation and defects in protein synthesis.[110,111] As described above, contribution from the diet suggests that measurement of urinary 8-oxoG renders this marker invalid. A study reported by Witt *et al.*[112] examined the effects of exercise and vitamin supplementation upon oxidative damage, as monitored by urinary 8-oxoGuo measurement. This is further supported as a suitable marker of oxidative stress by findings which show there to be a negligible contribution of urinary modified ribonucleosides from the diet.[113,114] Again, measurement of the deoxynucleoside avoids the involvement of any RNA derivatives.

It has therefore been proposed that urinary levels of 8-oxodG can be used to non-invasively monitor *in vivo* oxidative stress,[115,116] assuming an appropriate method of analysis exists, which takes the above points into account.

SIGNIFICANCE OF URINARY 8-oxodG MEASUREMENTS

The quantitation of 8-oxodG in urine has been used to assay *in vivo* oxidative DNA damage.[68]

These workers demonstrated a correlation between levels of urinary 8-oxodG and species-specific metabolic rate and longevity, consistent with the hypothesis of a steady-state level of oxidative damage. Urinary 8-oxodG has also been examined in relation to smoking, gender and body mass index.[83] The general finding, reviewed by Loft *et al.*,[117] was that metabolic rate appeared to be a factor accounting for inter-species and subject variations in 8-oxodG excretion and that smoking increased urinary levels of this lesion. Furthermore, this lesion and presumably others, has been shown to accumulate in tissue in an age-dependent manner, perhaps due to either a decrease in repair/antioxidant defence efficiency[118] or an increased rate of oxidant production.[22]

It has been shown that individuals with malignant disease (affecting breast, colon, lymphoma and teratoma) have a significantly higher excretion of 8-oxodG compared to healthy controls, hypothesised to be due to increased oxidative damage.[82,100] However, this increase may largely be due to the therapy which the patients are undergoing,[119] perhaps due to increased cell turnover. Elevated levels of urinary 8-oxodG and presumably DNA damage, as determined by HPLC, have been proposed as an explanation for the higher incidence of malignancy in patients with cystic fibrosis.[120] It is important to note that a rise in urinary 8-oxodG does not necessarily reflect more DNA damage, but can reflect a lowering or lower steady-state levels in tissue DNA, perhaps due to repair.[121] Thus far, few studies have attempted to correlate increased urinary oxidative biomarkers with disease, perhaps due to a possible multi-factorial explanation for elevated levels of oxidative stress in disease, or the drawbacks of analytical techniques available. In particular, strong data for the much postulated role of oxidative DNA damage in carcinogenesis[122] remains most elusive, although observational/epidemiological evidence is growing (reviewed by Poulsen and Loft[123]). It therefore seems reasonable to suggest that dietary antioxidants may play a role in cancer prevention, although Loft *et al.*[83] concluded that intake of antioxidants does not influence 8-oxodG excretion and, by implication, DNA damage.

DNA DAMAGE AND ANTIOXIDANTS

It has been postulated that prevention of damage to DNA, through antioxidant pathways, is an important approach to the prevention of carcinogenesis, suggesting that supplementation with antioxidants may significantly decrease levels of damage. However, whilst epidemiological studies suggest fruit and vegetables have protective effects against cancer, this may not be specifically due to antioxidants such as vitamins C and E.[124,125] Furthermore the experimental evidence for the benefits of antioxidant vitamins has not shown a consensus. Several groups have reported no effect of antioxidants, such as vitamin C or E, on oxidative DNA damage[126–128] and yet there remains evidence for a profound protective effect.[129–131] Such contradictory findings are exemplified in our recent report in which vitamin C supplementation displayed an apparent antioxidant effect, reducing 8-oxoG in lymphocyte DNA with a concomitant prooxidant effect, increasing levels of 8-oxoA in the same sample.[132] This novel finding, whilst surprising, maintained that vitamin C possesses an overall protective effect due to the different mutagenic abilities of 8-oxoG compared to 8-oxoA.[132] Further work, which has suggested a possible explanation for this dichotomy, measured levels of 8-oxodG in DNA, serum and urine[98] from the same supplemented subjects as described by Podmore *et al.*[132] The report described the reduction of DNA levels of 8-oxodG, whilst serum and urinary levels increased significantly. Additional support for this observation may be derived from two other reports which noted an appreciable, if non-significant, increase in urinary 8-oxodG of subjects with a diet high in fruit and vegetables[133] and those supplemented, twice daily, with 250 mg vitamin C.[134] Cooke *et al.*[98]

concluded by extending the adaptive response hypothesis of Rehmann *et al.*[134] providing experimental evidence that vitamin C may positively influence the repair of DNA and/or sanitisation of the deoxynucleotide pool. Measurement of 8-oxodG in all three biological matrices (DNA, serum and urine) clearly facilitated such a conclusion, reiterating the importance of 8-oxodG measurements other than in DNA. Example of other studies, in which dietary intervention modulated urinary 8-oxodG excretion, describe the reduction of urinary 8-oxodG levels upon a diet rich in Brussels sprouts.[135,136] Although potentially due to the antioxidant content of the Brussels sprouts, the authors postulate that the reduction seen might be due to the induction of xenobiotic metabolism enzymes by phytochemicals in cruciferous vegetables,[135] the consequence of which is removal of oxidative stress-inducing agents and hence reduction of the steady-state level.

There is, at present, no precedent to suggest what proportion of urinary 8-oxodG is derived from the deoxynucleotide pool or DNA. Therefore the increases in serum and urinary 8-oxodG may be derived from either source (or both). However, recent evidence from our laboratory may provide a clue. Our study, in which serum levels of 8-oxodG in the autoimmune disease, systemic lupus erythematosus (SLE), were examined, revealed an attenuated response to vitamin C supplementation, compared to control subjects (Evans *et al.*[‖]). A reduced purine 5′-nucleotidase activity[137] may account for this difference between the subject groups, assuming this enzyme acts upon modified, as well as native, mononucleotides. As illustrated in Figure 3, the purine 5′-nucleotidase is required to convert 5′,8-oxodGMP, derived from the nucleotide pool, to 8-oxodG, allowing excretion of the lesion. Given that this is the rate limiting step,[136] we speculate that processes requiring this activity to generate 8-oxodG could partly account for the quantitative differences in response between the SLE and control subjects.

