Oxidative Stress-Induced Carcinogenesis and Its Prevention
Guest Editor: Shinya Toyokuni

Sources of Extracellular, Oxidatively-Modified DNA Lesions: Implications for Their Measurement in Urine

Marcus S. Cooke[1,2,*], Paul T. Henderson[3], and Mark D. Evans[1]

[1]*Radiation and Oxidative Stress Section, Department of Cancer Studies and Molecular Medicine, Robert Kilpatrick Clinical Sciences Bilding, University of Leicester, LE2 7LX, UK*
[2]*Department of Genetics, Robert Kilpatrick Clinical Sciences Building, University of Leicester, Leicester, LE2 7LX, UK*
[3]*Department of Internal Medicine, University of California Davis Medical Center, Sacramento, CA, USA*

Received 23 April, 2009; Accepted 29 April, 2009

Summary **There is a robust mechanistic basis for the role of oxidation damage to DNA in the aetiology of various major diseases (cardiovascular, neurodegenerative, cancer). Robust, validated biomarkers are needed to measure oxidative damage in the context of molecular epidemiology, to clarify risks associated with oxidative stress, to improve our understanding of its role in health and disease and to test intervention strategies to ameliorate it. Of the urinary biomarkers for DNA oxidation, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) is the most studied. However, there are a number of factors which hamper our complete understanding of what meausrement of this lesion in urine actually represents. DNA repair is thought to be a major contributor to urinary 8-oxodG levels, although the precise pathway(s) has not been proven, plus possible contribution from cell turnover and diet are possible confounders. Most recently, evidence has arisen which suggests that nucleotide salvage of 8-oxodG and 8-oxoGua can contribute substantially to 8-oxoG levels in DNA and RNA, at least in rapidly dividing cells. This new observation may add an further confounder to the conclusion that 8-oxoGua or 8-oxodG, and its nucleobase equivalent 8-oxoguanine, concentrations in urine are simply a consequence of DNA repair. Further studies are required to define the relative contributions of metabolism, disease and diet to oxidised nucleic acids and their metabolites in urine in order to develop urinalyis as a better tool for understanding human disease.**

Key Words**: DNA damage, urine, oxidative stress, DNA repair, cell death**

Introduction

Normal cellular metabolism results in the continual production of reactive oxygen species (ROS). However, exposure to xenobiotics, ionising and nonionsing radiation etc. may lead to an increased production that overwhelms the antioxidant defences, leading to a condition of oxidative stress. Even normal levels of ROS production leads to modification of cellular biomolecules, such as DNA, lipids and proteins, for the most part, without detrimental effects upon the cell. However, under oxidative stress, levels of modification increase and this may have implications for cellular function.

*To whom correspondence should be addressed.
Tel: +44(0)116 2525825 Fax: +44 (0)116 2525832
E-mail: msc5@le.ac.uk

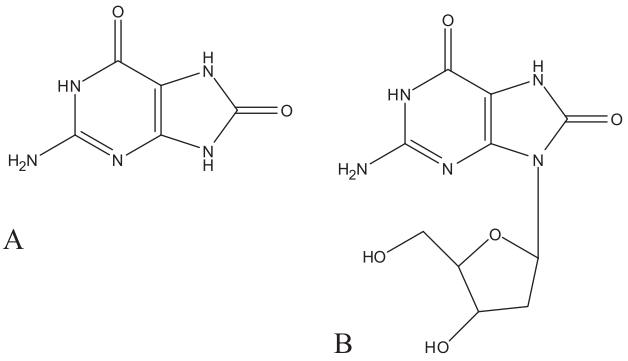

Fig. 1. Structures of (A) 8-oxo-7,8-dihydroguanine (8-oxoGua), and (B) 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG).

Perhaps most studied, is oxidative modification of DNA, and whilst the principle consequence of this might be mutations, it should not be forgotten that ROS-induced damage to DNA may have non-mutational effects, such as the acceleration of telomere shortening [*1*]. Over 30 nucleobase modifications have been described, of which 8-oxo-7,8-dihydroguanine (8-oxoGua, and its corresponding deoxyribonucleoside equivalent, 8-oxo-7,8-dihydro-2'-deoxyguanosine, 8-oxodG; Fig. 1), have received the greatest amount of attention [*2*], although the total number of DNA lesions (including sugar and phosphate modifications, and combinations thereof) is approaching 70 [*3*]. DNA may also be modified via reactive intermediates, arising from the interaction of ROS with lipids and proteins, leading to adducts such as 1,N^6-etheno-2'-deoxyadenosine (εdA), 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrimido[1,2-α]purin-10(3*H*)-one (M_1dG) and DNA-protein cross-links [*4*].

There is a rapidly growing body evidence which suggests that this plethora of lesions, arising from oxidative stress, may have an important role in the aetiology and/or pathogenesis of many diseases, such as cancer and aging (reviewed in Cooke *et al.* [*5*]). To fully understand the extent to which such DNA lesions are involved in disease, in order to develop diagnostic/prognostic methods and intervention strategies, methods for their analysis are essential. A wide variety of approaches have been employed to study oxidatively damaged DNA. However, findings from the European Standards Committee on Oxidative DNA Damage (ESCODD [*6*]), have suggested that a number of these techniques suffer from artefactual oxidation during sample workup and analysis (reviewed by Guetens *et al.* [*7*]). Of relevance to all the above techniques is the possibility of adventitious oxidation during sample storage and, with the exception of the cell-based methods, DNA extraction, although methods have been developed to minimise this risk [*8*].

Assessment of damage to DNA by methods requiring invasive procedures, e.g. blood samples or tissue biopsy, imposes severe limitations in large-scale human studies, requiring staff with specialist training, greater ethical scrutiny, and reducing the likelihood of consent. In constrast, examining the products of oxidatively generated damage to DNA in extracellular matrices offers a means by which oxidative stress may be assessed non-, or minimally, invasively, and circumvents DNA extraction and associated risk of artefact.

Methods of Analysis

Broadly, methods that have been applied to the study of oxidatively damaged DNA lesions in urine are either chromatographic [principally, HPLC-MS/MS; liquid chromatography pre-purification prior to GC-MS (i.e. HPLC-GC/MS), HPLC-EC, GC-MS], or immunoassay. Whilst a variety of lesions have been reported to be present in urine (Table 1), the majority of assays focus upon 8-oxodG as the analyte of choice, in part, for reasons that will be discussed later.

Chromatographic techniques

Whilst thymine (and thymidine) glycol was one of the first ROS-induced DNA lesions to be studied in urine [*9*, *10*], this was rapidly superceded by 8-oxodG, given the one thousand-fold greater sensitivity of 8-oxodG detection using HPLC-EC, compared to the UV detection of Tg, along with the predominance of 8-oxodG over Tg, in human urine [*11*]. A number of methods based upon column-switching HPLC with regular reverse phase (usually C18) separative columns and EC-detection for assay of 8-oxodG have been developed [*6*, *12–14*]. An approach shown to be successful in a wide variety of biological matrices (including DNA, urine, plasma, red blood cell extracts, cerebrospinal fluid, saliva, sweat, kidney dialysate, brain and muscle microdialysate, food (rat, mouse, monkey and human chow), *Caenorhabitbditis elegans*, cell culture medium and rat faeces), utilises an HPLC column comprising 'treated' carbon material, similar to that used for EC detector cells, together with Coularray, multichannel electrochemical detection [*15*].

At its simplest, column switching has meant that, following chromatographic separation of the urine's constituents, only the fraction containing the compound of interest (e.g. 8-oxodG) is applied to the final separation column and detector, either EC [*16*, *17*] or MS [*18*]; the remainder is diverted to waste. Benefits of mass spectrometry include: the use of isotopically-labelled internal standards, simplifying quantification and accounting for loss during sample workup (and potentially storage), differences in ionisation efficiencies due to matrix effects, and confirmation of analyte identity. The HPLC-MS/MS assay described by Weimann *et al.* [*18*] was the first report for the simultaneous analysis of the oxidised (8-oxoGua, 8-oxoguanosine and 8-oxodG) and native (Gua, guanosine and dG) moieties. Similarly, the

Table 1. DNA-derived markers of oxidative stress examined[1)] in human urine.

Nucleobase	Modification	Abbreviation
Gua	8-oxo-7,8-dihydroguanine	8-oxoGua
	8-oxo-7,8-dihydro-2'-deoxyguanosine	8-oxodG
	8-oxo-7,8-dihydroguanosine	8-oxoG
	Pyrimido[1,2-a]purin-10(3*H*)-one	M_1Gua
	3-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrimido[1,2-α]purin-10(3*H*)-one	M_1dG
	1, N^2-ethenoguanine	1, N^2-εGua
	N^2-3-ethenoguanine	N^2-3-εGua
Ade	8-oxo-7,8-dihydroadenine	8-oxoAde
	8-oxo-7,8-dihydro-2'-deoxyadenosine	8-oxodA
	1,N^6-etheno-2'-deoxyadenosine	εdA
Thy	Thymine glycol	Tg
	Thymidine glycol	dTg
	5-(hydroxymethyl)uracil	5-HMUra
	5-hydroxymethyl-2'-deoxyuridine	5-HMdUrd
	5-hydroxyuracil	5-OHUra
Cyt	3,N^4-ethenocytosine	εCyt
	3,N^4-etheno-2'-deoxycytidine	εdC

[1)] Examined, but not necessarily detected.

HPLC-GC/MS assay of Olinski's laboratory entails HPLC pre-purification of the compounds of interest, which again includes native compounds; 8-oxoGua, 8-oxodG, Gua, dG and 5-(hydroxymethyl)uracil (5-HMUra), [*19*, *20*], prior to derivatisation and GC-MS. Other urinary, oxidatively modified DNA (2'-deoxy)nucleobase products measured in urine include 5-hydroxyuracil (5-OHUra), 8-oxo-7,8-dihydroadenine (8-oxoAde) [*21*], 5-hydroxymethyl-2'-deoxyuridine (5-OHmUrd) [*22*], M_1dG [*23*], εdA [*24*] and 3,N^4-etheno-2'-deoxycytidine (εdC) [*25*].

Solid phase extraction (SPE) has been used for the analysis of urinary 8-oxodG by GC-MS [*26*] and HPLC-MS/MS, the latter of which minimised the incidence of interfering peaks reported by Lin *et al.* [*26*] in 10–20% of their urine samples [*27*]. The analysis of urinary 8-oxodA has been attempted previously [*28*], but levels were reported to be at, or below, the limit of detection for the HPLC-MS/MS assay (0.3 nM, 7.5 fmol injected, S/N = 3). A similar finding was noted with an SPE-based HPLC-MS/MS assay [*29*].

Whilst not as prevalent as methods for analysing primary DNA products of oxidation in urine, the analysis of secondary DNA products of oxidation reactions in urine is a rapidly growing area of interest. Several methods have been developed for the measurement of lipid peroxidation-induced etheno-DNA adducts in human urine. For example, HPLC-fluorescence detection of εdA [*30*]; isotope dilution-gas chromatography-negative ion chemical ionization/mass spectrometry methodology for 3,N^4-ethenocytosine (εCyt) and 3,N^4-ethenodeoxycytidine (εdC) [*31*, *32*]; HPLC MS/MS analysis of urinary εdA [*24*] and εdC [*33*] and a ^{32}P-postlabeling method has been reported for εdC in human urine [*25*].

Although not presently used for urinary analysis of DNA oxidation products, accelerator mass spectrometry (AMS) is an emerging technology for measuring radiocarbon-labeled nucleotides and nucleosides [*34–38*]. For radiocarbon, AMS has attomole sensitivity for DNA samples as small as a microgram. This technology is currently used with HPLC to measure pharmacokinetics and metabolism of xenobiotics [*39–42*] and, more recently, 8-oxodG metabolism [*36*].

