Oxidative Damage to DNA in Non-Malignant Disease: Biomarker or Biohazard?

Mark D. Evans* & Marcus S. Cooke[+]

Radiation & Oxidative Stress Group
Department of Cancer Studies & Molecular Medicine
and [+]Department of Genetics
University of Leicester, Leicester, U.K.

Running Head: DNA Oxidation and Non-Malignant Disease

*Address for Correspondence:
Radiation & Oxidative Stress Group
Department of Cancer Studies & Molecular Medicine
Level 1, RKCSB
University of Leicester
Leicester Royal Infirmary
University Hospitals of Leicester NHS Trust
Leicester LE2 7LX
United Kingdom.

Full Address: Radiation & Oxidative Stress Group, Department of Cancer Studies & Molecular Medicine, Level 1 RKCSB, University of Leicester, Leicester Royal Infirmary, University Hospitals of Leicester NHS Trust, Leicester LE2 7LX, U.K. Phone: +44(0)116 2525832. Fax: +44(0)116 2525888. e-mail: mde2@le.ac.uk.

Abstract.

Oxidative damage to DNA has been examined in many non-malignant conditions, in most cases for its utility as a marker of oxidative stress. Whilst this may prove useful, attempts to answer the question - why might oxidative damage be important in this disease? - would provide added value to the biomarker data, as well as give clues to pathogenesis and perhaps therapy. In this chapter, data from the scientific literature are considered broadly, where oxidative damage to DNA has been analysed either in tissues or in extracellular matrices, such as urine, in various groups of non-malignant disease. The lesion of primary focus is 8-hydroxy-2'-deoxyguanosine, only because this is the most widely measured lesion. By coupling biomarker information with the characteristics of the disease and a set of general mechanisms whereby DNA oxidation may be pathogenic (retrospectively derived from the literature examined), we can ascribe pathogenic roles for DNA oxidation in various diseases. Based on available experimental evidence, for a wide range of conditions, such mechanisms would include prominent roles for the induction of mitochondrial dysfunction, promotion of cytotoxicity and modulation of inflammatory responses. Our general conclusion is that, dependent on the disease, oxidative DNA damage may be a biomarker, biohazard or both of these.

Introduction

Reactive oxygen species (ROS) are ubiquitous by-products of aerobic metabolism and comprise a group of free radical and non-radical species, *e.g.* superoxide, hydrogen peroxide, hydroxyl radical and hypohalous acids, of varying reactivity. Although ROS have a number of defined physiological functions, it is their potentially detrimental effects, specifically on DNA, that are a focus of this chapter. There are numerous instances throughout the scientific literature where the origins of ROS, both endogenous and exogenous, have been described [1]. Similarly, the identities, mechanisms of formation and repair of products of DNA oxidation have recently been comprehensively reviewed [2].

Conceptually, it is straightforward to appreciate a role for DNA oxidation in the pathogenesis of cancer, for example via the induction of mutations through the mis-coding properties of DNA lesions. However, there still seems to be a lack of experimental evidence directly linking DNA oxidation and carcinogenesis. The links between chronic exposure to oxidants and carcinogenesis, of which DNA oxidation is a component, are a little firmer. Certain, initially non-malignant, chronic inflammatory conditions are associated with significant potential to progress to malignancy, and aside from the modulation of gene expression, chronic inflammation is likely to involve elevated production of ROS and increased oxidative damage to DNA. Examples of this scenario include associations between inflammatory bowel diseases, such as ulcerative colitis, Crohn's disease and colorectal cancer; *H. pylori* infection/gastritis and gastric cancer; chronic gastric reflux and oesophageal cancer; cirrhosis, hepatitis B/C infection and hepatocellular carcinoma. In fact, it would seem

that chronic inflammation can drive malignancy in several tissues and elevated levels of DNA oxidation products have been detected in both inflamed and malignant tissue.