Given the 8-oxodG sources described in this review, it would be pertinent to re-examine how vitamin C may modulate levels of 8-oxodG in both DNA and urine. Cooke *et al.*[98] postulated a number of explanations for the effects upon 8-oxodG following vitamin C supplementation:

1. Vitamin C acts as a prooxidant for guanine moieties not contained within DNA perhaps due to a particular cellular localisation of vitamin C. The increases seen in 8-oxodG would therefore be derived from the nucleotide pool *i.e.* oxidation of dGTP.
2. Equally, if vitamin C promotes the "purging" of 8-oxodG from the cell, again this would most likely come from the nucleotide pool. In the case of this and the above points, this would suggest that vitamin C is having some form of residual effect, detectable long after plasma values have returned to baseline and that it is the processing of these lesions which explains the delay between their removal and appearance in the urine.
3. Given that DNA levels of 8-oxoG are reduced by vitamin C[132] this would support the, largely *in vitro*, evidence for an activity which scavenges radicals,[1] preventing damage to DNA. In combination with steady-state repair, a decrease in DNA levels of 8-oxoG would be seen. This would not account for the concomitant increase in 8-oxoA.
4. Our favoured hypothesis suggests that vitamin C positively influences the removal of 8-oxodG from the DNA and/or nucleotide pool, most likely, via the upregulation of repair enzymes. Recent *in vitro* evidence from the authors' laboratory supports a role of vitamin C in both the activation of transcription factor binding and 8-oxodG removal (Holloway *et al.*[¶]). This may be

[‖] Evans, M.D., Cooke, M.S., Akil, M. and Lunec, J. Aberrant processing of 8-oxo-2′-deoxyguanosine in Systemic Lupus Erythematosus (Submitted).

[¶] Holloway. K., Cooke, M.S., Faux. S., Griffiths, H.R. and Lunec, J. (in preparation).

associated with vitamin C's prooxidant/redox properties, also accounting for the increase in DNA levels of 8-oxoA.

SUMMARY AND CONCLUSIONS

The most reported technique for the measurement of urinary 8-oxodG is HPLC-EC, however, more recently an ELISA-based method has increased in use. Whilst agreement between the HPLC and ELISA techniques is not established for baseline urinary levels, strong agreement is seen between laboratories using the same technique. Literature references to urinary 8-oxodG measurements have clearly illustrated the apparent involvement of oxidative stress in numerous pathological conditions and its generation following toxic insult. However, proof of a defined role is still absent. The question: "from where does urinary 8-oxodG derive?" is central to the meaningful interpretation of results derived from the above assays and an important prerequisite for proving a defined role. This review has addressed the question of source, suggesting that an endonuclease action and NER may yield 8-oxodG from DNA, whilst the hMTH system sanitises the nucleotide pool, giving rise to 8-oxodG. Furthermore cell death/turnover either through apoptosis or necrosis, may also contribute to the background levels of lesion. With the sources identified, it will be important to know what the relative contribution to total urinary 8-oxodG these processes represent and how their action may be modulated. Such a finding would show, in terms of cellular importance, how measurements of urinary 8-oxodG, as a marker of oxidative stress compares with DNA levels of 8-oxodG and DNA repair capacity. Significant work still needs to be performed to link more closely these three parameters and hence further elucidate the kinetics of 8-oxodG formation and clearance *in vivo*. Upon establishment of these details, urinary 8-oxodG measurements may become more than a reflection of generalised oxidative stress.

References

[1] B. Halliwell and J.M.C. Gutteridge (1999) *Free Radicals in Biology and Medicine*, Oxford, 3rd edn., Oxford, Clarendon Press.

[2] H. Wiseman, H. Kaur and B. Halliwell (1995) DNA damage and cancer, measurement and mechanism. *Cancer Letters*, **93**, 113–120.

[3] B. Halliwell (1989) Current status review, free radicals, reactive oxygen species and human disease, a critical evaluation with special reference to atherosclerosis. *British Journal of Experimental Pathology*, **70**, 737–757.

[4] S.P. Wolff, A. Garner and R.T. Dean (1986) Free radicals, lipids and protein degradation. *Trends in Biological Sciences*, **January**, 27–31.

[5] K. Frenkel, J. Karkoszka, E. Kim and E. Taioli (1993) Recognition of oxidised DNA bases by sera of patients with inflammatory diseases. *Free Radical Biology and Medicine*, **14**, 483–494.

[6] T.W. Kensler and B.G. Taffe (1986) Free radicals in tumour promotion. *Advances in Free Radical Biology and Medicine*, **2**, 347–387.

[7] R.A. Floyd (1990) The role of 8-hydroxyguanine in carcinogenesis. *Carcinogenesis*, **11**, 1447–1450.

[8] V.W. Bunker (1992) Free radicals, antioxidants and ageing. *Medical Laboratory Science*, **49**, 299–312.

[9] Y.A. Barnett and C.M. King (1995) An investigation of antioxidant status, DNA repair capacity and mutation as a function of age in humans. *Mutation Research*, **338**, 115–128.

[10] J. Lunec, K.E. Herbert, S. Blount, H.R. Griffiths and P. Emery (1994) 8-Hydroxydeoxyguanosine, a marker of oxidative DNA damage in systemic lupus erythematosus. *FEBS Letters*, **348**, 131–138.

[11] M.S. Cooke, N. Mistry, C. Wood, K.E. Herbert and J. Lunec (1997) Immunogenicity of DNA damaged by reactive oxygen species – implications for anti-DNA antibodies in *Lupus. Free Radical Biology and Medicine*, **22**, 151–159.