Immunoassay

Competitive enzyme-linked immunosorbent assay (ELISA) has received widespread, and growing, use for the analysis of lesions in extracellular matrices. Predominantly, 8-oxodG has been the lesion of choice for commercially available (e.g. from the Japanese Institute for the Control of Aging, JaICA), and custom made [*43*] ELISAs. The benefits of ELISA are (i) ease of use; (ii) no specialist (or indeed expensive) equipment is required; (iii) potential application to numerous extracellular matrices (serum [*44*], plasma [*45*], saliva [*46*, *47*], urine [*48*], CSF [*49*, *50*], cell culture medium [*51–53*] and sputum [*54*]; (iv) other than centrifugation of cloudy specimens, no pre-treatment of urine is required (v) high throughput. The kit from JaICA has, by far, received the most published use.

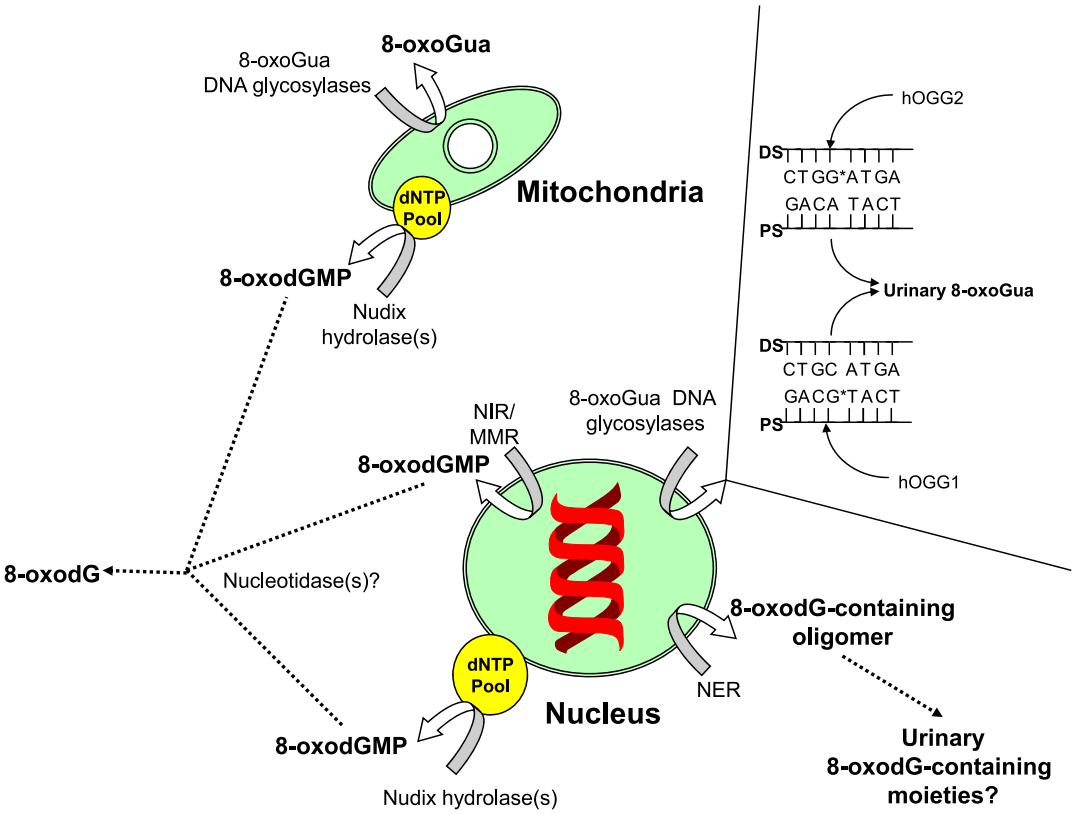

Fig. 2. DNA repair sources of extracellular 8-oxodG and 8-oxoGua. NER, nucleotide excision repair; hOGG1 and hOGG2, human 8-oxoguanine glycosylase (1 and 2); NIR, nucleotide incision repair; MMR, mis-match repair.

Sources of Extracellular, Oxidatively-Modified DNA Lesions

The possible sources of extracellular, oxidatively-modified, DNA lesions are: (i) DNA repair, (ii) diet, (iii) cell death/turnover, mitochondrial turnover and, most recently added to this list, (iv) cellular uptake and re-utilisation of damage products.

DNA repair

The DNA N-glycosylases responsible for removing modified bases are increasingly well defined [*2*, *55*]. The prevailing thought is that quantitatively, BER is by far the most important route for the removal of the majority of oxidatively-derived lesions. At its simplest, this process involves a specific enzyme, with a defined repertoire of substrate, removing the modified base, leaving an apurinic-apyrimidinic (AP) site. The subsequent fate of this AP site is proposed to be dependent upon whether the glycosylase also possesses an AP lyase activity, and may involve simply the removal of one nucleotide (short patch repair), or 2–6 nucleotides (long patch repair), followed by gap filling and ligation [*56*].

The glycosylase considered to have the primary activity in removing 8-oxoGua in human cells, is the human 8-oxoGua glycosylase 1 (hOGG1 [*57*, *58*]). This enzyme acts via short patch repair, and has a specificity for 8-oxoGua:Cyt pairs present in double-stranded DNA, i.e. formed in situ (Fig. 2). The activity of hOGG1 is complemented by another enzyme, denoted hOGG2, which appears to have similarities to prokaryotic endonuclease VII (Nei) enzymes. It has been reported that hOGG2, removes the 8-oxoGua from the nascent strand in 8-oxoGua:Ade or 8-oxoGua:Gua pairs, arising from misincorporation of 8-OH-dGTP (REF 50). Subsequent studies of eukaryotic NEI-like glycosylases (NEIL) suggest that while these enzymes, NEIL1 and NEIL2, can act on single stranded DNA and thus may have functions in DNA replication/transcription, OGG2 is possibly distinct from NEIL1 and NEIL2 on the basis of size and/or substrate specificity. However, it still would appaear that neither the protein nor the gene for OGG2 has been squenced, or formally identified as an existing, characterised, enzyme. Certainly 8-oxoGua appears to be a substrate for glycosylases other than OGG1 (NEIL1, NEIL2, NTH1) in various contexts, for example in single-stranded DNA, proximal to a 3'-end, or mispaired with Gua [*59*, *60*] (Fig. 2).

The contribution of OGG1 enzyme to levels of urinary 8-oxoGua has been examined in *OGG1* –/– mice, which

showed a 26% reduction in the deficient strain, compared to wild type, indicating that, in mice at least, OGG1 is a significant, but by no means unique, source of urinary 8-oxoGua [*61*]. The results clearly support the existence of back up DNA glycosylase(s), such as the Nei-like glycosylases [*62*], but which cannot entirely compensate for OGG1 deficiency.

In contrast, the presence of 2'-deoxyribonucleoside lesions in extracellular matrices is less well-defined, not least as there are no reports of a single DNA repair enzyme whose activity yields 8-oxodG. Based upon existing evidence, we suggest the following DNA repair routes (Fig. 2) as likely contributors to the presence of oxidatively-modified 2'-deoxyribonucleosides in urine:

Nudix hydrolases. Free deoxynucleotides have a greater propensity for oxidation compared to base-paired deoxynucleotides in DNA [*63*]. Furthermore, mitochondrial sources of dNTPs are larger than nuclear sources (*mitochondria*: 18.0 pmol dGTP/μg DNA versus *nuclear*: 8.0 pmol dGTP/μg DNA [*64*]). Taken together, these would suggest an imperative for preventing modified DNA precursors being incorporated into the genome. The best characterised enzyme which performs such a role is the 8-oxo-2'-deoxyguanosine triphosphatase (8-oxodGTPase [*65*]) activity of NUDT1 (aka MutT homologue, MTH1), hydrolysing 8-oxodGTP to 8-oxodGMP (Fig. 2). It has been suggested that further processing, perhaps by 5'(3')-nucleotidases, may give rise to 8-oxodG [*65*], which can be removed from the cell to ultimately appear in the urine. Recent data from the group of Harms-Ringdahl strongly imply that NUDT1 activity, and thus the nucleotide pool, represents a major source of extracellular 8-oxodG [*66*]. However, the roles of other Nudix hydrolases such as NUDT15 (MTH2) and NUDT5, which include 8-oxodGTP and 8-oxodGDP amongst their substrate repertoire respectively, remain to be defined [*67*, *68*].

Nucleotide excision repair (NER). Principally, NER removes bulky lesions, such as cyclobutane thymine dimers (T<>T), although there is there is some evidence for activity towards 8-oxodG (Fig. 2) [*69*]. Indeed, the rate of 8-oxoGua removal appears comparable to that for T<>T [69], the implication being that a lesion-containing oligomer, approximately 24–32 nucleotides long is then produced [*70*]. Galloway *et al.* [*71*] showed that, post-excision, T<>T-containing oligomers are subsequently degraded to lesion-containing 7-mers. What then happens to the oligomers is unknown, although, from the findings of Le Curieux and Hemminki [*72*], who detected the presence of urinary T<>T as a monophosphate nucleotide dimer, it may be implied that further processing does occur, the nature of which remains to be described. The situation for 8-oxodG is even less clear as a recent report failed to demonstrate 8-oxoGua-containing oligomers in urine [*73*] implying that further processing occurs, perhaps ultimately yielding 8-oxodG, or that they do not exist. However, under normal circumstances, when BER and NER are allowed to compete, NER appears to have a negligible role in the removal of 8-oxoGua [*74–78*]. Eliminating Ogg1 activity (*Ogg1*$^{-/-}$ mice) while reducing 8-oxoGua excretion by 26%, has no impact on the excretion of 8-oxodG compared to wild-type animals [*61*]. This perhaps suggests that other BER activities compensate for lost OGG1 activity, or if NER is contributing, it is either minimal even under these circumstances or 8-oxoGua-containing oligomers are not subject to further degradation. Results from experiments with XP cell lines have not entirely excluded the possibility of a role for NER in the repair of 8-oxoGua, however some XP proteins are also thought to play a role in BER, through modulating OGG1 activity, for example [*69*, *75*, *79–81*].

Nucleotide Incision Repair (NIR). This is a more recently described DNA repair pathway whose lesion repertoire is more oriented towards oxidised pyrimidines, rather than purines. However, dependent on the structural context, 8-oxoGua can be a substrate, when 8-oxo-Gua is present at the 3'-end of a DNA strand break, such a situation could occur from mis-incorporation of 8-oxodGMP during DNA synthesis or damage induced by ionising radiation, in this context 8-oxoGua is reistant to excision by OGG1. The 3'-5' exonuclease activity of Apn1, in *S. cerevisiae*, and APE-1, in humans, has been demostrated to fulfill this NIR activity towards 8-oxoGua in such a context [*82*, *83*]. An important issue with this pathway is that the initial product of NIR is a lesion-containing 2'-deoxyribonucleoside 3'-monophosphate, which could then be a substrate for a 5'(3')-nucleotidase activity, to yield 8-oxodG.

Mismatch Repair (MMR). The recognition of mis-incorporated 8-oxoGua either opposite G or A can render the lesion a substrate for MMR [*84*, *85*]. Recognition of the mispair by MutSα and MutLα heterodimeric complexes ultimately invokes a long-patch repair process in which exonuclease I, targeted to the nascent strand, sequentially removes 2'-deoxynucleotide monophosphates in the 5'-3' direction, again gnerating a lesion-containing that could be further degraded 8-oxodG by a 5'(3')-nucleotidase activity.