It seems that, initially at least, there is a more tenuous link between DNA oxidation and the pathogenesis of non-malignant conditions, despite the fact that markers of oxidative damage to DNA have been measured in many examples of this type of disease. Is it simply that some of these markers serve as convenient, established, markers of oxidative stress (an imbalance between oxidants and antioxidants in favour of the former)? Or does their occurrence imply something about a pathogenic role for oxidative DNA damage in these conditions? If we consider that oxidative DNA damage does have a pathogenic role in non-malignant disease, what general mechanisms may operate? (i), Damage to the genome may be so great that cells are unable to function, resulting in cytotoxicity, which could lead to the selective deletion of irreplaceable cell populations, *e.g.* in the CNS and/or pancreatic islet cells. Associated with this is more specific damage to mitochondria and the mitochondrial genome, leading to deficits in ATP production with consequences for mitochondrial/nuclear DNA repair and increased production of ROS, ultimately leading to compromised cell function and cell death. An associated factor, related to cell death is the induction of premature senescence in selected cell populations, as there is some evidence that oxidative damage can accelerate telomere shortening [3]. (ii), The cytotoxic impact of DNA damage may lead to release of cell components into the extracellular medium, this may become important in conditions where damaged material cannot be scavenged effectively, *e.g.* in autoimmune diseases such systemic lupus erythematosus. (iii), DNA damage may be sufficiently selective that coding regions of expressed genes are specifically damaged, or promoter regions, or

4

epigenetic processes such as DNA methylation are affected leading to alterations in gene expression. The impact of oxidative DNA damage on promoter function, for example, is relatively under-studied yet some more recent studies would suggest that this represents a new dimension to the pathogenic importance of oxidative DNA damage extending beyond mutagenicity [3, 4].

Measurement and importance of oxidative damage to DNA in non-malignant disease.

A plethora of DNA lesions arising from oxidative processes have been reported in the literature, encompassing various base and sugar modifications, strand breaks and more complex lesions, such as the bulkier adducts formed from the interaction of reactive aldehydes, derived from lipid peroxidation, with DNA [2]. Of this array of lesions, relatively few have received intense scrutiny, even in malignant disease. Of the simpler adducts, 8-hydroxy-7,8-dihydro-2'-deoxyguanosine (8-OH-dG) has received by far the most attention, despite elements of the analysis being fraught with problems, largely confined to its examination in extracted cellular DNA [5]. Deficits in the analysis of other lesions may have arisen because of the well-established literature for the analysis of 8-OH-dG, prompting investigators to focus upon this lesion at the expense of others, coupled with an apparent ease of 8-OH-dG formation/abundance. Additionally, despite attempts to search for and analyse multiple lesions, 8-OH-dG became the lesion of choice as few methodologies can detect multiple lesions. Levels of 8-OH-dG analysed in extracellular fluids, by chromatographic methods or immunoassay, have also received much attention, although the interpretation of changes or differences in these levels is not entirely straightforward. This is partly due to the fact that elevated levels in urine or other

extracellular matrices, could indicate enhanced oxidative damage to the genome, increased repair of lesions or even contributions from the diet and cell turnover. Notable progress is being made regarding the interpretation of extracellular levels of 8-OH-dG, with an emerging view that diet and cell turnover may have little role in contributing to urinary 8-OH-dG levels [6]. With this in mind, there are still several potential intracellular sources (repair activities) for this lesion, but if diet and cell turnover can be excluded, this presents an opportunity to re-interpret existing data in light of this new information. Whether these emerging findings are applicable to other urinary lesions derived from oxidative DNA damage (e.g. thymine glycol, 5-hydroxymethyluracil) remains to be fully established [7, 8].

There have been notably fewer studies measuring lesions derived from DNA oxidation other than 8-OH-dG, in disease, thus their biological role in this context is less well understood. Depending upon the particular conditions of DNA oxidation, the pattern of lesions produced varies and therefore whilst 8-OH-dG may be the most frequently studied, it is not necessarily the most abundant lesion under all circumstances. Furthermore, other lesions have potentially important biological consequences, and at a more basic level the potential effects of some of these lesions on mutational events, genome integrity, DNA replication and control of gene expression have received some study. Many of these lesions, which, for the purposes of this discussion include the formamidopyrimidines, thymine glycol, 5-hydroxymethyluracil (5-OHMeUra), 5-formyl uracil and 8,5'-cyclo-2'-deoxyadenosine (cyclo-dA), have been shown to possess mutagenic properties, but their effects can extend beyond this. Thymine glycol, while possessing some, albeit weak, mutagenic potential has the ability to block DNA replication, and could

therefore contribute to cytostasis and cell death. Weak replication blocking ability is also shown by 5-formyluracil, formed from oxidative modification the methyl group in thymine. There are two routes to the formation of 5-OHMeUra, and its potential biological effect is dependent upon the identity of the base from which it is formed (thymine or 5-methylcytosine). Firstly, oxidation of the thymine methyl group to yield 5-OHMeUra could have mutagenic consequences; additionally 5-OHMeUra can induce large or intermediate deletions in the genome. The formation of 5-OHMeUra via oxidation/deamination of 5-methylcytosine in CpG islands could modify gene expression, by interfering with the activities of sequence-specific DNA binding proteins involved in controlling gene expression via DNA methylation. It would seem that several different DNA oxidation products have the potential to modulate gene expression by affecting the ability of transcription factors to bind to promoter regions. 5-Formyluracil, 5-OHMeUra, cyclo-dA and 8-OH-dG have variously been shown to affect the DNA-binding properties of NFκB, Sp1 and AP-1, extending the potential pathogenic importance of these lesions beyond mutation [3, 9].