[12] H. Sies (1991) Introduction. In *Oxidative Stress – Oxidants and Antioxidants* (Ed. H. Sies), Academic Press, London, p. 1.

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

[14] M. Dizdaroglu (1991) Chemical determination of free radical-induced damage to DNA. *Free Radical Biology and Medicine*, **10**, 225–242.

[15] H. Kasai (1997) Analysis of a form of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutation Research*, **387**, 147–163.

[16] R.A. Floyd, J.J. Watson, P.K. Wong, D.H. Altmiller and R.C. Rickard (1986) Hydroxyl free radical adduct of deoxyguanosine, sensitive detection and mechanism of formation. *Free Radical Research*, **1**, 163–172.

[17] B. Halliwell and M. Dizdaroglu (1992) The measurement of oxidative damage to DNA by HPLC and GC/MS techniques. *Free Radical Research*, **16**, 75–87.

[18] U.-S. Devanaboyina and R.C. Gupta (1996) Sensitive detection of 8-hydroxy-2′-deoxyguanosine in DNA by ^{32}P-postlabelling assay and the basal levels in rat tissues. *Carcinogenesis*, **17**, 917–924.

[19] M. Cooke and K. Herbert (2000) Immunochemical detection of 8-oxodeoxyguanosine in DNA. In *A Handbook of Clinical Analysis* (Ed. J. Lunec) (in press).
[20] K.E. Herbert and J. Lunec (1999) Progress in the immunodetection of products of oxidative damage to DNA. In *DNA and Free Radicals, Techniques, Mechanisms and Applications* (Eds. O. I. Aruoma and B. Halliwell), OICA International, pp. 1–14.
[21] M. Pflaum, O. Will and B. Epe (1997) Determination of steady-state levels of oxidative DNA base modifications in mammalian cells by means of repair endonucleases. *Carcinogenesis*, **18**, 2225–2231.
[22] C.G. Fraga, M.K. Shigenaga, J.W. Park and B.N. Ames (1990) Oxidative damage to DNA during ageing, 8-hydroxy-2′-deoxyguanosine in rat organ DNA and urine. *Proceedings of the National Academy of Sciences, USA*, **87**, 4533–4537.
[23] Y. Kuchino, F. Mori, H. Kasai, S. Inoue, K. Miura, E. Ohtsuka and S. Nishimura (1987) Misreading of DNA templates containing 8-hydroxydeoxyguanosine at the modified base and at adjacent residues. *Nature*, **327**, 77–79.
[24] S. Shibutani, M. Takeshita and A.P. Grollman (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature*, **349**, 431–434.
[25] M.L. Wood, M. Dizdaroglu, E. Gajewski and J.M. Essignmann (1990) Mechanistic studies of ionising radiation and oxidative mutagenesis genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. *Biochemistry*, **29**, 7024–7032.
[26] K.C. Cheng, D.S. Cahill, H. Kasai, S. Nishimura and L.A. Loeb (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G→T and A→C substitutions. *Journal of Biological Chemistry*, **267**, 166–172.
[27] L.A. Lipscomb, M.E. Peek, M.L. Morningstar, S.M. Verghis, E. Miller, A. Rich, J.M. Essigmann and L.D. Williams (1995) X-ray structure of a DNA decamer containing 7,8-dihydro-8-oxoguanine. *Proceedings of the National Academy of Sciences, USA*, **92**, 719–723.
[28] M.L. Wood, A. Esteve, M.L. Morningstar, G.M. Kuziemk and J.M. Essigmann (1992) Genetic effects of oxidative DNA damage, comparative mutagenesis of 7,8-dihydro-8-oxoguanine and 7,8-dihydro-8-oxoadenine in *Escherichia coli*. *Nucleic Acids Research*, **20**, 6023–6032.
[29] A. Sancar (1995) DNA repair in humans. *Ann. Rev. Genetics*, **29**, 69–105.
[30] B. Demple and L. Harrison (1995) Repair of oxidative damage to DNA, enzymology and biology. *Annual Review of Biochemistry*, **63**, 915–948.
[31] H. Czeczot, B. Tudek, B. Lambert, J. Laval and S. Boiteux (1991) *Escherichia coli* Fpg protein and UvrABC endonuclease repair DNA damage induced by methylene blue plus visible light *in vivo* and *in vitro*. *Journal of Bacteriology*, **173**, 3419–3424.
[32] E. Muller, S. Boiteux, R.P. Cunningham and B. Epe (1991) Enzymatic recognition of DNA modifications induced by singlet oxygen and photosensitisers. *Nucleic Acids Research*, **18**, 5969–5973.
[33] S. Boiteux, E. Gajewski, J. Laval and M. Dizdaroglu (1992) Substrate specificity of the *Escherichia coli* Fpg protein (formamidopyrimidine – DNA glycosylase), excision of purine lesions in DNA produced by ionising radiation or photosensitisation. *Biochemistry*, **31**, 106–110.
[34] J. Tchou and A.P. Grollman (1993) Repair of DNA containing the oxidatively-damaged base, 8-oxoguanine. *Mutation Research*, **299**, 277-287.
[35] H.-S. Kim, Y.-W. Park, H. Kasai, S. Nishimura, C.-W. Park, K.-H. Choi and M.-H. Chung (1996) Induction of *E. coli* oh^{8}Gua endonuclease by oxidative stress: its significance in aerobic life. *Mutation Research*, **363**, 115–124.
[36] P.A. van der Kemp, D. Thomas, R. Barbey, R. De Oliveira and S. Boiteux (1996) Cloning and expression in *Escherichia coli* of the Ogg1 gene of *Saccharomyces cerevisiae*, which codes for a DNA glycosylase that excises 7,8-dihydro-8-oxoguanine and 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine. *Proceedings of the National Academy of Sciences, USA*, **93**, 5197–5202.
[37] P.-M. Girard, N. Guibourt and S. Boiteux (1997) The Ogg1 protein of *Saccharomyces cerevisiae*, a 7,8-dihydro-8-oxoguanine DNA glycosylase/AP lyase whose lysine 241 is a critical residue for catalytic activity. *Nucleic Acids Research*, **25**, 3204–3211.
[38] P.-M. Girard, C. D'Ham, J. Cadet and S. Boiteux (1998) Opposite base-dependent excision of 7,8-dihydro-8-oxoadenine by the Ogg1 protein of *Saccharomyces cerevisiae*. *Carcinogenesis*, **19**, 1299–1305.
[39] K. Arai, K. Morishita, K. Shinmura, T. Kohno, S.-R. Kim, T. Nohmi, M. Taniwaki, S. Ohwada and J. Yokota (1997) Cloning of a human homolog of the yeast OGG1 gene that is involved the repair of oxidative DNA damage. *Oncogene*, **14**, 2857–2861.
[40] R. Lu, H.M. Nash and G.L. Verdine (1997) A mammalian DNA repair enzyme that excises oxidatively damage guanines maps to a locus frequently lost in lung cancer. *Current Biology*, **7**, 397–407.
[41] T.A. Rosenquist, D.O. Zharkov and A.P. Grollman (1997) Cloning and characterisation of a mammalian 8-oxyguanine DNA glycosylase. *Proceedings of the National Academy of Sciences, USA*, **94**, 7429–7434.
[42] H. Aburatani, Y. Hippo, T. Takashima, C. Matsuba, T. Kodama, M. Takao, A. Yasui, K. Yamamoto, M. Asano, K. Fukasawa, T. Yoshinari, H. Inoue, E. Ohtsuka and S. Nishimura (1997) Cloning and characterisation of mammalian 8-hydroxyguanine-specific DNA glycosylase/apurinic, apyrimidinic lyase, a functional mutM homologue. *Cancer Research*, **57**, 2151–2156.
[43] M. Tani, K. Shinmura and T. Kohno (1998) Genomic structure and chromosomal localisation of the mouse *Ogg1* gene that is involved in the repair of 8-hydroxyguanine in DNA damage. *Mammalian Genome*, **9**, 32–37.
[44] T. Roldán-Arjona, Y.-F. Wei, K.C. Carter, A. Klungland, C. Anselmino, R.-P. Wang, M. Augustus and T. Lindahl (1997) Molecular cloning and functional expression of a human cDNA encoding for the antimutator enzyme 8-hydroxyguanine-DNA glycosylase. *Proceedings of the National Academy of Sciences, USA*, **94**, 8016–8020.
[45] J.P. Radicella, C. Dherin, C. Desmaze, M.S. Fox and S. Boiteux (1997) Cloning and characterisation of *hOGG1*, a human homolog of the OGG1 gene of *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences, USA*, **94**, 8010–8015.
[46] H.M. Nash, S.D. Bruner, O.D. Schärer, T. Kawate, T.A. Addona, E. Spooner, W.S. Lane and G.L. Verdine (1996) Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision DNA-repair protein superfamily. *Current Biology*, **6**, 968-980.
[47] S.D. Bruner, H.M. Nash, W.S. Lane and G.L. Verdine (1998) Repair of oxidatively damaged guanine in *Saccharomyces*