Endonuclease(s). A poorly characterised endonuclease has been reported by Bessho *et al.* [*86*] which, lacking a glycosylase activity, is predicted to give rise to 3',5'-8-oxodGDP as the putative product. We have previously proposed that this may be subsequently hydrolysed to 8-oxodG by nucleotidase(s).

As noted earlier, in addition to 8-oxodG, the following modified 2'-deoxyribonucleosides have been identified in

urine: dTg, 5-OHmUrd, M_1dG, εdA, and εdC, and their origins are even less clear.

Potential confounders

Diet. The majority of reports measuring oxidatively modified DNA lesions in urine, have focussed upon 2'-deoxyribonucleoside lesions, and 8-oxodG specifically. The reason for this is because early work in the field demonstrated that diet could affect levels of urinary 8-oxoGua, but not 8-oxodG. Whilst it was concluded that Tg was unaffected by diet, the raw data would suggest otherwise: gastric intubation of rats demonstrated that 44% of ingested [^{3}H]-Tg was recovered intact in the urine within 24 h ([*87*] and discussed in detail by Cooke *et al.* [*88*]). It is worth noting that the results from the HPLC-based assay used in the Park *et al.* [*89*] paper, examining urinary 8-oxoGua and 8-oxodG are perhaps questionable, as the assay utilised a monoclonal antibody which was not entirely specific for 8-oxodG. There would appear to be little human data on the subject of dietary contribution, not all of which agree with the animal findings. Gackowski *et al.* [*90*] have suggested that, in humans, neither 8-oxoGua, nor 8-oxodG, are affected by diet. However, pivotal to this study was the assumption that, like rats [*89*], humans reach a minimum level of 8-oxoGua excretion 2–3 days after switching to a nucleic acid-free diet. Similarly, it was assumed that a maximum level of 8-oxoGua excretion was reached 3–5 days after reverting to a normal diet. Additional sampling points throughout this study would make these conclusions more definitive.

In contrast, there is agreement between all studies showing that urinary 2'-deoxynucleoside lesions, are unaffected by diet. In rats, this was demonstrated by both the nucleic acid-free diet studies and intubation studies (only 1% of [^{3}H]-8-oxodG and 5% of [^{3}H]- thymidine glycol, dTg, appeared in the urine; [*91*] and [*87*], respectively). Cathcart *et al.* [*87*] suggest that the low levels of radioactivity probably derive from faecal contamination of the urine samples, the true values being closer to zero. In humans, the results of Gackowski [*90*], described above, concur with the studies in rats.

For completeness, it is worth considering ribonucleoside lesions, although there have been very few reports examining oxidatively-modified ribonucleosides in urine [*18*, *92*]. Such studies may be justified by reports which suggest a negligible contribution from diet to urinary modified ribonucleoside levels [*93*, *94*]. However, there are also data that indicate diet influences urinary levels of 8-oxoGuo, in rats at least (nucleic acid-free diet excreted 908 +/– 357 pmol of 8-oxoG/kg/day, compared to 2810 +/– 830 pmol of 8-oxodGuo/kg/day for those fed normal chow [*89*].

Urinary levels of another lesion, N^2-methyl-8-oxo-7,8-dihydroguanine, have been measured and noted to be elevated in ill and growing infants, initially suggesting a role for this oxidatively-modified tRNA component as a marker of oxidative stress [*95*]. However, its apparent absence in tRNA and reduction in urinary levels following administration of allopurinol, would suggest its production and appearance in urine is due to the action of xanthine dehydrogenase/oxidase, clearly ruling out its use as a biomarker of tRNA oxidation [*95*]. In contrast, 8-oxoGuo and 8-oxodG excretion was unaffected by administration of allopurinol [*95*], as was 8-oxoGua excretion (Helbock *et al.* unpublished results, refered to in [95]), further supporting the compounds are biomarkers of oxidatively generated damage.

Whilst oxidative modification of DNA bases, such as 8-oxoGua, may have a number of detrimental consequences for the cell [*1*], including mutation [*2*], the significance of oxidatively modified ribonucleosides is not so well defined [*1*], but may be associated with abnormal protein translation and defects in protein synthesis [*96–98*]. It is concluded that the source, and certainly the significance, of oxidatively modified ribonucleosides in urine is even less clear than for nucleobases and 2'-deoxyribonucleosides, particularly as some oxidised ribonucleosides may be products of cellular synthesis and may be considered endogenous. Further studies, preferably in humans, examining the source and significance of extracellular, oxidatively-modified ribonucleosides are clearly warranted. This is all the more important in the light of recent work describing the role of Y-box binding protein 1 and polynucleotide phosphorylase [*99*] in sequestering 8-oxoGua-containing transcripts (the possibility of RNA 'repair' is considered in greater detail in Evans and Cooke [*1*]) coupled with the observations that oxidised mRNA induces trnaslational errors [*100*].

Recent human studies investigating dietary contribution

Upon ingestion, nucleic acids are rapidly fragmented, by mastication combined with enzymic digestion and acid hydrolysis in the buccal cavity and gastro-intestinal tract (GIT [*101*]). Enzymes which can catalyse hydrolysis include DNase I, from salivary glands, the pancreas, liver and Paneth cells of the small intestine, may be present in high concentrations, combined with DNase II, whose optimal pH (4.6–5.5) makes it particularly suited to activity in the upper GIT [*101*]. The low pH of the stomach may allow depurination of DNA, generating free adenine and guanine, as *in vitro* studies have shown [*102*], although it is not clear whether this is the case for 8-oxodG, as the *N*-glycosidic bond appears to be very chemically stable [*103*].

Even though specific transport systems are reported to exist for bases and nucleosides, for healthy, well-nourished individuals, reliance on dietary sources of nucleosides to supplement salvage and *de novo* synthesis is negligible [*104*]. Furthermore, even in those tissues that do apparently

utilise dietary sources of nucleosides to supplement endogenous nucleotide synthesis, e.g. bone marrow and intestine, the contribution from diet is still regarded as minimal. Enterocytes may absorb some dietary nucleosides, but in the case of dietary purine nucleosides significant catabolism in the proximal small intestine results in complete degradation of these species to uric acid [*105*, *106*], this is in contrast to pyrimidines which do show some incorporation in cellular nucleic acids [*107*]. Collectively, it would appear unlikely that diet contributes to the levels of oxidatively-modified purines in urine, although the case for pyrimidine lesions may not be quite as clear. Nevertheless, a definitive exclusion of dietary contribution, is still required, and may have to be explored on a lesion-by-lesion basis.

Studies utilising radio-labelled lesions are preferred over those involving nucleic acid-free diets as the use of radio-labels provides a degree of stringency not afforded by nucleic acid-free diets (possible short-comings of nucleic acid-free diets have been discussed above). For example, the presence of labelled lesions in the urine/faeces, cross-contamination notwithstanding, can only come from the dietary source. However, for ethical reasons, radio-labelled lesions are to be avoided in human studies. To circumvent this issue, Cooke *et al.* [*20*] reported a feeding study using heavily damaged, stable isotopically-labelled [^{15}N]-DNA (98% incorporation) and discussed thoroughly the fate of ingested DNA. The [^{15}N]-DNA, had been irradiated with a dose of 50 kJ/m^2 UVC shown, by a previous study, to induce approximately 600 8-oxodG/10^5 dG [*108*].

Healthy, male volunteers were fed 5, 15 or 25 mg of the damaged [^{15}N]-DNA, and first void, mid-stream urine samples were collected for up to 14 days later. The presence of [$^{15}N_5$]-8-oxodG, and [$^{15}N_5$]-8-oxoGua in urine was investigated using HPLC-GC/MS [*90*]. Neither lesion was detected in any of the urine samples. To confirm that the absence of lesion was not a sensitivity issue, urinary [$^{15}N_5$]-dG and [$^{15}N_5$]-Gua, which should be present at significant levels, was also examined. Again, neither of these labelled compounds were detected in any of the urine samples. The authors also investigated whether any of the ingested [$^{15}N_5$]-Gua/dG had been degraded to [$^{15}N_5$]-uric acid, the final product of purine metabolism, prior to excretion, again, no labelled material was detected.

Unlike previous studies, which had only been performed in animals, the authors also examined the DNA from peripheral blood mononuclear cells (PBMC), collected for up to seven days following ingestion of labelled DNA, for possible incorporation of label. The justification for this derived from reports that phage DNA may appear in a diverse range of cell types, including PBMC, 24 h after ingestion by mice [*109*, *110*], implying that a proportion of DNA consumed is not entirely degraded, and may be both absorbed and integrated. The relevance of such findings to plant and animal DNA has been questioned [*101*]. Yet again, no [$^{15}N_5$]-labelled Gua, or [$^{15}N_5$]-8-oxoGua, was detected in the PBMC DNA. This result is consistent with previous findings, in mice and chickens, that dietary purines, are not incorporated into cellular nucleic acids [*107*]. No information on pyrimidine components was presented.

As with previous studies [*111*], Cooke *et al.* [*20*] could only presume that the isotopically labelled DNA components/adducts pass through the gastro-intestinal tract to appear in faeces. Nevertheless, these results, coupled with the findings of Gackowski *et al.* [*90*], provide the most compelling argument that diet is not a significant contributor to both urinary 8-oxoGua and 8-oxodG levels in human urine. This is consistent with the animal data relating to 2'-deoxyribonucleoside lesions [*111*], which includes dTg [*87*], but disagrees with early data for 8-oxoGua and Tg [*87*, *112*]. Species differences may be proposed to account for this discrepancy, although the focus of our attention must be upon the more relevant, human data. Earlier animal studies should be repeated using the highly specific and sensitive mass spectrometric techniques.

Cell death/turnover. It has been stated that urinary 8-oxodG does not reflect DNA repair, as it is not a product of base excision repair [*113*], rather it was a product of non-specific nucleases, acting upon DNA released during cell death, liberating dG which is subsequently oxidised (or indeed free 8-oxodG itself). Such a hypothesis did not leave scope for the potential existence of other, as yet undiscovered, repair pathways, which may yield 8-oxodG, and in any case, oxidation of dG in the systemic circulations does not appear to occur (discussed below, under Issues of artefact and stability). Whilst repair processes, whose reaction product may be 8-oxodG, have subsequently been described (see above), the possible contribution from cell turnover remains to be addressed. Indeed, most of the evidence against a contribution from cell turnover is anecdotal. For example, there exists a number of reports in which urinary 8-oxodG has been measured in patients undergoing chemotherapy, in which cytotoxic agents are used to cause (cancer) cell death. No increases in urinary 8-oxodG were noted in the studies of both Faure *et al.* [*114*] and Erhola *et al.* [*115*], despite evidence of extensive cell death i.e. significant increases (p<0.01) in urinary uric acid (a biochemical index of cell turnover); and reduction in tumour mass. Furthermore any reported increases in urinary 8-oxodG following chemo- or radiotherapy have been attributed entirely to DNA repair [*116*, *117*]. Thus far, the most decisive argument against the contribution of cell death to urinary levels of 8-oxodG and 8-oxoGua comes from a report from the Olinski group [*118*]. This study revealed that a statistically significant (p = 0.0003) increase (60%) in 8-oxoGua was observed 24 h after infusion of the

cisplatin, a period when no loss of blood cells was reported. In the "nadir days", when the most distinct cell death can be observed, urinary excretion of 8-oxoGua dropped significantly in comparison with the aforementioned time point. The implication being that cell death does not appear to contribute to urinary 8-oxoGua (and 8-oxodG) in humans.