In terms of the sub-cellular sources of lesions, the nucleus, deoxynucleotide pools and mitochondria are logical locations. Mitochondrial DNA would be a particular target of interest largely because, although mitochondria are endowed with their own set of repair enzymes, mitochondrial DNA is more exposed than nuclear DNA and lies close to an important site for potential ROS production. The detrimental impact of damage to mitochondrial DNA is particularly acute in those cells dependent on mitochondrial function, such as neuronal cells or cardiomyocytes, and indeed studies would indicate a role for such damage in the pathogenesis of Alzheimer's disease or heart failure, for example [10]. Figure 1 considers the potential sources of DNA oxidation products.

As an analytical target, the popularity of 8-OH-dG relies on an abundant literature concerning analysis and biological properties, as well as the importance ascribed by the presence of multiple DNA repair pathways for this lesion. Whether the repair pathways are extensive simply because they have been studied more closely or because this lesion is peculiarly genotoxic is not entirely clear. Certainly, 8-OH-dG has been widely analysed in tissues and extracellular matrices in a number of non-malignant conditions, although a limited number of studies have examined more than one lesion. Thus, a major focus of this chapter is on 8-OH-dG, simply because of the relatively abundant literature devoted to it, a situation not shared by most of the other lesions resulting from DNA oxidation. In the following sections, we discuss a range of non-malignant conditions and also refer the reader to a recent review article which considers many of the conditions discussed herein and contains numerous literature citations relating to particular diseases [2].

Autoimmune disease

Inflammation is an important component of the pathology of systemic and tissue-specific autoimmune diseases, e.g. rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis, Sjogren's syndrome, Behcet's disease. The majority of the studies of DNA oxidation in autoimmune disease are confined to RA and SLE, with analysis being conducted predominantly in peripheral blood mononuclear cells (PBMC), with some analysis of urinary 8-OH-dG levels [11, 12]. It is presently difficult to assign exact roles for DNA oxidation in the pathogenesis of these conditions, largely because, beyond using lesion analysis as a biomarker of oxidative stress, studies examining functional impacts of these lesions on immune cell function or to explain damage to affected organ systems are lacking. Those studies

that have measured levels of 8-OH-dG, show elevated levels of this lesion in PBMC from patients with RA and SLE (also Behcet's disease and vasculitis), plus depending on the particular study, either very low or unchanged urinary excretion of 8-OH-dG in SLE and elevated excretion of 8-OH-dG in RA, compared to healthy subjects. Elevated excretion has been taken as indicative of increased oxidative stress in RA while the reports of very low levels of 8-OH-dG in urine from SLE patients has been taken as evidence of abnormal repair of 8-OH-dG, rather than a low level of oxidative damage. Despite this, functional measurements of specific enzyme activities for the repair of oxidative DNA damage in SLE seem to be minimal. Although not specific for oxidative stress, elevated levels of DNA strand breaks, in PBMC from RA patients have also been reported, again the functional significance of this is unclear.

In the case of RA, the hyperplastic tissue in the synovial joint, the pannus, is subject to an oxidative burden which is most likely accompanied by oxidative DNA damage. Indeed the ability of cells in the pannus to deal with DNA damage may be compromised by evidence of somatic mutations in p53 (transition mutations characteristic of oxidative damage) and potential abnormalities in mismatch repair, the latter accompanied by elevated levels of microsatellite instability. Thus, cell populations in the pannus are presented with opportunities to evade DNA repair and for mutations to be expressed. Collectively, there are several consequences of this for certain cell populations, including increased or decreased cell death, acquisition of growth advantages, or enhancement of a pro-inflammatory environment, since p53 is reported to be involved in controlling the expression of IL-6 for example. The recent demonstration that oxidatively-modified mitochondrial DNA (mtDNA) has pro-inflammatory properties, and can induce arthritis in a mouse model, coupled with the

presence of mtDNA and 8-OH-dG in synovial fluids of RA patients, implies that this material could help perpetuate a pro-inflammatory environment in the RA joint [13].

