8-Oxo-7,8-dihydro-2’-deoxyguanosine: Reduce, Re-use, Recycle?

Marcus S. Cooke1* and Mark D. Evans

Radiation and Oxidative Stress Group, Dept. Cancer Studies and Molecular Medicine,
1 & Dept. Genetics, Robert Kilpatrick Clinical Sciences Building, University of
Leicester, Leicester, LE2 7LX, UK.

* To whom correspondence should be addressed. E-mail: msc5@le.ac.uk.

ACCEPTED IN PROCEEDINGS OF THE NATIONAL ACADEMY OF
SCIENCES, USA
Despite a variety of antioxidant defenses, cellular production of oxidants, such as reactive oxygen species (ROS), leads to a ‘background’ level of damage to the cell. Should the balance between oxidants and antioxidants shift in favor of the former, a condition of oxidative stress arises, which leads to widespread modification of molecules such as lipids and proteins. Nucleic acids, and their precursor (deoxy)ribonucleotide pools are particular targets, with over 70 damage products described, whose presence can have important implications for cell function (1). For example, in addition to mutation, oxidatively-modified DNA can lead to alterations in cell signalling and gene expression, promote microsatellite instability and accelerate telomere shortening (2), as a result oxidative stress has been implicated as a factor in a wide variety of pathological conditions, such as cancer, cardiovascular disease, aging and neurodegenerative diseases (3). The most widely studied product of DNA oxidation is 8-oxo-7,8-dihydro-2’-deoxyguanosine (8-oxodG), and its nucleobase equivalent 8-oxo-7,8-dihydrguanine (8-oxoGua). Well established methods are available for the assessment of this biomarker of oxidative stress in nuclear or mitochondrial DNA (4), as well as in extracellular matrices, such as urine (5). The prevailing view is that extracellular 8-oxodG is principally a product of Nudix hydrolase and 5’-nucleotidase activities resulting in sanitization of 8-oxodGTP from deoxyribonucleotide pools, whereas 8-oxoGua derives from the action of base excision repair enzymes, such as human 8-oxoguanine DNA glycosylase (hOGG1; Fig. 1). In a recent issue of PNAS, Hah et al. (6) utilized accelerator mass spectrometry (AMS), a technique with exquisite sensitivity, to experimentally examine previously difficult questions concerning the metabolic fate of 8-oxodG, using more realistic levels of substrates than had previously been feasible.
This work describes evidence for an apparently futile series of events in which extracellular 8-oxodG cycles through uptake, introduction into the deoxyribonucleotide pool, potential incorporation into nucleic acids, or removal by Nudix hydrolases, with implied further processing by nucleotidases and subsequent excretion into the extracellular milieu (Fig.2). Such a finding is counter-intuitive as it would be expected that, given the plethora of repair systems to ensure that oxidatively-modified bases do not persist in DNA, once removed, DNA repair products would not be substrates for reincorporation into nucleic acids.

The proposed salvage metabolism suggested by the authors is in some places in agreement, and in others at odds, with existing literature. On the basis that phosphorylation of 8-oxodGMP to 8-oxodGDP by guanylate kinase (normally involved in phosphorylation of GMP and dGMP to their corresponding dinucleotides) does not occur (7), the authors speculate upon a route that circumvents this apparent impediment to the ‘re-utilisation’ of 8-oxodG. This route hinges on the conversion of ribonucleotides to deoxyribonucleotides by ribonucleotide reductase, specifically 8-oxoGDP to 8-oxodGDP. However, in 1999 Hayakawa et al. (8) implied that ribonucleotide reductase may serve as a ‘gatekeeper’ to specifically exclude the influx of species such as 8-oxoGDP into the 2’-deoxyribonucleotide pool, albeit not preventing their possible entry into RNA, the consequences of which are discussed elsewhere (9). It is therefore vital that the ability of ribonucleotide reductase to use 8-oxoGDP as a substrate is re-evaluated. Two other enzyme activities important in the metabolic route suggested by the authors are purine nucleoside phosphorylase (PNPase) and hypoxanthine-guanine phosphoribosyltransferase (HGPRTase). While HGPRTase is reported to be able to synthesise 8-oxoGMP from 8-oxoGua and 5-phospho-D-ribosyl-1-pyrophosphate (10), PNPase is reported to be inactive towards
8-oxodG (11) and, as with ribonucleotide reductase, a critical re-evaluation of the activity of PNPase towards 8-oxodG is warranted. The phosphorylation of 8-oxodG to 8-oxodGMP by deoxynucleoside kinase is reported not to happen (11) and even if it were to do so, evidence suggests that further metabolism of 8-oxodGMP would not occur.