Is there any evidence to suggest that 8-oxodG may be liberated from DNA whilst in the systemic circulation? Perhaps. It has been reported that rat liver homogenates can release 8-oxodG from oxidatively-modified DNA [*119*]. The authors suggest that, during DNA degradation, most of the 8-oxodG, present in DNA, is released by enzymes, and subsequently appears in the urine, implying this to be a major source. However, they do acknowledge their system does not accurately represent the *in vivo* situation, not least as it is unrealistic—multiple, uncompartmentalised nucleases acting upon circulating DNA, particularly as the majority of cell death in healthy humans will be via apoptosis.

It has been suggested by Cooke *et al.* [*120*] that were cell death to be the primary source of urinary lesions, the ratio between native and modified, in urine, would be similar to that seen in cells (reportedly anywhere between one 8-oxodG/10^5 and one 8-oxodG/10^7 dG [*121*], although the recommendation from ESCODD is that the level in lymphocytes is likely to be somewhere between 4.2 and 0.3 8-oxodG per 10^6 dG [*8*]. In fact the ratio appears to be very close. For the 2'-deoxyribonucleoside: 28 (+/– 2) 8-oxodG: 12 (+/– 2) dG nmol/24 h, although not as close for the nucleobase: 136 +/– 12 8-oxoGua: 1931 (+/– 182) Gua nmol/24 h [*18*]. Neither ratio even approaches that seen in cells. Of course, were dG or Gua to be metabolised upon release from DNA, as reported by Shi *et al.* [*119*], this could account for the closeness of the ratio. Indeed this suggestion might be supported by the findings of Bialkowski *et al.* [*103*], who noted that the *N*-glycosidic bond is more stable for 8-oxodG, than dG. Nevertheless, Weimann *et al.* [*73*], in a study examining oligonucleotides in urine, concluded that the limited excretion of oligonucleotides into urine argues against oligonucleotides, or indeed nucleosides, originating from cell death [*73*]. On balance, these results suggest that the contribution of cell death to urinary 8-oxodG levels is minimal, but clearly further work needs to be performed.

Uptake and re-utilisation. In contrast to 8-oxoG in DNA, little information is available on the mutagenic potential and cellular responses to the presence of free 8-oxodG. The cellular metabolism of 8-oxodG is the subject of an emerging and sometimes conflicting literature. In human cells, dG predominantly undergoes phosphorolysis by purine nucleoside phosphorylase (PNP) to form the free nucleobase Gua and 2'-deoxyribose-1'-phosphate. The resulting free nucleobase is a substrate for hypoxanthine-guanine phosphoribosyltransferase (HGPRT), resulting in formation of GMP. Two additional phosphorylation steps afford GTP, which serves as a substrate for RNA polymerase-dependent incorporation into RNA. This pathway can also feed into DNA synthesis, since GDP can be reduced to dGDP by ribonucleotide reductase (RR) by replacement of the 2'-OH with a hydrogen atom. This pathway is represented schematically by:

$$dG \rightarrow Gua \rightarrow GMP \rightarrow GDP \rightarrow GTP \rightarrow RNA$$

and

$$dG \rightarrow Gua \rightarrow GMP \rightarrow GDP \rightarrow dGDP \rightarrow dGTP \rightarrow DNA$$

Alternatively, cellular deoxycytidine kinase (dCK) or mitochondrial deoxyguanosine kinase (dGK) phosphorylate dG to form dGMP, which can then be serially phosphorylated to dGTP prior to incorporation into DNA during replication or repair synthesis:

$$dG \rightarrow dGMP \rightarrow dGDP \rightarrow dGTP \rightarrow DNA.$$

Based upon the known nucleotide salvage pathways for dG, 8-oxodG may also be metabolized by two distinct pathways.

(1) If 8-oxodG is a substrate for PNP, the nucleobase portion of 8-oxodG can be hydrolyzed from the 2'-deoxyribose ring. The resulting 8-oxoGua is a substrate for nucleotide salvage, resulting in the ribonucleotide and 2'-deoxyribonucleotide triphosphates 8-oxoGTP and 8-oxodGTP, respectively, which contribute to RNA and DNA synthesis.

(2) Direct mono-, di- and triphosphorylation of 8-oxodG would result in formation of 8-oxodGTP, a substrate for DNA synthesis [*122–128*]. Both possible pathways reflect the known metabolism of the canonical 2'-deoxynucleoside dG described above [*129–131*]. However, nucleotide salavage of 8-oxodG is complicated by contributions from BER via OGG1 and OGG2 and nucleotide pool "cleansing" by nudix hydrolases and 5'-nucleotidase activity as shown in Fig. 3.

In an effort to determine whether 8-oxodG is a substrate for the human nucleotide salvage pathways that utilize dG, Henderson and coworkers measured [^{14}C]8-oxodG metabolism in MCF-7 cells [*35*]. The [^{14}C]8-oxodG was labeled on the nucleobase in order to probe both possible nucleotide salvage mechanisms. AMS was required for the study since the levels of ^{14}C-labeled metabolites were too low be be detected by other mass spectrometry methods and well below the limit of detection for liquid scintillation counting. The ability of cells dosed with extracellular 8-oxodG to import 8-oxodG and employ either PNP-dependent nucleotide salvage or direct phosphorylation for nucleic acid

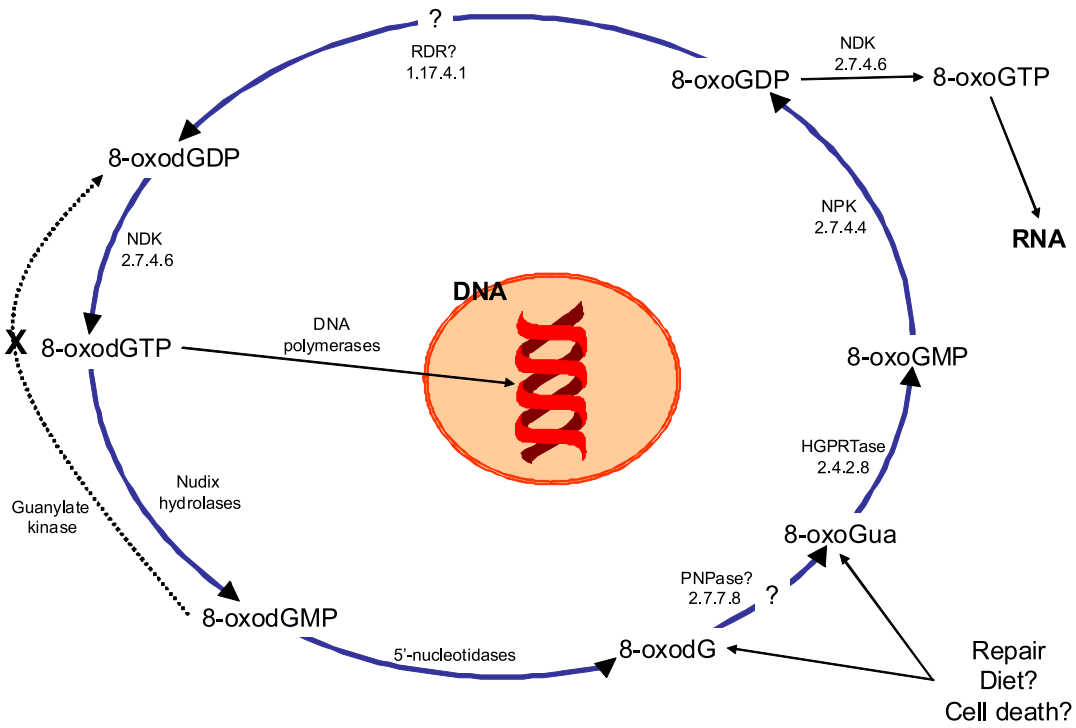

Fig. 3. Potential fate of extracellular 8-oxodG, and 8-oxoGua, by metabolic salvage pathways. Neither 8-oxodG, nor 8-oxodGMP can be phosphorylated, as they are not substrates for deoxynucleoside kinase, or guanylate kinase, respectively. Therefore the alternative pathway, discussed by Hah *et al.* [*35*], relies upon degradation of extracellular 8-oxodG to 8-oxoGua, perhaps by purine nucleoside phosphorylase (PNP). 6-Hydroxypurine phosphoribosyltransferase (HGPRTase) can catalyse the formation of 8-oxoguanosine monophosphate, providing a route for the oxidised moiety, via nucleoside-phosphate kinase (NPK) and nucleoside-diphosphate kinase (NDK), to be incorporated into RNA. In this model ribonucleoside-diphosphate reductase (RDR) is responsible for the conversion of 8-oxoguanine-containing ribonucleotides to deoxyribonucleotide equivalents. Nucleoside-diphosphate kinase then catalyses the phosphorylation of 8-oxodGDP to 8-oxo-7,8-dihydro-2'-deoxyguanosine triphosphate, a substrate for DNA polymerases for incorporation into DNA. Potential sources of extracellular 8-oxodG and 8-oxoGua are indicated (diet, death, DNA repair) contributions from which may have profound implications for the cell, and our understanding of what measurement of these biomarkers of oxidative stress really means.

synthesis was investigated. Incorporation of exogoenous 8-oxodG was observed at a concentration of approximately one 8-oxoG nucleobase per 10^7 nucleotides in DNA and one 8-oxoG per 10^6 nucleotides in RNA, in cells exposed to concentrations of 8-oxodG approximating those present in human plasma (~100 fmol per mL) [*89*]. Resulting levels of 8-oxoGua incorporated into DNA and RNA, were at concentrations within an order of magnitude of those typically reported for mammalian cells as measured by HPLC-EC [*8*].

These initial observations were accompanied by inhibitor-based experiments aimed at determining the mechanism of incorporation. The [^{14}C]8-oxodG experiments were repeated in the presence of inhibitors of PNP, RR and dCK [*132*]. The inhibition of PNP almost completely abrogated incorporation of radiocarbon into both DNA and RNA, indicating PNP as a gatekeeper of 8-oxodG catabolism. Inhibition of RR resulted in reduced radiocarbon incorporation into DNA, but had little effect on 8-oxoG incorporation into RNA, indicating that 8-oxoGDP is a substrate for RR, which leads to incorporation of the nucleobase portion of the exogenous 8-oxodG into DNA. Inhibition of dCK had no effect on radiocarbon incorporation into DNA and RNA compared to controls with no inhibitor, supporting, at best, a minimal contribution by direct phosphorylation of 8-oxodG to the overall metabolism, which is in contrast to catabolism of dG [*132*].

An intriguing possible consequence of nucleotide salvage for DNA and nucleotide pool repair pathways is the production of both 8-oxodG and 8-oxoGua that may ultimately be excreted in urine. Prevention of the incorporation of 8-oxodG into DNA or RNA derives from the activity of MTH1 and 5'-nucleotidase. Whether 8-oxodGMP is a substrate for cellular dGMP nucleotidase activity remains unanswered.

The recently published data on exogenous 8-oxodG is in partial agreement with the literature. For example, Zeng *et al.* [*133*] demonstrated that HGPRT can catalyse the formation of 8-oxoGuo monophosphate from 8-oxoGua,

which is consistent with PNP-dependent nucleotide salvage of 8-oxodG. However, this process has not been specifically demonstrated to occur *in vivo*, even in a cell culture system. The extracellular addition of 400 μM 8-oxodG to a number of leukaemic cell lines (KG-1, H9, CEM-CM3, Molt-4), in which hOGG1 activity is absent or low, results in cytotoxicity and the accumulation of 8-oxoGua in their nuclear DNA [*134*, *135*], consistent with PNP-mediated cleavage of the glycosidic bond of 8-oxodG. However, this increase 8-oxoGua does not appear to be due to the direct incorporation of the extracellular 8-oxodG [*136*]. However, this study reported the use of tritium-labeled 8-oxodG in which the radioactive label resided on the 2'-deoxyribose ring, which would be precluded from incorporation into DNA unless there was direct phosphorylation by dCK or dGK. It has previously been reported that 8-oxodG is not a substrate for deoxynucleoside kinase [*137*] or guanylate kinase [*65*], which are not required by the PNP-mediated pathway for incorporation of 8-oxoGua into DNA or RNA. The report by Kim et al claimed that the 8-oxodG was not substantially degraded to 8-oxoGua, supporting the notion that the fate of 8-oxodG, once present following repair, is excreted, intact, into extracellular compartments [*136*]. However, we would have to question the biological relevence of using an extracellular concentration of 400 μM 8-oxodG. For example, Henderson and coworkers demonstrated that increasing concentrations of 8-oxodG in the cell culture medium above approximately 2 μM resulted in saturation of nucleotide salvage resulting little or no additional incorporation into DNA with increased 8-oxodG concentrations in the media [*35*].