cerevisiae by an alternative pathway. *Current Biology*, **8**, 393–403.

[48] T.K. Hazra, T. Izumi, L. Maidt, R.A. Floyd and S. Mitra (1998) The presence of two distinct 8-oxoguanine repair enzymes in human cells, their potential complementary role in preventing mutation. *Nucleic Acids Research*, **26**, 5116–5122.

[49] S. Chevillard, J.P. Radicella, C. Levalois, J. Lebeau, M.-F. Poupon, S. Oudard, B. Dutrillaux and S. Boiteux (1998) Mutations in *OGG1*, a gene involved in the repair of oxidative DNA damage, are found in human lung and kidney tumours. *Oncogene*, **16**, 3083–3086.

[50] Y. Tsurudome, T. Hirano, H. Yamato, I. Tanaka, M. Sagai, H. Hirano, N. Nagata, H. Itoh and H. Kasai (1999) Changes in levels of 8-hydroxyguanine in DNA, its repair and *OGG1* mRNA in rat lungs after intratracheal administration of diesel exhaust particles. *Carcinogenesis*, **20**, 1573–1576.

[51] H.-S. Lee, Y.-S. Lee, H.-S. Kim, J.-Y. Choi, H.M. Hassan and M.-H. Chung (1998) Mechanism of regulation of 8-hydroxyguanine endonuclease by oxidative stress: roles of FNR, ArcA and Fur. *Free Radical Biology and Medicine*, **24**, 1193–1201.

[52] T. Bessho, K. Tano, H. Kasai, E. Ohtsuka and S. Nishimura (1993) Evidence for two DNA repair enzymes for 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in human cells. *Journal of Biological Chemistry*, **268**, 19 416–19 421.

[53] T. Bessho, R. Roy, K. Yamamoto, S. Nishimura, K. Tano and S. Mitra (1993) Repair of 8-hydroxyguanine in DNA by mammalian N-methyl purine-DNA glycosylase. *Proceedings of the National Academy of Sciences, USA*, **90**, 8901–8904.

[54] J.-C. Huang, D.S. Hsu, A. Kazantsev and A. Sancar (1994) Substrate spectrum of human excinuclease, repair of abasic sites, methylated bases, mismatches and bulky adducts. *Proceedings of the National Academy of Sciences, USA*, **91**, 12 213–12 217.

[55] G. Dianov, C. Bischoff, J. Piotrowski and V.A. Bohr (1998) Repair pathways for processing of 8-oxoguanine in DNA by mammalian cell extracts. *Journal of Biological Chemistry*, **273**, 33 811–33 816.