These recent findings may have profound implications for measurement of 8-oxo(d)G, and potentially other DNA modifications, in both DNA and urine. It is conceivable that as 8-oxodG represents only a minor structural modification of dG, it can be utilised as a substrate for many endogenous metabolic pathways. However, it might be a significant oversight to assume that such a fate does not apply to other biomarkers of DNA damage, oxidatively-derived or otherwise. Indeed, there is evidence to suggest that bromodeoxyxycytidine can be taken up by dividing cells and, following deamination and phosphorylation, and/or vice versa, is present in the deoxyribonucleoside triphosphate pool as a substrate for DNA synthesis (12). A further consequence of the findings of Hah et al. (6) is to ascribe even greater importance upon the activities of Nudix-type enzymes. These enzymes would appear to be the true gatekeepers ensuring that 8-oxodGTP is excluded from the genome, irrespective of whether it is derived from oxidation of dGTP in situ in the nucleotide pool, or from the 'piggy-backing' of extracellular 8-oxo(d)G onto the metabolic processes for native nucleobases and deoxyribonucleosides.

The authors also comment on a hypothesis that has received limited discussion, or experimental examination in the literature, that of further oxidation of 8-oxodG. The authors provide interesting preliminary evidence that, under conditions of on-going oxidative stress, 8-oxodG appears to be oxidised further. The chemical feasibility of this process has been known for a sometime and the nature of several of
the products identified. However, demonstration of their formation in a cellular system, in the context of free 2'-deoxyribonucleosides has been lacking. Those laboratories which examine urinary 8-oxodG now have to ask the question “how much material is missed due to further oxidation and what is the implication of this for biomarker studies?”

Whilst it is certain that 8-oxodG is present extracellularly in vivo, having been measured in plasma (13) and cerebrospinal fluid (14) for example, its provenance is not entirely clear. In addition to the DNA repair process described above, cell turnover and the diet are possible sources of 8-oxodG/8-oxoGua and, whilst their contribution is considered to be minimal (15, 16), they have not been ruled out entirely. Indeed significant contribution from the latter two routes probably negates the utility of measuring this lesion in urine, under any circumstances. The findings of Hah et al. (6), now extend this caveat to include assessment of nuclear, and potentially mitochondrial, 8-oxodG. Such measurements would therefore not be uniquely reflective of cellular oxidative stress, but nonetheless would represent a DNA damage burden, and hence risk/threat, to the cell.

If 8-oxodG is almost exclusively derived from DNA repair, either direct repair of DNA or, perhaps more likely, sanitisation of nucleotide pools, then the possibility of urinary 8-oxodG being used as a phenotypic marker of selected repair activities is also likely to be out of the question if salvage or loss of 8-oxodG by further oxidation are significant processes. This then restores 8-oxodG to its original context as simply a generalised marker of oxidative stress.

Whilst the data of Hah et al. (6) most certainly demonstrate the potential for extracellular 8-oxodG to be incorporated into cellular DNA, the references we cite above add to the debate and give a fuller picture of the possible processes surrounding
this interesting area. For researchers studying nucleic acid-derived biomarkers of oxidative stress, it is interesting to note that of 10.8 pmol extracellular, radio-labelled 8-oxodG added, ~8 % was localised on or inside the cells, of which only ~1.0 % became incorporated into DNA, with the remaining ~92 % being in the medium. Presumably this distribution reflects, not only incorporation, but also removal of radiocarbon-labelled 8-oxodG derivatives from DNA (and possibly RNA) and the ribo- and also deoxyribonucleotide triphosphate pools. Perturbation of these repair pathways could lead to greater incorporation into DNA. Other questions raised include: (i) determining the extent to which this phenomenon occurs in vivo, especially in the body comprised of largely non-replicating cells, indeed in non-replicating cells, most incorporation is likely to be into RNA. (ii) How big a contribution is salvage to 8-oxodGTP in the deoxyribonucleotide pool, compared to direct oxidation of dGTP (and other precursors) and what fraction of 8-oxodG in cellular DNA is derived from the deoxyribonucleotide pools, following salvage? (iii) What are typical concentrations of extra-, or indeed intra-, cellular 8-oxodG?

Nevertheless, and of broadest significance, these data should be borne in mind when interpreting the measurement of 8-oxodG, or indeed 8-oxoGua, in DNA, and extracellular matrices, such as urine.


Figure legends

Fig. 1. Origin of extracellular 8-oxo-7,8-dihydro-2’-deoxyguanosine (8-oxodG), and its nucleobase equivalent, 8-oxo-7,8-dihydro-guanine (8-oxoGua) via the action of Nudix hydrolase(s) towards the deoxyribonucleotide triphosphate pool (dNTP pool), and 8-oxoGua DNA glycosylases, e.g. human 8-oxoGua DNA glycosylase 1, towards DNA. Modified from (16).

Fig. 2. Potential fate of extracellular 8-oxo-7,8-dihydro-2’-deoxyguanosine (8-oxodG), and 8-oxo-7,8-dihydro-guanine (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. (5), 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.
Figure 1
Figure 2