Kim *et al.* [*136*] provided evidence concurring with an earlier report [*65*] that 8-oxodG is not phosphorylated to 8-oxodGMP. The authors hypothesise that the cytotoxicity of 8-oxodG is, in part at least, via error-prone DNA synthesis, mediated by DNA polymerase β [*136*], implying that cytosolic 8-oxodG, whilst not a substrate for (re-) incorporation into DNA, may not be as benign as previously thought. Alternative explanation is that very high concentrations, 8-oxodG interferes with feedback inhibited control of dGTP concentrations via PNP-mediated proceses, resulting in toxic levels of dGTP in the cells dosed with 400 μM 8-oxodG.

Issues of artefact and stability

The potential exists, as for DNA, that urinary lesions may arise from the artefactual oxidation of nucleobases, or 2'-deoxyribonucleosides, following exposure to metabolic enzymes, or other oxidising species, after release into the systemic circulation, or in the urine. However, Shigenaga *et al.* [*111*] showed that dG is not artefactually oxidised in the systemic circulation, or following incubation with microsomal enzymes. Equally, 8-oxodG, Tg and dTg are not subject to degradation upon release into the systemic circulation ([*87*, *111*], the stability of 8-oxoGua not having been examined. Experimental studies with 8-oxodG injected intravenously have shown that recovery is almost complete in urine in pigs, implying that further metabolism in the systemic circulation is an unlikely event [*138*].

Furthermore, as significant concentrations of hydrogen peroxide have been reported to be present in urine [*26*, *139*]. Lin *et al.* (2004) examined the potential for 8-oxodG to be generated from dG, present in the urine. In a recent study combinations of dG (1 nmol) and hydrogen peroxide (50 or 100 μM), were incubated in a number of urine samples, for 24 h, at room temperature [*26*]. The authors concluded that hydrogen peroxide has a negligible effect upon 8-oxodG production, under these circumstances, agreeing with previous results from Ames' laboratory. Storage of urine samples at –20°C does not lead to a decrease in urinary 8-oxodG concentrations, when analysed over a 15 year period [*140*], making urine an ideal matrix to store in biobanks for future analysis.

Whilst the above provide evidence of stability in extracellular matrices, it is worth noting that 8-oxodG is more prone to oxidation than dG, due to its lower redox potential [*141*, *142*] and is preferentially oxidised, even in the presence of an excess of Gua [*143*, *144*]. By the same token, oxidation of Thy to Tg is less likely to occur, than Gua to 8-oxoGua, due to the lower oxidation potential of Gua, compared to Thy. However, it would appear that most of these studies have examined the oxidation of 8-oxoGua *in situ* in DNA [*145*], rather than as a post-excision product of DNA repair, which may alter the likelihood of oxidation and its 'oxidisability'. In contrast, M_1G and M_1dG both appear to undergo further oxidative metabolism in rat liver cytosol, with the nucleobase adduct being a better substrate for such enzymic oxidation than the deoxyribonucleoside adduct [*146*, *147*]. There is also some evidence to suggest that M_1G is further oxidised when administered intravenously, although M_1dG was not examined [*146*].

Overall, it would appear that 8-oxodG, Tg and dTg, and probably 8-oxoGua, are not formed artefactually *in vivo*, in biological matrices of mammals, including humans, and there is no published evidence for their degradation upon release. In contrast, there is strong evidence that M_1G and M_1dG may undergo oxidation, either enzymically, or in the systemic circulation.

Conclusions

There is a great deal of evidence, albeit largely circumstantial, implicating a role for oxidatively damaged DNA in various diseases, in particular cancer [*5*]. Of the biomarkers of oxidatively damaged DNA, urinary 8-oxodG has received perhaps the most interest. The benefits of using urine as a matrix in which to study oxidatively-modified

DNA include:

1. Non-invasive, with ramifications for gaining ethical approval and access to vulnerable groups.
2. Samples are easily collected (low biological hazard, no pre-processing prior to storage, small volumes), stored (stable at –80°C for >10 y, with no 'special' treatments or preservatives) and transported.
3. Stability of 8-oxodG in urine allows access to samples from previous studies/biobanks.
4. Applicable to the study of large sample size, as many of the assays are semi-automated and with potentially high throughput.

Measurement of this biomarker is also the closest to validation, which includes understanding the provenence of 8-oxodG in urine, evaluation of confounding factors and work towards inter-laboratory consensus. However, an important caveat with validation of urinary biomarkers, is to not simply apply the findings for one lesion, to all the others. For example, we have noted herein that the literature indicates that diet may contribute to levels of urinary 8-oxoGua, but not urinary Tg. To some extent, therefore, all lesions need to be indipendetly validated, drawing on the experience of 8-oxo(d)G.

To accelerate this process, the European Standards Committee of Urinary (DNA) Lesion Analysis (ESCULA) has been formed, which now represents an international group of >25 laboratories dedicated to the validation of urinary biomarkers of DNA damage (further information, including how to apply for membership, can be found at http://escula.org).

Acknowledgments

MSC and MDE are partners of ECNIS (Environmental Cancer Risk, Nutrition and Individual Susceptibility), a network of excellence operating within the European Union 6th Framework Program, Priority 5: "Food Quality and Safety" (Contract No 513943). PTH was supported by National Institutes of Health Grants RR13461, CA93373, the California Breast Cancer Research Program Grant 9KB-0179 and the Knapp Family Fund.

References

[1] Evans, M.D. and Cooke, M.S.: Factors contributing to the outcome of oxidative damage to nucleic acids. *Bioessays*, **26**, 533–542, 2004.

[2] Evans, M.D., Dizdaroglu, M., and Cooke, M.S.: Oxidative DNA damage and disease: induction, repair and significance. *Mutat. Res.*, **567**, 1–61, 2004.

[3] Dizdaroglu, M.: Chemical determination of free radical-induced damage to DNA. *Free Radic. Biol. Med.*, **10**, 225–242, 1991.

[4] Evans, M. and Cooke, M.: Lipid- and protein-mediated oxidative damage to DNA, in *Oxidative Stress, Disease and Cancer*, ed. By Singh, K.K., Imperial College Press, London, pp. 201–252, 2006.

[5] Cooke, M.S., Olinski, R., and Evans, M.D.: Does measurement of oxidative damage to DNA have clinical significance? *Clin. Chim. Acta*, **365**, 30–49, 2006.

[6] Pilger, A., Germadnik, D., Schaffer, A., Theiler, A., Pils, P., Sluka, F., Winker, N., and Rüdiger, H.W.: 8-Hydroxydeoxyguanosine in leukocyte DNA and urine of quartz-exposed workers and patients with silicosis. *Int. Arch. Occup. Environ. Health*, **73**, 305–310, 2000.

[7] Guetens, G., De Boeck, G., Highley, M., van Oosterom, A.T., and de Bruijn, E.A.: Oxidative DNA damage: biological significance and methods of analysis. *Crit. Rev. Clin. Lab. Sci.*, **39**, 331–457, 2002.

[8] Gedik, C.M. and Collins, A.: Establishing the background level of base oxidation in human lymphocyte DNA: results of an interlaboratory validation study. *FASEB J.*, **19**, 82–84, 2005.

[9] Hariharan, P.V. and Cerutti, P.A.: Excision of damaged thymine residues from gamma-irradiated poly(dA-dT) by crude extracts of Escherichia coli. *Proc. Natl. Acad. Sci. USA*, **71**, 3532–3536, 1974.

[10] Cao, E.H. and Wang, J.J.: Oxidative damage to DNA: levels of thymine glycol and thymidine glycol in neoplastic human urines. *Carcinogenesis*, **14**, 1359–1362, 1993.

[11] Bergtold, D.S., Simic, M.G., Alessio, H., and Cutler, R.G.: Urine biomarkers for oxidative DNA damage. *Basic Life Sci.*, **49**, 483–489, 1988.

[12] Tagesson, C., Chabiuk, D., Axelson, O., Barański, B., Palus, J., and Wyszyńska, K.: Increased urinary excretion of the oxidative DNA adduct, 8-hydroxydeoxyguanosine, as a possible early indicator of occupational cancer hazards in the asbestos, rubber, and azo-dye industries. *Pol. J. Occup. Med. Environ. Health*, **6**, 357–368, 1993.

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

[14] Kasai, H., Svoboda, P., Yamasaki, S., and Kawai, K.: Simultaneous determination of 8-hydroxydeoyguanosine, a marker of oxidative stress, and creatinine, a standardization compound, in urine. *Ind. Health*, **43**, 333–336, 2005.

[15] Bogdanov, M.B., Beal, M.F., McCabe, D.R., Griffin, R.M., and Matson, W.R.: A carbon column-based liquid chromatography electrochemical approach to routine 8-hydroxy-2'-deoxyguanosine measurements in urine and other biologic matrices: a one-year evaluation of methods. *Free Radic. Biol. Med.*, **27**, 647–666, 1999.

[16] Kasai, H.: A new automated method to analyze urinary 8-hydroxydeoxyguanosine by a high-performance liquid chromatography-electrochemical detector system. *J. Radiat. Res. (Tokyo)*, **44**, 185–189, 2003.

[17] Loft, S., Poulsen, H.E., Vistisen, K., and Knudsen, L.E.: Increased urinary excretion of 8-oxo-2'-deoxyguanosine, a

biomarker of oxidative DNA damage, in urban bus drivers. *Mutat. Res.*, **441**, 11–19, 1999.

[18] Weimann, A., Belling, D., and Poulsen, H.E.: Quantification of 8-oxo-guanine and guanine as the nucleobase, nucleoside and deoxynucleoside forms in human urine by high-performance liquid chromatography-electrospray tandem mass spectrometry. *Nucleic Acids Res.*, **30**, E7, 2002.

[19] Rozalski, R., Siomek, A., Gackowski, D., Foksinski, M., Gran, C., Klungland, A., and Olinski, R.: Diet is not responsible for the presence of several oxidatively damaged DNA lesions in mouse urine. *Free Radic. Res.*, **38**, 1201–1205, 2004.

[20] Cooke, M.S., Evans, M.D., Dove, R., Rozalski, R., Gackowski, D., Siomek, A., Lunec, J., and Olinski, R.: DNA repair is responsible for the presence of oxidatively damaged DNA lesions in urine. *Mutat. Res.*, **574**, 58–66, 2005.

[21] Ravanat, J.L., Guicherd, P., Tuce, Z., and Cadet, J.: Simultaneous determination of five oxidative DNA lesions in human urine. *Chem. Res. Toxicol.*, **12**, 802–808, 1999.

[22] Harman, S.M., Liang, L., Tsitouras, P.D., Gucciardo, F., Heward, C.B., Reaven, P.D., Ping, W., Ahmed, A., and Cutler, R.G.: Urinary excretion of three nucleic acid oxidation adducts and isoprostane F(2)alpha measured by liquid chromatography-mass spectrometry in smokers, ex-smokers, and nonsmokers. *Free Radic. Biol. Med.*, **35**, 1301–1309, 2003.