Modification of the known autoantigen, the ribonucleoprotein Ro60, in SLE by the lipid peroxidation end product 4-hydroxy-nonenal to enable neo-antigen formation and facilitate epitope spreading, links oxidative stress in this condition directly with the pathology. This apparently intimate involvement of oxidative stress with the pathology in SLE is supported further by reports of mice models, where antioxidants can significantly suppress mortality in the NZB x NZW F1 lupus-prone mouse and a recent report implicating oxidative stress as an early event in the development of lupus-like symptoms in the Nrf2-deficient mouse (Nrf2 is a key transcription factor involved in cellular response to oxidative stress). Oxidative modification of DNA has been shown to render it more immunogenic and also markedly increase its antigenicity for anti-dsDNA antibodies characteristic of SLE [14]. This may provide a mechanism for enhancement of selected autoantigen production which, coupled with reports of elevated apoptosis and/or poor scavenging of apoptotic debris and enhanced oxidative stress in SLE perhaps leading to enhanced cell turnover, provides a route to expose the immune system to oxidatively-modified nucleic acid. Autoantibodies specifically recognising 8-OH-dG, and other lesions (5-hydroxymethyl-2'-deoxyuridine), have been isolated from SLE patients, providing support that oxidatively modified DNA may play a role in this disease [15]. Despite this evidence, the question still remains whether damage is introduced into DNA before or after cell death, either route may be possible in light of suggestions of DNA repair abnormalities in SLE; a direct demonstration of a repair deficit for oxidative DNA damage in SLE-derived neutrophils has been reported.

Neurological disease

Oxidatively damaged DNA has been measured in several neurodegenerative conditions, most notably Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Friedrich's ataxia (FA), and Huntington's disease (HD) [16,17]. However, whilst oxidative stress, and more particularly oxidative DNA damage, may be a feature of some neurodegenerative diseases, there is not universal agreement as to its importance. Neuronal cells would seem to be particular targets for oxidative injury because of their high metabolic activity/oxygen consumption, reliance on mitochondrial function, relatively high unsaturated lipid content and transition metal ion availability. The functional impairment and death of neuronal cells has particular importance, given the potential severity their loss can have upon numerous physiological functions such as cognition, memory, learning, motor functions etc. A pathogenic mechanism for oxidative DNA damage in neurodegeneration is cytotoxicity, especially depletion of selected populations of neuronal cells, e.g. in the substantia nigra of Parkinsonian brains. It is this selectivity, and subsequent manifestation of symptoms, that is the intriguing aspect of oxidative damage in the CNS. The importance of mitochondria to the viability of neuronal cells makes the genome of these organelles a particularly crucial target for ROS, and is a recurrent theme in the pathogenesis of several major neurological disorders. Oxidative damage to mtDNA, leading to mutation or deletion events, the impairment of ATP production and increased ROS generation can have direct implications for damage to, and repair of, nuclear DNA (nDNA), in addition to the pivotal role of mitochondria in apoptosis. The validity of these generic concepts is reinforced by an examination of oxidative DNA damage analysis in selected neurodegenerative conditions:

(a). Alzheimer's disease (AD): Increased levels of oxidative damage to nDNA and mtDNA have been measured in selected regions of the brain in AD patients, as well as in PBMC. Whilst several of these studies have examined 8-OH-dG in particular, using immunostaining for example, others have shown elevated levels of various purine and pyrimidine lesions using chromatographic analyses [18]. Lower levels of extracellular 8-OH-dG in cerebrospinal fluid (CSF) from AD patients, compared to healthy subjects, coupled with elevated levels in cellular DNA, also suggests poor repair and persistence of oxidative DNA damage in AD. Damage to mitochondria in AD, and resultant neuronal dysfunction, is considered important for reasons discussed above, although it is not clear whether increased mitochondrial production of ROS and subsequent mitochondrial damage is an early or exacerbating neurodegenerative event in AD. Alterations in the expression of components of the mitochondrial electron transport chain have been observed in AD, along with mutations/deletions in the mitochondrial genome and correlations with elevated levels of 8-OH-dG. This is also interesting when taken in the context of the observation that mitochondrial hOGG1 (human 8-oxo-guanine DNA glycosylase) function may be impaired in regions of the AD brain. It would seem that initial irreversible damage could precipitate a vicious cycle of worsening mitochondrial function and subsequent loss of cellular integrity.