[56] A.D. Scott, M. Neishabury, D.H. Jones, S.H. Reed, S. Boiteux and R. Waters (1999) Spontaneous mutation, oxidative DNA damage and the roles of base and nucleotide excision repair in the yeast *Saccharomyces cerevisiae*. *Yeast*, **15**, 205–218.

[57] M. Jaiswal, L.J. Lipinski, V.A. Bohr and S.J. Mazur (1998) Efficient *in vitro* repair of 7-hydro-8-oxodeoxyguanosine by human cell extracts: involvement of multiple pathways. *Nucleic Acids Research*, **26**, 2184–2191.

[58] L.J. Lipinski, N. Hoehr, S.J. Mazur, G.L. Dianov, S. Sentürker, M. Dizdaroglu and V.A. Bohr (1999) Repair of oxidative DNA base lesions induced by fluorescent light is defective in xeroderma pigmentosum group A cells. *Nucleic Acids Research*, **27**, 3153–3158.

[59] A. Sancar and M.-S. Tang (1993) Nucleotide excision repair. *Photochemistry and Photobiology*, **57**, 905–921.

[60] M.H. Chung, H.-S. Kim, E. Ohtsuka, H. Kasai, F. Yamamoto and S. Nishimura (1991) An endonuclease activity in human polymorphonuclear neutrophils that removes 8-hydroxyguanine residues from DNA. *Biochemistry Biophysics Research Communications*, **178**, 1472–1478.

[61] J.T. Reardon, T. Bessho, H.C. Kung, P.H. Bolton and A. Sancar (1997) *In vitro* repair of oxidative DNA damage by human nucleotide excision repair system, possible explanation for neurodegeneration in Xeroderma pigmentosum patients. *Proceedings of the National Academy of Sciences, USA*, **294**, 9463–9468.

[62] M.L. Michaels and J.H. Miller (1992) The GO system protects organisms from the mutagenic effects of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-oxoguanine). *Journal of Bacteriology*, **174**, 6321–6325.

[63] J.C. Huang, D.L. Svoboda, J.T. Reardon and A. Sancar (1992) Human nucleotide excision nuclease removes thymine dimers from DNA incising the 22nd phosphodiester bond 5′ and the 6th phosphodiester bond 3′ to the photodimer. *Proceedings of the National Academy of Sciences, USA*, **89**, 3664–3668.

[64] A.M. Galloway, M. Liuzzi and M.C. Paterson (1994) Metabolic processing of cyclobutyl pyrimidine dimers and (6–4) photoproducts in UV-treated human cells. Evidence for distinct excision-repair pathways. *Journal of Biological Chemistry*, **269**, 974–980.

[65] J. Holmes, S. Clark and P. Modrich (1992) Strand-specific mismatch repair correction in nuclear extracts of human and *Drosophila melanogaster* cell lines. *Proceedings of the National Academy of Sciences, USA*, **87**, 5837–5841, 1992.

[66] J.P. McGoldrick, Y.C. Yeh, M. Solomon, J.M. Essigman and A.-L. Lu (1995) Characterisation of a mammalian homolog of the *Escherichia coli* Mut Y mismatch repair protein. *Molecular Cellular Biology*, **15**, 989–996.

[67] Q.-M. Zhang, N. Ishikawa, T. Nakahra and S. Yonei (1998) *Escherichia coli* Mut Y protein has a guanine-DNA glycosylase that acts on 7,8-dihydro-8,-oxoguanine, guanine mispair to prevent spontaneous GC → CG transversions. *Nucleic Acids Research*, **26**, 4669–4675.

[68] M.M. Slupska, C. Baikalov, W.M. Luther, J.-H. Chiang, Y.-F. Wei and J.H. Miller (1996) Cloning and sequencing a human homolog (hMYH) of the *Eseherichia coli* mut Y gene whose function is required for the repair of oxidative DNA damage. *Journal of Bacteriology*, **178**, 3885–3892.

[69] J.-Y. Mo, H. Maki and M. Sekiguchi (1992) Hydrolytic elimination of a mutagenic nucleotide, 8-oxodGTP, by human 18-kilodalton protein, sanitisation of the nucleotide pool. *Proceedings of the National Academy of Sciences, USA*, **89**, 11 021–11 025.

[70] K. Bialkowski and K.S. Kasprzak (1998) A novel assay of 8-oxo-2′-deoxyguanosine 5′-triphosphate pyrophosphohydrolase (8-oxo-dGTPase) activity in cultured cells and its use for evaluation of cadmium(II) inhibition of this activity. *Nucleic Acids Research*, **26**, 3194–3201.

[71] H. Maki and M. Sekiguchi (1992) Mut T protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. *Nature*, **355**, 273–275.

[72] G. Wani and S.M. D'Ambrosio (1995) Cell type-specific expression of human 8-oxo-7,8-dihydroguanosine triphosphatase in normal breast and skin tissues *in vivo*. *Carcinogenesis*, **16**, 277–283.

[73] M. Kobayashi, Y. Ohara-Nemoto, M. Kaneko, H. Hayakawa, M. Sekiguchi and K. Yamamoto (1998) Potential of *Escherichia coli* GTP cyclohydrolase II for hydrolysing 8-oxo-dGTP, a mutagenic substrate for DNA synthesis. *Journal of Biological Chemistry*, **273**, 26 394–26 399.

[74] H. Hayakawa, A. Taketomi, K. Sakumi, M. Kuwano and M. Sekiguchi (1995) Generation and elimination of 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-triphosphate, a mutagenic substrate for DNA synthesis, in human cells. *Biochemistry*, **34**, 89–95.

[75] R.K. Bestwick, G.L. Moffett and C.K. Mathews (1982) Selective expansion of mitochondrial nucleoside

triphosphate pools in antimetabolite-treated HeLa cells. *Journal of Biological Chemistry*, **257**, 9300–9304.