[23] Hoberg, A.M., Otteneder, M., Marnett, L.J., and Poulsen, H.E.: Measurement of the malondialdehyde-2'-deoxyguanosine adduct in human urine by immuno-extraction and liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. *J. Mass. Spectrom.*, **39**, 38–42, 2004.

[24] Hillestrøm, P.R., Hoberg, A.M., Weimann, A., and Poulsen, H.E.: Quantification of 1,N6-etheno-2'-deoxyadenosine in human urine by column-switching LC/APCI-MS/MS. *Free Radic. Biol. Med.*, **36**, 1383–1392, 2004.

[25] Sun, X., Karlsson, A., Bartsch, H., and Nair, J.: New ultrasensitive 32P-postlabelling method for the analysis of 3,N4-etheno-2'-deoxycytidine in human urine. *Biomarkers*, **11**, 329–340, 2006.

[26] Lin, H.S., Jenner, A.M., Ong, C.N., Huang, S.H., Whiteman, M., and Halliwell, B.: A high-throughput and sensitive methodology for the quantification of urinary 8-hydroxy-2'-deoxyguanosine: measurement with gas chromatography-mass spectrometry after single solid-phase extraction. *Biochem. J.*, **380**, 541–548, 2004.

[27] Cooke, M.S., Singh, R., Hall, G.K., Mistry, V., Duarte, T.L., Farmer, P.B., and Evans, M.D.: Evaluation of enzyme-linked immunosorbent assay and liquid chromatography-tandem mass spectrometry methodology for the analysis of 8-oxo-7,8-dihydro-2'-deoxyguanosine in saliva and urine. *Free Radic. Biol. Med.*, **41**, 1829–1836, 2006.

[28] Weimann, A., Belling, D., and Poulsen, H.E.: Measurement of 8-oxo-2'-deoxyguanosine and 8-oxo-2'-deoxyadenosine in DNA and human urine by high performance liquid chromatography- electrospray tandem mass spectrometry. *Free Radic. Biol. Med.*, **30**, 757–764, 2001.

[29] Evans, M.D., Singh, R., Mistry, V., Sandhu, K., Farmer, P.B., and Cooke, M.S.: Analysis of urinary 8-oxo-7,8-dihydropurine-2'-deoxyribonucleosides by LC-MS/MS and improved ELISA. *Free Radic. Res.*, **42**, 831–840, 2008.

[30] Nair, J., Barbin, A., Velic, I., and Bartsch, H.: Etheno DNA-base adducts from endogenous reactive species. *Mutat. Res.*, **424**, 59–69, 1999.

[31] Chen, H.J., Hong, C.L., Wu, C.F., and Chiu, W.L.: Effect of cigarette smoking on urinary 3,N4-ethenocytosine levels measured by gas chromatography/mass spectrometry. *Toxicol. Sci.*, **76**, 321–327, 2003.

[32] Chen, H.J., Wu, C.F., Hong, C.L., and Chang, C.M.: Urinary excretion of 3,N4-etheno-2'-deoxycytidine in humans as a biomarker of oxidative stress: association with cigarette smoking. *Chem. Res. Toxicol.*, **17**, 896–903, 2004.

[33] Hillestrøm, P.R., Weimann, A., and Poulsen, H.E.: Quantification of urinary etheno-DNA adducts by column-switching LC/APCI-MS/MS. *J. Am. Soc. Mass Spectrom.*, **17**, 605–610, 2006.

[34] Coldwell, K.E., Cutts, S.M., Ognibene, T.J., Henderson, P.T., and Phillips, D.R.: Detection of Adriamycin-DNA adducts by accelerator mass spectrometry at clinically relevant Adriamycin concentrations. *Nucleic Acids Res.*, **36**, e100, 2008.

[35] Hah, S.S., Mundt, J.M., Kim, H.M., Sumbad, R.A., Turteltaub, K.W., and Henderson, P.T.: Measurement of 7,8-dihydro-8-oxo-2'-deoxyguanosine metabolism in MCF-7 cells at low concentrations using accelerator mass spectrometry. *Proc. Natl. Acad. Sci. USA*, **104**, 11203–11208, 2007.

[36] Hah, S.S., Sumbad, R.A., de Vere White, R.W., Turteltaub, K.W., and Henderson, P.T.: Characterization of oxaliplatin-DNA adduct formation in DNA and differentiation of cancer cell drug sensitivity at microdose concentrations. *Chem. Res. Toxicol.*, **20**, 1745–1751, 2007.

[37] Tompkins, E.M., Farmer, P.B., Lamb, J.H., Jukes, R., Dingley, K., Ubick, E., Turteltaub, K.W., Martin, E.A., and Brown, K.: A novel 14C-postlabeling assay using accelerator mass spectrometry for the detection of O6-methyldeoxyguanosine adducts. *Rapid Commun. Mass Spectrom.*, **20**, 883–891, 2006.

[38] Goldman, R., Day, B.W., Carver, T.A., Mauthe, R.J., Turteltaub, K.W., and Shields, P.G.: Quantitation of benzo[a]pyrene-DNA adducts by postlabeling with 14C-acetic anhydride and accelerator mass spectrometry. *Chem. Biol. Interact.*, **126**, 171–183, 2000.

[39] Sandhu, P., Vogel, J.S., Rose, M.J., Ubick, E.A., Brunner, J.E., Wallace, M.A., Adelsberger, J.K., Baker, M.P., Henderson, P.T., Pearson, P.G., and Baillie, T.A.: Evaluation of microdosing strategies for studies in preclinical drug development: demonstration of linear pharmacokinetics in dogs of a nucleoside analog over a 50-fold dose range. *Drug Metab. Dispos.*, **32**, 1254–1259, 2004.

[40] Li, Y., Shawgo, R.S., Tyler, B., Henderson, P.T., Vogel, J.S., Rosenberg, A., Storm, P.B., Langer, R., Brem, H., and Cima, M.J.: *In vivo* release from a drug delivery MEMS device. *J. Control Release*, **100**, 211–219, 2004.

[41] Knutson, C.G., Skipper, P.L., Liberman, R.G., Tannenbaum,

S.R., and Marnett, L.J.: Monitoring *in vivo* metabolism and elimination of the endogenous DNA adduct, M1dG {3-(2-deoxy-beta-D-erythro-pentofuranosyl)pyrimido[1,2-alpha]purin-10(3H)-one}, by accelerator mass spectrometry. *Chem. Res. Toxicol.*, **21**, 1290–1294, 2008.

[42] Clifford, A.J., Arjomand, A., Dueker, S.R., Schneider, P.D., Buchholz, B.A., and Vogel, J.S.: The dynamics of folic acid metabolism in an adult given a small tracer dose of 14C-folic acid. *Adv. Exp. Med. Biol.*, **445**, 239–251, 1998.

[43] Chiou, C.C., Chang, P.-Y., Chan, E.-C., Wu, T.-L., Tsao, K.-C., and Wu, J.T.: Urinary 8-hydroxydeoxyguanosine and its analogs as DNA markers of oxidative stress: development of an ELISA and measurement in both bladder and prostate cancers. *Clinica. Chimica. Acta*, **334**, 87–94, 2003.

[44] Breton, J., Sichel, F., Bianchini, F., and Prevost, V.: Measurement of 8-hydroxy-2'-deoxyguanosine by a commercially available ELISA test: comparison with HPLC/electrochemical detection in calf thymus DNA and determination in human serum. *Anal. Lett.*, **36**, 123–134, 2003.

[45] Tope, A.M. and Panemangalore, M.: Assessment of oxidative stress due to exposure to pesticides in plasma and urine of traditional limited-resource farm workers: formation of the DNA-adduct 8-hydroxy-2-deoxy-guanosine (8-OHdG). *J. Environ. Sci. Health B*, **42**, 151–155, 2007.

[46] Sawamoto, Y., Sugano, N., Tanaka, H., and Ito, K.: Detection of periodontopathic bacteria and an oxidative stress marker in saliva from periodontitis patients. *Oral Microbiol. Immunol.*, **20**, 216–220, 2005.

[47] Sugano, N., Yokoyama, K., Oshikawa, M., Kumagai, K., Takane, M., Tanaka, H., and Ito, K.: Detection of Streptococcus anginosus and 8-hydroxydeoxyguanosine in saliva. *J. Oral Sci.*, **45**, 181–184, 2003.

[48] Yoshida, R., Ogawa, Y., and Kasai, H.: Urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine values measured by an ELISA correlated well with measurements by high-performance liquid chromatography with electrochemical detection. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 1076–1081, 2002.

[49] Ihara, Y., Takata, H., Tanabe, Y., Nobukuni, K., and Hayabara, T.: Influence of repetitive transcranial magnetic stimulation on disease severity and oxidative stress markers in the cerebrospinal fluid of patients with spinocerebellar degeneration. *Neurol. Res.*, **27**, 310–313, 2005.

[50] Kikuchi, A., Takeda, A., Onodera, H., Kimpara, T., Hisanaga, K., Sato, N., Nunomura, A., Castellani, R.J., Perry, G., Smith, M.A., and Itoyama, Y.: Systemic increase of oxidative nucleic acid damage in Parkinson's disease and multiple system atrophy. *Neurobiol. Dis.*, **9**, 244–248, 2002.

[51] Cooke, M.S., Patel, K., Ahmad, J., Holloway, K., Evans, M.D., and Lunec, J.: Monoclonal antibody to single-stranded DNA: a potential tool for DNA repair studies. *Biochem. Biophys. Res. Commun.*, **284**, 232–238, 2001.

[52] Haghdoost, S., Czene, S., Naslund, I., Skog, S., and Harms-Ringdahl, M.: Extracellular 8-oxo-dG as a sensitive parameter for oxidative stress *in vivo* and *in vitro*. *Free Radic. Res.*, **39**, 153–162, 2005.

[53] Kantha, S.S., Wada, S., Tanaka, H., Takeuchi, M., Watabe, S., and Ochi, H.: Carnosine sustains the retention of cell morphology in continuous fibroblast culture subjected to nutritional insult. *Biochem. Biophys. Res. Commun.*, **223**, 278–282, 1996.

[54] Kulkarni, N., Cooke, M.S., and Grigg, J.: Neutrophils in induced sputum from healthy children: role of interleukin-8 and oxidative stress. *Respir. Med.*, **101**, 2108–2112, 2007.

[55] Cooke, M.S., Evans, M.D., Dizdaroglu, M., and Lunec, J.: Oxidative DNA damage: mechanisms, mutation, and disease. *Faseb. J.*, **17**, 1195–1214, 2003.

[56] Fortini, P., Parlanti, E., Sidorkina, O.M., Laval, J., and Dogliotti, E.: The type of DNA glycosylase determines the base excision repair pathway in mammalian cells. *J. Biol. Chem.*, **274**, 15230–15236, 1999.

[57] Arai, K., Morishita, K., Shinmura, K., Kohno, T., Kim, S.R., Nohmi, T., Taniwaki, M., Ohwada, S., and Yokota, J.: Cloning of a human homolog of the yeast OGG1 gene that is involved in the repair of oxidative DNA damage. *Oncogene*, **14**, 2857–2861, 1997.

[58] Rosenquist, T.A., Zharkov, D.O., and Grollman, A.P.: Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. *Proc. Natl. Acad. Sci. USA*, **94**, 7429–7434, 1997.

[59] Parsons, J.L., Zharkov, D.O., and Dianov, G.L.: NEIL1 excises 3' end proximal oxidative DNA lesions resistant to cleavage by NTH1 and OGG1. *Nucleic. Acids Res.*, **33**, 4849–4856, 2005.