A recent report would indicate that the impact of oxidative DNA damage in the brain extends beyond neurodegenerative disease and into that syndrome to which we will all eventually succumb, ageing [4]. At least in the context of neuronal cells, this study suggests that the ageing brain is, perhaps not surprisingly, accompanied by alterations in gene expression. What is interesting is that there may be a specific role for oxidative DNA damage in the down-regulation of selected genes via oxidative

modification to promoter regions, which, as well as being more susceptible to damage because of their high GC content are also less likely to be repaired. What makes the promoter regions of particular genes more susceptible to damage compared to others awaits further study.

(b). Parkinson's disease (PD): Oxidative stress is associated with PD, demonstrated by increased levels of markers of protein, lipid and nucleic acid oxidation, along with deficits in antioxidant function. In particular, increased 8-OH-dG levels in cells of the substantia nigra, the region of the brain defective in PD, have been observed, as well as reports of increased levels of 8-OH-dG in CSF [19]. As with AD, defects in mitochondrial function and damage to mtDNA are apparent in PD, which could play a role in the selective destruction of cells of the substantia nigra. Dementia with Lewy bodies (DLB), is the second most common pathological subgroup of dementia after AD, sharing clinical similarities to PD and AD. Markers of oxidative stress are also elevated in this condition, including ring-opened purines and pyrimidine oxidation products, and higher levels of 8-OH-dG in the substantia nigra of DLB brains, albeit to a lesser extent than in PD.

(c). Amyotrophic lateral sclerosis (ALS; motor-neurone disease): Oxidative damage may play a role in both sporadic and familial ALS; increased levels of 8-OH-dG are found in the motor cortex from sporadic ALS brains and spinal cord from both sporadic and familial ALS. There is also significantly increased urinary excretion of 8-OH-dG in ALS, suggestive of increased oxidative stress. The selective neuronal degeneration observed in ALS may again include a pathogenic role for defective

mitochondrial function. Deficits in mtDNA repair of oxidative damage has been noted particularly in large motor neurones of the lumbar spinal cord in sporadic ALS.

(d). Huntington's disease (HD) and Friedreich's Ataxia (FA): The genetic defects in HD (expansion of CAG repeats in exon 1 of the huntingtin gene) and FA (expansion of a GAA repeat in intron 1 of the FRDA gene, coding for the protein frataxin) are known. Expression of the aberrant proteins in both of these conditions affects mitochondrial electron transport, although at different points. Their effects could ultimately be similar however, and it would seem feasible that consequential oxidative damage to DNA plays a role in the pathology, including promoting a progressive deterioration of mitochondrial function and cell death. Frataxin is involved in the regulation of mitochondrial iron content and the accumulation of mitochondrial iron in FA could further enhance oxidative damage. There is markedly increased excretion of 8-OH-dG in FA patients, supporting increased DNA oxidation in this condition. While not all reports in the literature are supportive of a role for oxidative damage in HD, there would seem to be some defects in mitochondrial function, along with evidence of regional specificity in the distribution of oxidative DNA damage to mtDNA in the brain of patients with HD, suggesting such damage may be important [20].

Cardiovascular disease

Oxidative DNA damage has been analysed primarily in atherosclerosis, in plaque tissue itself, in lymphocytes from patients with atherosclerosis or those at risk for the disease. Whilst many studies support elevated levels of lesions, particularly 8-OH-dG, these lesions have usually been measured in well established disease, thus roles for

oxidative DNA damage as an early event in cardiovascular disease (CVD) is not particularly developed. As with the neurodegenerative conditions discussed above, an important target for oxidative DNA damage in CVD would seem to be mitochondria, with death of selected cell populations being an eventual outcome, e.g. in the case of cardiomyocyte injury and heart failure [21]. The mitochondria of ischaemic hearts display abnormalities in oxidative phosphorylation, as well as an increased prevalence of the common 5 kbp deletion in the mitochondrial genome. This deletion also appears more frequently in cells of the atherosclerotic plaque and even in PBMC. It has been suggested that oxidative stress may contribute to this, and other, mtDNA deletion events and its occurrence in PBMC suggests a more global oxidative stress in the vasculature of these patients [22]. The common mtDNA deletion also accumulates with age in normal hearts, suggesting deficits in mitochondrial function accompany the ageing vasculature (as well as the ageing CNS). In atherosclerosis, increased levels of oxidised DNA lesions may serve as markers of increased oxidative stress in plaques, but what function may DNA oxidation have in the development of the plaque? Mitochondrial damage and its influence on nDNA may be one pathological role, via induction of apoptosis or necrosis, for example, perhaps enabling an accumulation of cellular debris, enhancement of inflammatory cell influx, the formation of fibrotic tissue and plaque instability/rupture. Interestingly, some suggestions from the literature would indicate that carcinogenesis and atherosclerosis may share common molecular processes, with a potential pathogenic role for DNA damage [23] In this case, this raises the possibility of (oxidative) DNA damage inducing mutation, conferring survival/growth advantages and assisting the clonal expansion of selected vascular smooth muscle cells, for example [24]. This latter area and that of the examination of somatic mutations in CVD, is receiving notable

