Available online at www.sciencedirect.com
SCIENCE DIRECT®
Experimental and Toxicologic Pathology 57 (2005) 161–169
EXPERIMENTAL AND TOXICOLOGIC PATHOLOGY
www.elsevier.de/etp

Oxidative DNA modifications

Henrik E. Poulsen*

Department of Clinical Pharmacology Q7642, Rigshospitalet, 9 Blegdamsvej, DK-2100 Copenhagen, Denmark

Received 25 April 2005

Abstract

Oxidative DNA modifications are frequent in mammalian DNA and have been suggested an important mechanism in carcinogenesis, diabetes and ageing. The foundations for this suggestion are:

Evidence for the importance of oxidative DNA modifications in cancer development is: high levels of oxidative lesions in cancer tissue; highly conserved and specific DNA repair systems targeting oxidative lesions; high levels of oxidative DNA lesions in oxidative DNA repair knock-out animals; defective repair of oxidative lesions in cancer-prone progeria syndromes; reduced cancer incidence in populations with high dietary antioxidant intake; and increased oxidative stress to DNA in tobacco smokers. Conflicting evidence for a relation between oxidative stress to DNA and cancer is: disagreement about the true levels and occurrence of the oxidative lesions in vivo; failure to identify the localization of oxidative lesions in important genes, e.g. tumor suppressor and oncogenes; lack of evidence that the oxidative lesions induce mutations in vivo; no cancer development in animals knocked-out for specific DNA repair enzymes in spite of high tissue levels of oxidative lesions; and unchanged cancer rates after antioxidant interventions in large clinical controlled and randomized trials.

The rate of DNA oxidation has been estimated from urinary excretion of repair products and it is evident that if these lesions were not repaired, a large part of DNA would be oxidized to a degree not compatible with living.

The methodologies by which oxidative DNA modifications are measured cover a wide and different range, advantages and disadvantages will be presented. One particular problem is artificial oxidation, and methods to prevent such artifacts will be presented together with results from a large interlaboratory standardization program. The methodology by which the lesions can be measured is complicated and prone to artifacts during DNA isolation, digestion, derivatization and maybe even during the separation procedure proper prior to detection. A large effort from 20+ laboratories supported by a grant from the EU has reduced artifacts considerably and work towards interlaboratory standardization of the methodology is in progress. The presently agreed "normal" levels of the most frequent known lesion 8-oxodG is about 5 per million dG's in DNA.

A comprehensive evaluation of the evidence, from chemistry to clinical and epidemiological trials, linking oxidative modifications to cancer will be given. Finally, an estimate of the quantitative role oxidative DNA modifications play among the multiplicity of other insults is given.

*Tel.: +45 3545 7671; fax: +45 3545 2745.
E-mail address: hepo@rh.dk.

0940-2993/$ - see front matter
doi:10.1016/j.etp.2005.05.015

While there is no question that all of these oxidative mechanisms do exist, quantitative data on their importance for the human situation do not exist. Prospective human studies that can provide such quantitative data on different mechanisms are underway.

Keywords: Oxidative stress; DNA repair; DNA damage; Aging; Defense system

Introduction

Cells are constantly exposed to oxidants from metabolic and other biochemical reactions as well as external factors. Oxidative modifications of DNA are abundant, mutagenic and thought to be important in carcinogenesis and aging as supported by experimental studies in animals and in vitro (Ames et al., 1995; Loft and Poulsen, 1996; Wiseman and Halliwell, 1996). Indeed cells are constantly exposed to oxidants from both physiological processes, such as mitochondrial respiration (Chance et al., 1979), and pathophysiological conditions such as inflammation, ischemia/reperfusion, foreign compound metabolism and radiation (Ames et al., 1995). The bodily defences against oxidants include an extensive system of antioxidant enzymes and radical scavengers and chain breakers, of which many are nutritionally dependent. Failure of the system of enzymatic, endogenous and nutritional antioxidants may lead to mutagenic oxidative DNA damage as well as deregulation of cell cycle control, resulting in carcinogenesis. Fortunately, damage to DNA is also repaired with high efficiency in the cells in the body (Demple and Harrison, 1994; Laval, 1994). DNA repair enzymes, such as glycosylases, including *OGG1* and also nucleotide excision repair enzymes, including *ERCC1*, appear highly active in order to prevent oxidative damage from resulting in mutations. However, there is limited knowledge about the regulation of these genes in relation to oxidative stress and DNA damage.

DNA damage

The cellular DNA is subject to a wide variety of modifications induced by normal cellular functions as well as by external agents. These agents induce damage either by formation of reactive oxygen species (ROS) or by direct damage in the form of purine dimers, cross-links, strand breaks, or adduct formation.

DNA damages are considered as the most serious ROS-induced cellular modifications as DNA is not synthesized de novo but copied and hence the modifications can induce mutations and genetic instability. Nearly 100 different free radical modifications in DNA have been identified (von Sonnentag, 1987; Dizdaroglu, 1992), classified as base- or deoxyribose lesions, strand breaks or cross-links. By measuring excretion of some of the DNA repair products in the urine, it has been estimated that without repair accumulation of DNA oxidations would double in 60–190 days and result in oxidation of 1% of the DNA bases in 8 years (Poulsen et al., 1998a).