[60] Dou, H., Mitra, S., and Hazra, T.K.: Repair of oxidized bases in DNA bubble structures by human DNA glycosylases NEIL1 and NEIL2. *J. Biol. Chem.*, **278**, 49679–49684, 2003.

[61] Rozalski, R., Siomek, A., Gackowski, D., Foksinski, M., Gran, C., Klungland, A., and Olinski, R.: Substantial decrease of urinary 8-oxo-7,8-dihydroguanine, a product of the base excision repair pathway, in DNA glycosylase defective mice. *Int. J. Biochem. Cell Biol.*, **37**, 1331–1336, 2005.

[62] Morland, I., Rolseth, V., Luna, L., Rognes, T., Bjoras, M., and Seeberg, E.: Human DNA glycosylases of the bacterial Fpg/MutM superfamily: an alternative pathway for the repair of 8-oxoguanine and other oxidation products in DNA. *Nucleic Acids Res.*, **30**, 4926–4936, 2002.

[63] Mo, J.Y., Maki, H., and Sekiguchi, M.: Hydrolytic elimination of a mutagenic nucleotide, 8-oxodGTP, by human 18-kilodalton protein: sanitization of nucleotide pool. *Proc. Natl. Acad. Sci. USA*, **89**, 11021–11025, 1992.

[64] Bestwick, R.K., Moffett, G.L., and Mathews, C.K.: Selective expansion of mitochondrial nucleoside triphosphate pools in antimetabolite-treated HeLa cells. *J. Biol. Chem.*, **257**, 9300–9304, 1982.

[65] Hayakawa, H., Taketomi, A., Sakumi, K., Kuwano, M., and Sekiguchi, M.: 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, 1995.

[66] Haghdoost, S., Sjolander, L., Czene, S., and Harms-Ringdahl, M.: The nucleotide pool is a significant target for oxidative stress. *Free Radic. Biol. Med.*, **41**, 620–626, 2006.

[67] Cai, J.P., Ishibashi, T., Takagi, Y., Hayakawa, H., and Sekiguchi, M.: Mouse MTH2 protein which prevents muta-

tions caused by 8-oxoguanine nucleotides. *Biochem. Biophys. Res. Commun.*, **305**, 1073–1077, 2003.

[68] Ishibashi, T., Hayakawa, H., and Sekiguchi, M.: A novel mechanism for preventing mutations caused by oxidation of guanine nucleotides. *EMBO Rep.*, **4**, 479–483, 2003.

[69] Reardon, J.T., Bessho, T., Kung, H.C., Bolton, P.H., and Sancar, A.: *In vitro* repair of oxidative DNA damage by human nucleotide excision repair system: possible explanation for neurodegeneration in xeroderma pigmentosum patients. *Proc. Natl. Acad. Sci. USA*, **94**, 9463–9468, 1997.

[70] Sancar, A., Lindsey-Boltz, L.A., Unsal-Kacmaz, K., and Linn, S.: Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu. Rev. Biochem.*, **73**, 39–85, 2004.

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

[72] Le Curieux, F. and Hemminki, K.: Cyclobutane thymidine dimers are present in human urine following sun exposure: quantitation using 32P-postlabeling and high-performance liquid chromatography. *J. Invest. Dermatol.*, **117**, 263–268, 2001.

[73] Weimann, A., Riis, B., and Poulsen, H.E.: Oligonucleotides in human urine do not contain 8-oxo-7,8-dihydrodeoxyguanosine. *Free Radic. Biol. Med.*, **36**, 1378–1382, 2004.

[74] Dianov, G., Bischoff, C., Piotrowski, J., and Bohr, V.A.: Repair pathways for processing of 8-oxoguanine in DNA by mammalian cell extracts. *J. Biol. Chem.*, **273**, 33811–33816, 1998.

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

[76] Cappelli, E., Degan, P., and Frosina, G.: Comparative repair of the endogenous lesions 8-oxo-7,8-dihydroguanine (8-oxoG), uracil and abasic site by mammalian cell extracts: 8-oxoG is poorly repaired by human cell extracts. *Carcinogenesis*, **21**, 1135–1141, 2000.

[77] Pascucci, B., Maga, G., Hubscher, U., Bjoras, M., Seeberg, E., Hickson, I.D., Villani, G., Giordano, C., Cellai, L., and Dogliotti, E.: Reconstitution of the base excision repair pathway for 7,8-dihydro-8-oxoguanine with purified human proteins. *Nucleic. Acids Res.*, **30**, 2124–2130, 2002.

[78] Sokhansanj, B.A., Rodrigue, G.R., Fitch, J.P., and Wilson, D.M. 3rd.: A quantitative model of human DNA base excision repair. I. Mechanistic insights. *Nucleic Acids Res.*, **30**, 1817–1825, 2002.

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

[80] Klein, J.C., Bleeker, M.J., Saris, C.P., Roelen, H.C., Brugghe, H.F., van den Elst, H., van der Marel, G.A., van Boom, J.H., Westra, J.G., Kriek, E., and et al.: Repair and replication of plasmids with site-specific 8-oxodG and 8-AAFdG residues in normal and repair-deficient human cells. *Nucleic Acids Res.*, **20**, 4437–4443, 1992.

[81] Bernardes de Jesus, B.M., Bjoras, M., Coin, F., and Egly, J.M.: Dissection of the molecular defects caused by pathogenic mutations in the DNA repair factor XPC. *Mol. Cell Biol.*, **28**, 7225–7235, 2008.

[82] Ishchenko, A.A., Yang, X., Ramotar, D., and Saparbaev, M.: The 3'->5' exonuclease of Apn1 provides an alternative pathway to repair 7,8-dihydro-8-oxodeoxyguanosine in Saccharomyces cerevisiae. *Mol. Cell Biol.*, **25**, 6380–6390, 2005.

[83] Parsons, J.L., Dianova, I.I., and Dianov, G.L.: APE1-dependent repair of DNA single-strand breaks containing 3'-end 8-oxoguanine. *Nucleic Acids Res.*, **33**, 2204–2209, 2005.

[84] Colussi, C., Parlanti, E., Degan, P., Aquilina, G., Barnes, D., Macpherson, P., Karran, P., Crescenzi, M., Dogliotti, E., and Bignami, M.: The mammalian mismatch repair pathway removes DNA 8-oxodGMP incorporated from the oxidized dNTP pool. *Curr Biol.*, **12**, 912–918, 2002.

[85] Russo, M.T., De Luca, G., Degan, P., and Bignami, M.: Different DNA repair strategies to combat the threat from 8-oxoguanine. *Mutat. Res.*, **614**, 69–76, 2007.

[86] Bessho, T., Tano, K., Kasai, H., Ohtsuka, E., and Nishimura, S.: Evidence for two DNA repair enzymes for 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in human cells. *J. Biol. Chem.*, **268**, 19416–19421, 1993.

[87] Cathcart, R., Schwiers, E., Saul, R.L., and Ames, B.N.: Thymine glycol and thymidine glycol in human and rat urine: a possible assay for oxidative DNA damage. *Proc. Natl. Acad. Sci. USA*, **81**, 5633–5637, 1984.

[88] Cooke, M.S., Lunec, J., and Evans, M.D.: Progress in the analysis of urinary oxidative DNA damage. *Free Radic. Biol. Med.*, **33**, 1601–1614, 2002.

[89] Park, E.M., Shigenaga, M.K., Degan, P., Korn, T.S., Kitzler, J.W., Wehr, C.M., Kolachana, P., and Ames, B.N.: Assay of excised oxidative DNA lesions: isolation of 8-oxoguanine and its nucleoside derivatives from biological fluids with a monoclonal antibody column. *Proc. Natl. Acad. Sci. USA*, **89**, 3375–3379, 1992.

[90] Gackowski, D., Rozalski, R., Roszkowski, K., Jawien, A., Foksinski, M., and Olinski, R.: 8-Oxo-7,8-dihydroguanine and 8-oxo-7,8-dihydro-2'-deoxyguanosine levels in human urine do not depend on diet. *Free Radic. Res.*, **35**, 825–832, 2001.

[91] Shigenaga, M.K., Park, J.W., Cundy, K.C., Gimeno, C.J., and Ames, B.N.: *In vivo* oxidative DNA damage: measurement of 8-hydroxy-2'-deoxyguanosine in DNA and urine by high-performance liquid chromatography with electrochemical detection. *Methods Enzymol.*, **186**, 521–530, 1990.

[92] Witt, E.H., Reznick, A.Z., Viguie, C.A., Starke-Reed, P., and Packer, L.: Exercise, oxidative damage and effects of antioxidant manipulation. *J. Nutr.*, **122**, 766–773, 1992.

[93] Schoch, G., Heller-Schoch, G., Muller, J., Heddrich, M., and Gruttner, R.: Determination of RNA-metabolism as indicator of nutritional status. *Klin. Padiatr.*, **194**, 317–319, 1982.

[94] Gehrke, C.W., Kuo, K.C., Waalkes, T.P., and Borek, E.:

Patterns of urinary excretion of modified nucleosides. *Cancer Res.*, **39**, 1150–1153, 1979.

[95] Helbock, H.J., Thompson, J., Yeo, H., and Ames, B.N.: N2-methyl-8-oxoguanine: a tRNA urinary metabolite—role of xanthine oxidase. *Free Radic. Biol. Med.*, **20**, 475–481, 1996.

[96] Zhang, J., Perry, G., Smith, M.A., Robertson, D., Olson, S.J., Graham, D.G., and Montine, T.J.: Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. *Am. J. Pathol.*, **154**, 1423–1429, 1999.

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

[98] Shan, X., Chang, Y., and Lin, C.L.: Messenger RNA oxidation is an early event preceding cell death and causes reduced protein expression. *FASEB J.*, **21**, 2753–2764, 2007.

[99] Hayakawa, H. and Sekiguchi, M.: Human polynucleotide phosphorylase protein in response to oxidative stress. *Biochemistry*, **45**, 6749–6755, 2006.

[100] Tanaka, M., Chock, P.B., and Stadtman, E.R.: Oxidized messenger RNA induces translation errors. *Proc. Natl. Acad. Sci. USA*, **104**, 66–71, 2007.

[101] Beever, D. and Kemp, C.: Safety issues associated with the DNA in animal feed derived from genetically modified crops. A review of scientific and regulatory procedures. *Nutr. Abst. Rev. Series B, Livestock Feeds and Feeding*, **70**, 175–182, 2000.

[102] Klinedinst, D.K. and Drinkwater, N.R.: Mutagenesis by apurinic sites in normal and ataxia telangiectasia human lymphoblastoid cells. *Mol. Carcinog.*, **6**, 32–42, 1992.

[103] Bialkowski, K., Cysewski, P., and Olinski, R.: Effect of 2'-deoxyguanosine oxidation at C8 position on N-glycosidic bond stability. *Z. Naturforsch. C*, **51**, 119–122, 1996.

[104] Grimble, G.K.: Essential and conditionally essential nutrients in clinical nutrition. *Nutr. Res. Rev.*, **6**, 97–119, 1993.

[105] Boza, J.J., Jahoor, F., and Reeds, P.J.: Ribonucleic acid nucleotides in maternal and fetal tissues derive almost exclusively from synthesis de novo in pregnant mice. *J. Nutr.*, **126**, 1749–1758, 1996.

[106] Mohamedali, K.A., Guicherit, O.M., Kellems, R.E., and Rudolph, F.B.: The highest levels of purine catabolic enzymes in mice are present in the proximal small intestine. *J. Biol. Chem.*, **268**, 23728–23733, 1993.

[107] Berthold, H.K., Crain, P.F., Gouni, I., Reeds, P.J., and Klein, P.D.: Evidence for incorporation of intact dietary pyrimidine (but not purine) nucleosides into hepatic RNA. *Proc. Natl. Acad. Sci. USA*, **92**, 10123–10127, 1995.