research attention, not to the detriment of the widely accepted inflammation hypothesis of atherosclerosis, but as a complement to this. Oxidative damage to DNA in the pathogenesis of cardiovascular disease evidently extends beyond heart failure and atherosclerosis, having implications for the pathogenesis of stroke, ischaemia-reperfusion injury and pre-eclampsia.

Diabetes

In both type 1 and type 2 diabetes there are many reports of elevated oxidative stress, including markers of DNA oxidation (predominantly 8-OH-dG, but some studies have measured oxidised pyrimidines and ring-opened purines), in both extracellular matrices, peripheral blood cells and in rodent models of diabetes. As with many other studies, these lesions have been used primarily markers of oxidative stress. There are close mechanistic links between hyperglycaemia, the oxidative stress seen in this disease and, by implication, oxidative DNA damage. The latter is thought to be involved in the development of several of the important complications of diabetes, *e.g.* peripheral vascular disease, cardiomyopathy, nephropathy, and renal pathology [25]. The most likely effect of this oxidative DNA damage is a role in induction of cell death, and perhaps negative effects upon gene expression. Of direct relevance to the production of insulin, oxidative DNA damage probably plays a role in the destruction of insulin-secreting pancreatic islet β-cells in both type 1 and type 2 diabetes. Several reports do again imply an important role for mitochondrial damage, not only to the metabolically active β-cells, but also to the tissues integral to the manifestation of diabetic complications, *e.g.* there are significant correlations between tissue levels of 8-OH-dG and ~5 kbp deletions in mtDNA from muscle of patients with non-insulin dependent diabetes mellitus and in renal tissues of diabetic rats [26].

Conclusions, DNA Oxidation – biomarker, biohazard … or both?

A pathogenic role for oxidatively-damaged DNA in non-malignant disease is perhaps, for some, difficult to conceive. In part this is because in many conditions where oxidative DNA damage has been measured, little consideration has been given to pathogenic roles for such damage. It is perfectly valid to use measures of oxidative DNA damage as markers of on-going oxidative stress, although the utility of such measurements would be strengthened by parallel assessment of other markers such as indices of protein/lipid oxidation or assessment of antioxidant status. The relative importance of DNA oxidation in disease pathogenesis is another area worthy of consideration. The measurement of DNA oxidation products in various disease states has outstripped work on their pathogenic significance, as is the, perhaps understandable, pre-occupation with 8-OH-dG. This pre-occupation probably results from the quantity and quality of the information (8-OH-dG is the subject of most of the reports measuring oxidative DNA damage and is regarded as a biologically important lesion) passed on to the wider medical research community from those laboratories concerned with the measurement of DNA oxidation. Compared to 8-OH-dG, there have relatively few studies examining other products of DNA oxidation, in terms of their measurement in disease and biological impact, and redressing the balance would be welcome. Evidently, there is greater added value to the assessment of markers of oxidative DNA damage if pathogenic roles can be ascribed to this damage. Although some laboratories have gone beyond simply measuring oxidative DNA damage, many studies do not follow-up with experimental investigations as to why such damage may be important for in disease leaving conclusions to be purely conjecture by the authors. This is an area that is undergoing some expansion and we see this as necessary in order for the field to catch up with the plethora of reports

using oxidative DNA damage simply as a biomarker. Figure 2 summarises potential pathological roles of oxidative DNA damage in the various disease states mentioned in this chapter.

What is evident to us, is that nuclear DNA is not necessarily the most important target genome, and we also suggest that a viewpoint solely linking oxidative DNA damage with mutation is perhaps naive. The effects of oxidative DNA damage on epigenetic phenomena, and the control of gene expression is, we feel, set to develop over the coming years and provide renewed impetus and relevance to the free radical field. At this stage, our conclusion is perhaps predictable: oxidative DNA damage in non-malignant conditions can represent both a biohazard and a biomarker. However the extent to which the damage extends beyond being a biomarker to play a role in the pathology, and also the timing of damage in relation to the pathogenesis of the disease, also appears to depend on the condition studied.