[76] D. Kang, J.-I. Nishida, A. Iyama, Y. Nakabeppu, M. Furuichi, T. Fujiwara, M. Sekiguchi and K. Takeshige (1995) Intracellular localisation of 8-oxo-dGTPase in human cells, with special reference to the role of mitochondria. *Journal of Biological Chemistry*, **270**, 14 659–14 665.

[77] H. Kamiya and H. Kasai (1995) Formation of 2-hydroxydeoxyadenosine triphosphate, an oxidatively damage nucleotide, and its incorporation by DNA polymerases. *Journal of Biological Chemistry*, **270**, 19 446–19 450.

[78] K. Fujikawa, H. Kamiya and H. Kasai (1998) The mutations induced by oxidatively damaged nucleotides, 5-formyl-dUTP and 5-hydroxyl-dCTP, in *Escherichia coli*. *Nucleic Acids Research*, **26**, 4582–4587.

[79] K. Fujikawa, H. Kamiya, H. Yakushiji, Y. Fujii, Y. Nakabeppu and H. Kasai (1999) The oxidised forms of dATP are substrates for the human MutT homologue, the hMTH1 protein. *Journal of Biological Chemistry*, **274**, 18 201–18 205.

[80] T. Lindhal (1993) Instability and decay of the primary structure of DNA. *Nature*, **362**, 709–715.

[81] M.K. Shigenaga, C.J. Gimeno and B.N. Ames (1989) Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of *in vivo* oxidative DNA damage. *Proceedings of the National Academy of Sciences, USA*, **86**, 9697–9701.

[82] C. Tagesson, M. Källberg and P. Leanderson (1992) Determination of urinary 8-hydroxydeoxyguanosine by coupled-column high-performance liquid chromatography with electrochemical detection, a noninvasive assay for *in vivo* oxidative DNA damage in humans. *Toxicological Methods*, **1**, 242–251.

[83] S. Loft, K. Vistisen, M. Ewertz, A. Tjonneland, K. Overvad and H.E. Poulsen (1992) Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans, influence of smoking, gender and body mass index. *Carcinogenesis*, **13**, 2241–2247.

[84] M.B. Bogdanov, M.F. Beal, D.R. McCabe, R.M. Griffin and W. Matson (1999) A carbon column based LCEC approach to routine 8-hydroxy-2′-deoxyguanosine measurements in urine and other biological matrices. *Free Radical Biology and Medicine*, **27**, 647–666.

[85] P. Degan, M.K. Shigenaga, E.-M. Park, P.E. Alperin and B.N. Ames (1991) Immunoaffinity isolation of urinary 8-hydroxy-2′-deoxyguanosine and 8-hydroxyguanine and quantitation of 8-hydroxy-2′-deoxyguanosine in DNA by polyclonal antibodies. *Carcinogenesis*, **12**, 865–871.

[86] E.M. Park, M.K. Shigenaga, P. Degan, T.S. Korn, J.W. Kitzler, C.M. Weher, P. Kolachana and B.N. Ames (1992) Assay of excised oxidative DNA lesions, isolation of 8-oxoguanine and its nucleoside derivatives from biological fluids with a monoclonal antibody column. *Proceedings of the National Academy of Sciences, USA*, **89**, 3375–3379.

[87] M. Erhola, S. Toyokuni, K. Okada, T. Tanaka, H. Hiai, H. Ochi, K. Uchida, T. Osawa, M.M. Neiminen, H. Alho and P. Kellokumpu-Lehtinen (1997) Biomarker evidence of DNA oxidation in lung cancer patients, association of urinary 8-hydroxy-2′-deoxyguanosine excretion with radiotherapy, chemotherapy and response to treatment. *FEBS Letters*, **409**, 287–291.

[88] M.D. Evans, M.S. Cooke, I.D. Podmore, Q. Zheng, K.E. Herbert and J. Lunec (1999) Discrepancies in the measurement of UVC-induced 8-oxo-2′deoxyguanosine, implications for the analysis of oxidative DNA damage. *Biochemical Biophysical Research Communications*, **259**, 374–378.

[89] A.J.R. Teixeira, M.R. Ferreira, W.J. van Dijk, G. van de Werken and A.P.J.M. de Jong (1995) Analysis of 8-hydroxy-2′-deoxyguanosine in rat urine and liver DNA by stable isotope-dilution gas chromatography/mass spectrometry. *Analytical Biochemistry*, **226**, 307–319.

[90] I. Holmberg, P. Stål and M. Hamberg (1999) Quantitative determination of 8-hydroxydeoxyguanosine in human urine by isotope dilution mass spectrometry, normal levels in hemochromatosis. *Free Radical Biology and Medicine*, **26**, 129–135.

[91] J.-L. Ravanat, P. Guicherd, Z. Tuce and J. Cadet (1999) Simultaneous determination of five oxidative DNA lesions in human urine. *Chemical Research in Toxicology*, **12**, 802–808.

[92] J.-L. Ravanat, B. Duretz, A. Guiller, T. Douki and J. Cadet (1998) Isotope dilution high-performance liquid chromatography-electrospray tandem mass spectrometry assay for the measurement of 8-oxo-7,8-dihydro-2′-deoxyguanosine in biological samples. *Journal of Chromatography*, **715**, 349–356.

[93] P.V. Hariharan and P.A. Cerutti (1974) Excision of damaged thymine residues from gamma-irradiated poly(dA-dT) by crude extracts of *Escherichia coli*. *Proceedings of the National Academy of Sciences, USA*, **71**, 3532–3536.

[94] D.S. Bergtold, M.G. Simic, H. Alessio and R.G. Cutler (1988) Urine biomarkers for oxidative DNA damage. In *Oxygen Radicals in Biology and Medicine* (Eds. M G. Simic, K. A. Taylor, J. F. Ward and C. Von Sonntag), New York, Plenum, pp. 483–490.

[95] R. Cathcart, E. Schwiers, R.L. Saul and B.N. Ames (1984) Thymine glycol and thymidine glycol in human and rat urine. A possible assay for oxidative DNA damage. *Proceedings of the National Academy of Sciences, USA*, **81**, 5633–5637.