[108] Evans, M.D., Cooke, M.S., Podmore, I.D., Zheng, Q., Herbert, K.E., and Lunec, J.: Discrepancies in the measurement of UVC-induced 8-oxo-2'-deoxyguanosine: implications for the analysis of oxidative DNA damage. *Biochem. Biophys. Res. Commun.*, **259**, 374–378, 1999.

[109] Schubbert, R., Hohlweg, U., Renz, D., and Doerfler, W.: On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. *Mol. Gen. Genet.*, **259**, 569–576, 1998.

[110] Schubbert, R., Renz, D., Schmitz, B., and Doerfler, W.: Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. *Proc. Natl. Acad. Sci. USA*, **94**, 961–966, 1997.

[111] Shigenaga, M.K., Gimeno, C.J., and Ames, B.N.: Urinary 8-hydroxy-2'-deoxyguanosine as a biological marker of *in vivo* oxidative DNA damage. *Proc. Natl. Acad. Sci. USA*, **86**, 9697–9701, 1989.

[112] Fraga, C.G., Shigenaga, M.K., Park, J.W., Degan, P., and Ames, B.N.: Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. *Proc. Natl. Acad. Sci. USA*, **87**, 4533–4537, 1990.

[113] Lindahl, T.: Instability and decay of the primary structure of DNA. *Nature*, **362**, 709–715, 1993.

[114] Faure, H., Mousseau, M., Cadet, J., Guimier, C., Tripier, M., Hida, H., and Favier, A.: Urine 8-oxo-7,8-dihydro-2-deoxyguanosine vs. 5-(hydroxymethyl) uracil as DNA oxidation marker in adriamycin-treated patients. *Free Radic. Res.*, **28**, 377–382, 1998.

[115] Erhola, M., Toyokuni, S., Okada, K., Tanaka, T., Hiai, H., Ochi, H., Uchida, K., Osawa, T., Nieminen, M.M., Alho, H., and Kellokumpu-Lehtinen, P.: 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 Lett.*, **409**, 287–291, 1997.

[116] Haghdoost, S., Svoboda, P., Naslund, I., Harms-Ringdahl, M., Tilikides, A., and Skog, S.: Can 8-oxo-dG be used as a predictor for individual radiosensitivity? *Int. J. Radiat. Oncol. Biol. Phys.*, **50**, 405–410, 2001.

[117] Bergman, V., Leanderson, P., Starkhammar, H., and Tagesson, C.: Urinary excretion of 8-hydroxydeoxyguanosine and malondialdehyde after high dose radiochemotherapy preceding stem cell transplantation. *Free Radic. Biol. Med.*, **36**, 300–306, 2004.

[118] Siomek, A., Tujakowski, J., Gackowski, D., Rozalski, R., Foksinski, M., Dziaman, T., Roszkowski, K., and Olinski, R.: Severe oxidatively damaged DNA after cisplatin treatment of cancer patients. *Int. J. Cancer*, **119**, 2228–2230, 2006.

[119] Shi, M., Takeshita, H., Komatsu, M., Xu, B., Aoyama, K., and Takeuchi, T.: Generation of 8-hydroxydeoxyguanosine from DNA using rat liver homogenates. *Cancer Sci.*, **96**, 13–18, 2005.

[120] Cooke, M.S., Evans, M.D., and Lunec, J.: DNA repair: insights from urinary lesion analysis. *Free Radic. Res.*, **36**, 929–932, 2002.

[121] Helbock, H.J., Beckman, K.B., Shigenaga, M.K., Walter, P.B., Woodall, A.A., Yeo, H.C., and Ames, B.N.: DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. *Proc. Natl. Acad. Sci. USA*, **95**, 288–293, 1998.

[122] Pavlov, Y.I., Minnick, D.T., Izuta, S., and Kunkel, T.A.: DNA replication fidelity with 8-oxodeoxyguanosine triphosphate. *Biochemistry*, **33**, 4695–4701, 1994.

[123] Kamath-Loeb, A.S., Hizi, A., Kasai, H., and Loeb, L.A.: Incorporation of the guanosine triphosphate analogs 8-oxo-dGTP and 8-NH2-dGTP by reverse transcriptases and mammalian DNA polymerases. *J. Biol. Chem.*, **272**, 5892–5898, 1997.

[124] Einolf, H.J., Schnetz-Boutaud, N., and Guengerich, F.P.: Steady-state and pre-steady-state kinetic analysis of 8-oxo-7,8-dihydroguanosine triphosphate incorporation and extension by replicative and repair DNA polymerases. *Biochemistry*, **37**, 13300–13312, 1998.

[125] Macpherson, P., Barone, F., Maga, G., Mazzei, F., Karran, P., and Bignami, M.: 8-oxoguanine incorporation into DNA repeats *in vitro* and mismatch recognition by MutSalpha. *Nucleic Acids Res.*, **33**, 5094–5105, 2005.

[126] Hanes, J.W., Thal, D.M., and Johnson, K.A.: Incorporation and replication of 8-oxo-deoxyguanosine by the human mitochondrial DNA polymerase. *J. Biol. Chem.*, **281**, 36241–36248, 2006.

[127] Brown, J.A., Duym, W.W., Fowler, J.D., and Suo, Z.: Single-turnover kinetic analysis of the mutagenic potential of 8-oxo-7,8-dihydro-2'-deoxyguanosine during gap-filling synthesis catalyzed by human DNA polymerases lambda and beta. *J. Mol. Biol.*, **367**, 1258–1269, 2007.

[128] Shimizu, M., Gruz, P., Kamiya, H., Masutani, C., Xu, Y., Usui, Y., Sugiyama, H., Harashima, H., Hanaoka, F., and Nohmi, T.: Efficient and erroneous incorporation of oxidized DNA precursors by human DNA polymerase eta. *Biochemistry*, **46**, 5515–5522, 2007.

[129] Arner, E.S. and Eriksson, S.: Mammalian deoxyribonucleoside kinases. *Pharmacol. Ther.*, **67**, 155–186, 1995.

[130] Van Rompay, A.R., Johansson, M., and Karlsson, A.: Phosphorylation of nucleosides and nucleoside analogs by mammalian nucleoside monophosphate kinases. *Pharmacol. Ther*, **87**, 189–198, 2000.

[131] Mathews, C.K.: DNA precursor metabolism and genomic stability. *FASEB J.*, **20**, 1300–1314, 2006.

[132] Mundt, J.M., Hah, S.S., Sumbad, R.A., Schramm, V., and Henderson, P.T.: Incorporation of extracellular 8-oxodG into DNA and RNA requires purine nucleoside phosphorylase in MCF-7 cells. *Nucleic. Acids Res.*, **36**, 228–236, 2008.

[133] Zeng, H.H., Choi, J.Y., and Chung, M.H.: 8-Hydroxyguanine, a product from damaged DNA can be used in the DNA salvage pathway. *Chin. Chem. Lett.*, **14**, 529–532, 2003.

[134] Hyun, J.W., Choi, J.Y., Zeng, H.H., Lee, Y.S., Kim, H.S., Yoon, S.H., and Chung, M.H.: Leukemic cell line, KG-1 has a functional loss of hOGG1 enzyme due to a point mutation and 8-hydroxydeoxyguanosine can kill KG-1. *Oncogene*, **19**, 4476–4479, 2000.

[135] Hyun, J.W., Jung, Y.C., Kim, H.S., Choi, E.Y., Kim, J.E., Yoon, B.H., Yoon, S.H., Lee, Y.S., Choi, J., You, H.J., and Chung, M.H.: 8-hydroxydeoxyguanosine causes death of human leukemia cells deficient in 8-oxoguanine glycosylase 1 activity by inducing apoptosis. *Mol. Cancer Res.*, **1**, 290–299, 2003.

[136] Kim, J.E., Hyun, J.W., Hayakawa, H., Choi, S., Choi, J., and Chung, M.H.: Exogenous 8-oxo-dG is not utilized for nucleotide synthesis but enhances the accumulation of 8-oxo-Gua in DNA through error-prone DNA synthesis. *Mutat. Res.*, **596**, 128–136, 2006.

[137] Kim, J.E. and Chung, M.H.: 8-Oxo-7,8-dihydro-2'-deoxyguanosine is not salvaged for DNA synthesis in human leukemic U937 cells. *Free Radic. Res.*, **40**, 461–466, 2006.

[138] Loft, S., Larsen, P.N., Rasmussen, A., Fischer-Nielsen, A., Bondesen, S., Kirkegaard, P., Rasmussen, L.S., Ejlersen, E., Tornoe, K., Bergholdt, R., and *et al.*: Oxidative DNA damage after transplantation of the liver and small intestine in pigs. *Transplantation*, **59**, 16–20, 1995.

[139] Long, L.H., Evans, P.J., and Halliwell, B.: Hydrogen peroxide in human urine: implications for antioxidant defense and redox regulation. *Biochem. Biophys. Res. Commun.*, **262**, 605–609, 1999.

[140] Loft, S., Svoboda, P., Kasai, H., Tjonneland, A., Vogel, U., Moller, P., Overvad, K., and Raaschou-Nielsen, O.: Prospective study of 8-oxo-7,8-dihydro-2'-deoxyguanosine excretion and the risk of lung cancer. *Carcinogenesis*, **27**, 1245–1250, 2006.

[141] Prat, F., Houk, K.N., and Foote, C.S.: Effect of guanine stacking on oxidation of 8-oxoguanine in B-DNA. *J. Am. Chem. Soc.*, **120**, 845–846, 1998.

[142] Yanagawa, H., Ogawa, Y., and Ueno, M.: Redox ribonucleosides. Isolation and characterization of 5-hydroxyuridine, 8-hydroxyguanosine, and 8-hydroxyadenosine from Torula yeast RNA. *J. Biol. Chem.*, **267**, 13320–13326, 1992.

[143] Uppu, R.M., Cueto, R., Squadrito, G.L., Salgo, M.G., and Pryor, W.A.: Competitive reactions of peroxynitrite with 2'-deoxyguanosine and 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxodG): relevance to the formation of 8-oxodG in DNA exposed to peroxynitrite. *Free Radic. Biol. Med.*, **21**, 407–411, 1996.

[144] Tretyakova, N.Y., Niles, J.C., Burney, S., Wishnok, J.S., and Tannenbaum, S.R.: Peroxynitrite-induced reactions of synthetic oligonucleotides containing 8-oxoguanine. *Chem. Res. Toxicol.*, **12**, 459–466, 1999.

[145] Matter, B., Malejka-Giganti, D., Csallany, A.S., and Tretyakova, N.: Quantitative analysis of the oxidative DNA lesion, 2,2-diamino-4-(2-deoxy-beta-D-erythro-pentofuranosyl)amino]-5(2H)-oxazolon e (oxazolone), *in vitro* and *in vivo* by isotope dilution-capillary HPLC-ESI-MS/MS. *Nucleic Acids Res.*, **34**, 5449–5460, 2006.

[146] Knutson, C.G., Akingbade, D., Crews, B.C., Voehler, M., Stec, D.F., and Marnett, L.J.: Metabolism *in Vitro* and *in Vivo* of the DNA Base Adduct, M1G. *Chem. Res. Toxicol.*, **20**, 550–557, 2007.

[147] Otteneder, M.B., Knutson, C.G., Daniels, J.S., Hashim, M., Crews, B.C., Remmel, R.P., Wang, H., Rizzo, C., and Marnett, L.J.: *In vivo* oxidative metabolism of a major peroxidation-derived DNA adduct, M1dG. *Proc. Natl. Acad. Sci. USA*, **103**, 6665–6669, 2006.