References

1. Halliwell B, Gutteridge JMC: Free Radical Biology and Medicine, ed 3. Oxford, Oxford University Press, 1999.

2. Evans MD, Dizdaroglu M, Cooke MS: oxidative DNA damage and disease: induction, repair and significance. Mut Res: Rev Mut Res 2004; 567: 1-61.

3. Evans MD, Cooke MS: Factors contributing to the outcome of oxidative damage to nucleic acids. BioEssays 2004; 26: 533-542.

4. Lu T, Pan Y, Kao S-Y, Li C, Kohane I, Chan C, Yankner BA: Gene regulation and DNA damage in the ageing human brain. Nature 2004; 429; 883-891.

5. ESCODD (European Standards Committee on Oxidative DNA Damage): Comparative analysis of baseline 8-oxo-7,8-dihydroguanine in mammalian cell DNA, by different methods in different laboratories: an approach to consensus. Carcinogenesis, 2002; 23: 2129-2133.

6. Cooke MS, Evans MD, Dove R, Rozalski R, Gackowski D, Siomek A, Lunec J, Olinski R: DNA repair is responsible for the presence of oxidatively damaged DNA lesions in urine. Mut Res Fundam Mol Mech Mutagen 2005; 574: 58-66. 16.

7. Cooke MS, Lunec J, Evans MD: Progress in the analysis of urinary oxidative DNA damage. Free Radical Biol Med 2002; 33: 1601-1614.

8. Siomek A, Gackowski D, Foksinski M, Gran C, Klungland A, Olinski R: Diet is not responsible for the presence of several oxidatively damaged DNA lesions in mouse urine. Free Radical Res 2004; 38: 1201-1205.

9. Rogstad DK, Liu P, Burdzy A, Lin SS, Sowers, LC: Endogenous DNA lesions can inhibit the binding of the AP-1 (c-Jun) transcription factor. Biochemistry 2002; 41: 8093-8102.

10. Kang D, Hamasaki N: Alterations of mitochondrial DNA in common diseases and disease states: aging, neurodegeneration, heart failure, diabetes, and cancer. Curr Med Chem 2005; 12: 129-441.

11. Bashir S, Harris G, Denman MA, Blake DR, , Winyard PG: Oxidative DNA damage and cellular sensitivity to oxidative stress in human autoimmune diseases. Ann Rheum Dis 1993; 52: 659-666.

12. Evans MD, Cooke MS, Akil M, Samanta A, Lunec J: Aberrant processing of oxidative DNA damage in systemic lupus erythematosus. Biochem. Biophys. Res. Comms 2000; 273: 894-898.

13. Collins LV, Hajizadeh S, Holme E, Jonsson IM, Tarkowski A: Endogenously oxidized mitochondrial DNA induces in vivo and in vitro inflammatory responses. J Leukoc Biol 2004; 75: 995-1000.

14. Cooke MS, Mistry N, Wood C, Herbert KE, Lunec J: Immunogenicity of DNA damaged by reactive oxygen species – implications for anti-DNA antibodies in lupus. Free Radical Biol Med 1997; 22: 151-159.

15. Frenkel K, Karkoszka J, Kim E, Taioli E: Recognition of oxidized DNA bases by sera of patients with inflammatory diseases. Free Radical Biol Med 1993; 14: 483-494.

16. Cooke MS: Neurodegenerative disease and the repair of oxidatively damaged DNA; in Beal MF, Lang AE, Ludolph A (eds): Neurodegenerative Diseases. Cambridge University Press, Cambridge, 2005.

17. Sayre L M, Smith MA, Perry, G: Chemistry and biochemistry of oxidative stress in neurodegenerative disease. Curr Med Chem 2001; 8: 721-738.

18. Migliore L, Fontana I, Colognato R, Coppede F, Siciliano G, Murri, L: Searching for the role and the most suitable biomarkers of oxidative stress in Alzheimer's disease and in other neurodegenerative diseases. Neurobiol Ageing 2005; 26: 587-595.

19. Alam ZI, Jenner A, Daniel SE, Lees AJ, Cairns N, Marsden CD, Jenner P, Halliwell B: Oxidative DNA damage in the Parkinsonian brain: An apparent selective increase in 8-hydroxyguanine levels in substantia nigra. J Neurochem 1997; 69: 1196-1203.