[96] S. Lagorio, C. Tagesson, F. Forastiere, I. Iavarone, O. Axelson and A. Carere (1984) Exposure to benzene and urinary concentrations of 8-hydroxydeoxyguanosine, a biological marker of oxidative damage to DNA. *Occupational and Environmental Medicine*, **51**, 739–743.

[97] T. Yamamoto, K.-I. Hosokawa, T. Tamura, H. Kanno, M. Urabe and H. Honjo (1996) Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels in women with or without gynaecologic cancer. *Journal of Obstetric and Gynaecological Research*, **22**, 359–363.

[98] M.S. Cooke, M.D. Evans, I.D. Podmore, K.E. Herbert, N. Mistry, P. Mistry, P.T. Hickenbotham, A. Hussieni, H.R. Griffiths and J. Lunec (1998) Novel repair action of vitamin C upon *in vivo* oxidative DNA damage. *FEBS Letters*, **363**, 363–367.

[99] D. Germadnik, A. Pilger and H.W. Rüdiger (1997) Assay for the determination of urinary 8-hydroxy-2′-deoxyguanosine by high-performance liquid chromatography with electrochemical detection. *Journal of Chromatography B*, **689**, 399–403.

[100] C. Tagesson, M. Källberg and G. Wingren (1996) Urinary malondialdehyde and 8-hydroxydeoxyguanosine as potential markers of oxidative stress in industrial art glass workers. *International Archives of Occupational and Environmental Health*, **69**, 5–13.

[101] H.E. Poulsen, S. Loft, H. Prieme, K. Vistisen, J. Lykkesfeldt, K. Nyyssonen and J.T. Salonen (1998) Oxidative DNA damage *in vivo*, relationship to age, plasma antioxidants, drug metabolism, glutathione-S-transferase activity and urinary creatinine excretion. *Free Radical Research*, **29**, 565–571.

[102] S. Toyokuni, T. Tanaka, Y. Hattori, Y. Nishiyama, A. Yoshida, K. Uchida, H. Hiai, H. Ochi and T. Osawa (1997) Quantitative immunohistochemical determination of 8-hydroxy-2′-deoxyguanosine by a monoclonal antibody N45. 1, its application to ferric nitrilotriacetate-induced renal carcinogenesis model. *Laboratory Investigation*, **76**, 365–374.

[103] N.U. Ahmed, M. Ueda, O. Nikaido, T. Osawa and M. Ichihashi (1999) High levels of 8-hydroxy-2′-deoxyguanosine appear in normal human epidermis after a single dose of ultraviolet radiation. *British Journal of Dermatology*, **140**, 226–231.

[104] H. Tsuboi, K. Kouda, H. Takeuchi, M. Takigawa, Y. Masamoto, M. Takeuchi and H. Ochi (1998) 8-Hydroxydeoxyguanosine in urine as an index of oxidative damage to DNA in the evaluation of atopic dermatitis. *British Journal of Dermatology*, **138**, 1033–1035.

[105] S. Takahashi, M. Hirose, S. Tamano, M. Ozaki, S.-I. Orita, T. Ito, M. Takeuchi, H. Ochi, S. Fukada, H. Kasai and T. Shirai (1998) Immunohistochemical detection of 8-hydroxy-2′-deoxyguanosine in paraffin-embedded sections of rat liver after carbon tetrachloride treatment. *Toxicological Pathology*, **26**, 247–252.

[106] H.L. Witherall, R.A. Hiatt, M. Replogle and J. Parsonnet (1998) *Helicobacter pylori* and urinary excretion of 8-hydroxy-2-deoxyguanosine, an oxidative DNA adduct. *Cancer Epidemiology Biomarkers and prevention*, **7**, 91–96.

[107] J. Leinonen, T. Lehtimäki, S. Toyokuni, K. Okada, T. Tanaka, H. Hiai, H. Ochi, P. Laippala, V. Rantalaiho, O. Wirta, A. Pasternack and H. Alho (1997) New biomarker evidence of oxidative DNA damage in patients with non-insulin diabetes mellitus. *FEBS Letters*, **417**, 150–152.

[108] S.S. Kantha, S.-I. Wada, H. Tanaka, M. Takeuchi, S. Watabe and H. Ochi (1996) Carnosine sustains the retention of cell morphology in continuous fibroblast culture subjected to nutritional insult. *Biochemical and Biophysical Research Communications*, **223**, 278–282.

[109] S. Loft and H.E. Poulsen (1996) Cancer risk and oxidative DNA damage in man. *Journal of Molecular Medicine*, **74**, 297–312.

[110] J. Zhang, G. Perry, M.A. Smith, D. Robertson, S.J. Olson, D.G. Graham and T.J. Montine (1999) Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in *Substantia Nigra* neurons. *American Journal of Pathology*, **154**, 1423–1429.

[111] A. Nunomura, G. Perry, M.A. Pappolla, R. Wade, K. Hirai, S. Chiba and M.A. Smith (1999) RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. *Journal of Neuroscience*, **19**, 1959–1964.

[112] E.H. Witt, A.Z. Reznick, C.A. Viguie, P. Starke-Reed and L. Packer (1992) Exercise, oxidative damage and effects of antioxidant manipulation. *Journal of Nutrition*, **122**, 766–773.

[113] C.W. Gehrke, K.C. Kuo, T.P. Waalkes and E. Borek (1979) Patterns of urinary excretion of modified nucleosides. *Cancer Research*, **39**, 1150–1153.

[114] G. Schoch, G. Heller-Schoch, J. Muller, M. Heddrich and R. Gruttner (1982) Determination of RNA metabolism as an indicator of nutritional status. *Klinische Padiatrie*, **194**, 317–319.

[115] K.C. Cundy, R. Kohen and B.N. Ames (1989) Determination of 8-hydroxydeoxyguanosine in human urine. A possible assay for *in vivo* oxidative DNA damage. In *Oxygen Radicals in Biology and Medicine* (Eds. M.G. Simic, K.A. Taylor, J.F. Ward and C. Von Sonntag), New York, Plenum, pp. 479–482.