20. Schapira AH: Mitochondrial involvement in Parkinson's disease, Huntington's disease, hereditary spastic paraplegia and Friedreich's ataxia. Biochem Biophys Acta 1999; 1410: 159-170.

21. Tsutsui H: Oxidative stress in heart failure: the role of mitochondria. Intern Med 2001; 12: 1177-1182

22. Botto N, Berti S, Manfredi S, Al-Jabri A, Federici C, Clerico A, Ciofini E, Biagini A, Andreassi, MG: Detection of mtDNA with 4977 bp deletion in blood cells and atherosclerotic lesions of patients with coronary artery disease. Mutat Res 2005; 570: 81-88.

23. Ross JS, Stagliano NE, Donovan MJ, Breitbart RE, Ginsburg GS: Atherosclerosis and cancer: Common molecular pathways of disease development and progression. Ann NY Acad Sci 2001; 947: 271-293.

24. Andreassi MG, Botto N: Genetic instability, DNA damage and atherosclerosis. Cell Cycle 2003; 2; 224-227.

25. Hinokio Y, Suzuki S, Hirai M, Chiba M, Hirai A, Toyota T: Oxidative DNA damage in diabetes mellitus: its association with diabetic complications. Diabetologia 1999; 42: 995-998.

26. Suzuki S, Hinokio Y, Komatu K, Ohtomo M, Onoda M, Hirai S, Hirai M, Hirai A, Chiba M, Kasuga S, Akai H, Toyota T: Oxidative damage to mitochondrial DNA and its relationship to diabetic complications. Diabetes Res Clin Pract 1999; 45: 161-168.

Figure Legends

Figure 1: Potential sources and targets of intra- and extracellular DNA oxidation products.

Antioxidants can counteract DNA oxidation and include low molecular weight species, *e.g.* vitamin C, vitamin E and glutathione, as well as high molecular weight antioxidants, *e.g.* catalase, glutathione peroxidase and superoxide dismutase. 2'-deoxynucleotide triphosphates oxidised in the nucleotide (dNTP) pools act as a potential source for mis-incorporation of oxidation products into DNA, 8-oxo-2'-deoxyguanosine triphosphate, for example, can be removed from the cell via the action of human mutT homologue (hMTH1). nDNA = nuclear DNA; mtDNA = mitochondrial DNA.

Figure 2: Summary of pathological impacts of oxidative damage to DNA in non-malignant conditions, with references to specific evidence in particular disease states.

Oxidant-mediated alteration in gene expression would have numerous impacts on cell function and survival and thus may overarch the other phenomena in the figure. The role of somatic mutation in atherosclerosis is still tentative. AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; FA, Friedreich's ataxia; HD, Huntington's disease; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; PD, Parkinson's disease; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.
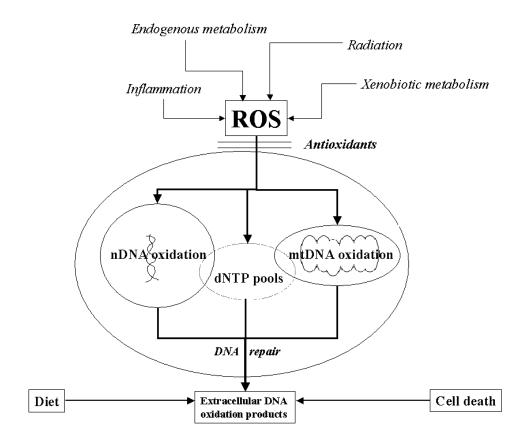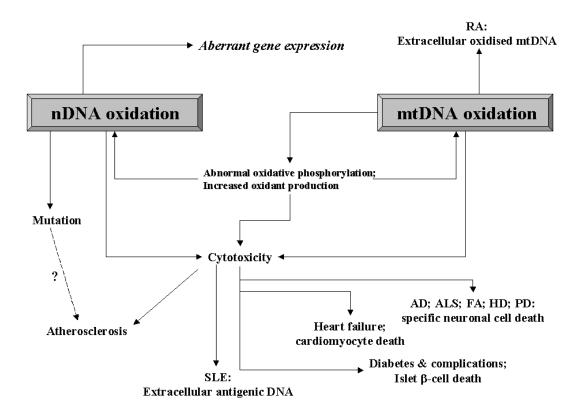
Figure 1

Figure 2