[116] M.G. Simic (1992) Urinary biomarkers and the rate of DNA damage in carcinogenesis and anticarcinogenesis. *Mutation Research*, **267**, 277–290.

[117] S. Loft, A. Fischer-Nielsen, I.B. Jeding, K. Vistisen and H.E. Poulsen (1993) 8-Hydroxydeoxyguanosine as a urinary biomarker of oxidative DNA damage. *Journal of Toxicology and Environmental Health*, **40**, 391–404.

[118] M. Roth, L.R. Emmons, M. Haner, H.J. Muller and J.M. Boyle (1989) Age-related decrease in an early step of DNA-repair of normal human lymphocytes exposed to ultraviolet-irradiation. *Experimental Cell Research*, **180**, 171–177.

[119] P. Sangeetha, U.N. Das, R. Koratkar and P. Suryaprabha (1990) Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. *Free Radical Biology and Medicine*, **8**, 15–19.

[120] R.K. Brown, A. McBurney, J. Lunec and F.J. Kelly (1995) Oxidative damage to DNA in patients with cystic fibrosis. *Free Radical Biology and Medicine*, **18**, 801–806.

[121] K. Okamura, T. Doi, M. Sakurai, K. Hamada, Y. Yoshioka, S. Sumida and Y. Sugawa-Katayama (1997) Effect of endurance exercise on the tissue 8-hydroxydeoxyguanosine content in dogs. *Free Radical Research*, **26**, 523–528.

[122] B.N. Ames, L.S. Gold and W.C. Willett (1995) The causes and prevention of cancer. *Proceedings of the National Academy of Sciences, USA*, **92**, 5258–5265.

[123] H.L. Poulsen and S. Loft (1998) Role of oxidative DNA damage in cancer initiation and promotion. *European Journal of Cancer Prevention*, **7**, 9–16.

[124] G. Block (1991) Vitamin C and cancer prevention, the epidemiologic evidence. *American Journal of Clinical Nutrition*, **53**, 270S–282S.

[125] B.N. Ames, M.K. Shigenaga and T.M. Hagen (1993) Oxidants, antioxidants, and the degenerative diseases of ageing. *Proceedings of the National Academy of Sciences, USA*, **90**, 7915–7922.

[126] H. Priemé, S. Loft, S. Nyyssönen, J.T. Salonen and H.E. Poulsen (1997) No effect of supplementation with vitamin E, ascorbic acid, or coenzyme Q10 on oxidative DNA damage estimated by 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion in smokers. *American Journal of Clinical Nutrition*, **65**, 503–507.

[127] H. Daube, G. Scherer, K. Riedel, T. Ruppert, A.R. Tricker, P. Rosenbaum and F. Adlkofer (1997) DNA adducts in human placenta in relation to tobacco smoke exposure and plasma antioxidant status. *Journal of Cancer Research and Clinical Oncology*, **123**, 141–151.

[128] M. Fenech, I. Dreosti and C. Aitken (1997) Vitamin E supplements and their effect on vitamin E status in blood and genetic damage rate in peripheral blood lymphocytes. *Carcinogenesis*, **18**, 359–364.

[129] C.G. Fraga, P.A. Motchnik, M.K. Shigenaga, H.J. Helbock, R.A. Jacob and B.N. Ames (1991) Ascorbic acid protects against endogenous oxidative DNA

damage in human sperm. *Proceedings of the National Academy of Sciences, USA*, **88**, 11 003–11 006.

[130] S.J. Duthie, A. Ma, M.A. Ross and A.R. Collins (1996) Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Research*, **56**, 1291–1295.

[131] D. Zhang, S. Okada, Y. Yu, P. Zheng, R. Yamaguchi and H. Kasai (1997) Vitamin E inhibits apoptosis, DNA modification and cancer incidence by iron mediated peroxidation in Wistar rat kidney. *Cancer Research*, **57**, 2410–2414.

[132] I.D. Podmore, H.R. Griffiths, K.E. Herbert, N. Mistry, P. Mistry and J. Lunec, (1998) Vitamin C exhibits prooxidant properties. *Nature*, **395**, 231–232; I.D. Podmore, H.R. Griffiths, K.E. Herbert, N. Mistry, P. Mistry and J. Lunec (1998) Does vitamin C have a pro-oxidant effect? *Nature*, **395**, 231–232.

[133] M.G.L. Hertog, A. Vries, A. Ocké, A. Schouten, H. Bas Bueno-de-Mesquita and H. Vehagen (1997) Oxidative DNA damage in humans, comparison between high and low habitual fruit and vegetable consumption. *Biomarkers*, **2**, 259–262.

[134] A. Rehman, C.S. Collis, M. Yang, M. Kelly, A.T. Diplock, B. Halliwell and C. Rice-Evans (1998) The effects of iron and vitamin C co-supplementation on oxidative damage to DNA in healthy volunteers. *Biochemical Biophysical Research Communications*, **246**, 293–298.

[135] H. Verhagen, H.E. Poulsen, S. Loft, G. van Poppel, M.I Willems and P.J. van Bladeren (1995) Reduction in oxidative DNA-damage in humans by Brussels sprouts. *Carcinogenesis*, **16**, 969–970.

[136] X.-S. Deng, J. Tuo, H.E. Poulsen and S. Loft (1998) Prevention of oxidative DNA damage in rats by Brussels sprouts. *Free Radical Research*, **28**, 323–333.

[137] J.N. Stolk, D.G.M. de Koning, A.H. Pennings, R.A. De Abreu, L.B.A. nav der Putte and A.M.T. Boerbooms (1999) Reduced purine 5′-nucleotidase activity in lymphocytes of patients with systemic lupus erythematosus, results of a pilot study. *Annals of Rheumatic Diseases*, **58**, 122–125.