Despite antioxidant defense systems and extensive DNA repair of oxidatively modified DNA, complete protection from the noxious effects of ROS cannot be provided. The oxygen species are thus suspected to be implicated in a number of diseases including cancer, atherosclerosis, diabetes, several neurodegenerative disorders, and stressors like noice and university examinations (Ames, 2001; Vasavada and Agarwal, 2005; Masalkar and Abhang, 2005; Carlsson et al., 2005; Schulze and Lee, 2005; Sullivan and Brown, 2005; Drost et al., 2005; Maguire-Zeiss et al., 2005; Sato et al., 2005; Sivonova et al., 2004; Liu et al., 2005; Behl, 2005).

Analysis of oxidized DNA and excreted repair products

High-performance (or pressure) liquid chromatography (HPLC) is particularly suited for small water-soluble molecules and proteins. Most used for analysis of DNA fragments is the reverse-phase HPLC. Quantification by electrochemical detection (Loft et al., 1992) or mass spectrometry (Weimann et al., 2001, 2002) is preferred. Regarding mass spectrometry with triple quadropoles, it must be emphasized that unknown urinary substances similar in mass to 8-oxodG need to be separated from 8-oxodG by elaborate chromatography, just as is the case with electrochemical detection.

Following derivatization it is possible to use the gas chromatography separation procedure coupled with mass spectrometry to measure oxidized DNA products. However, this method has with few exceptions not been used for urinary measurements of DNA oxidation products, but has been the method used for estimation of tissue DNA, actually the modified bases after hydrolysis, and other DNA oxidation products in tissue. For urine measurements by gas chromatography–mass spectrometry (GC–MS) a semi-preparative HPLC procedure was applied, followed by hydrolysis, derivatization and then GC–MS analysis (Pourcelot et al., 1999; Faure et al., 1998).

GC–MS used for quantification of oxidative DNA products in tissue has been criticized for errors due to artificial oxidation, however, provided that sufficient precautions are taken; this can be avoided and results similar to those from HPLC-ECD can be provided regarding 8-oxodG in DNA (Rodriguez et al., 2000). Presumably, this is also valid for other oxidative DNA products, but needs to be validated. In case of 8-oxodA the validity has been questioned (Poulsen et al., 1998b) regarding 8-oxodA in an experiment with vitamin C and vitamin E intervention (Podmore et al., 1998) and using HPLC-MS/MS it seems likely that the high reported 8-oxodA values relates to artificial oxidation (Weimann et al., 2001). Many of the problems regarding artificial oxidation relate to the very high content of non-oxidized dG in DNA hydrolysates, about 1,000,000 times higher than 8-oxodG. This means that oxidation of only a very minute fraction of dG gives deleterious artifacts from a quantitative aspect. For urine measurements, the levels of oxidized and non-oxidized nucleosides are similar and would a priori not present a problem of the same magnitude. For these reasons most groups have abandoned the use of GC–MS for tissue analysis.

The use of a specific antibody could be the basis for a fast and effective methodology to measure 8-oxodG. However, it has proven difficult to produce an antibody with sufficient specificity for analysis in urine. Several publications have appeared (Erhola et al., 1997; Leinonen et al., 1997; Tsuboi et al., 1998; Thompson et al., 1999). However, although some characterization of the antibody and epitope is given, it appears not to be tested against the many different DNA and RNA products in urine (Schram, 1998). Furthermore, testing against the present method of choice HPLC-ECD, GC–MS or HPLC-MSMS has only been stated without data, and at the present time the data have not been made available in the literature (Erhola et al., 1997). One particular problem with the immunologically based assays may relate to the high number of DNA/RNA fragments/metabolites and similar substances excreted into urine. In case of RNA products, high concentrations of very similar chemical substances are excreted into urine (Schram, 1998). A similar myriad of DNA products undoubtedly is also excreted. Together this may make it very difficult to produce a specific antibody. A commercially available kit tested out against the three-dimensional HPLC-ECD shoved clear non-specificity (Prieme et al., 1996). Until clear demonstration of close correlation to the verified HPLC-ECD or HPLC-MS/MS methods, the use of immunologically based methods for quantification of 8-oxodG in urine cannot be recommended. A very comprehensive and detailed review of all the methodologies has been published by Guetens et al. (2002).

Interpretation of urinary excretion and tissue levels of 8-oxodG

The urinary excretion of 8-oxodG in pigs following i/v injection follows simple kinetics with a half-life of a about 2.5 h, a clearance of about 4 mL min^{-1} kg BW^{-1} and a volume of distribution close to 1 L kg^{-1} BW (Loft et al., 1995), and moreover the urinary excretion rate corresponded to the infusion rate. After liver transplantation we observed an increased urinary excretion of 8-oxodG and in a vena cava clamp experiment the excretion was temporarily reduced. These experiments indicate that steady state between formation and urinary excretion is obtained rapidly. Collectively, these data indicate that the 8-oxodG in the urine mainly originates from genomic DNA. However, on a more detailed level the contribution of 8-oxodG from the nucleotide pool cell turnover, cell death, and from inflammatory cells is unknown. Presently, neither direct nor indirect data from the in-vivo situation are available.

Accepting that the contribution of nuclear DNA reflects the oxidation of nuclear DNA, the urinary excretion is a reflection of the average total oxidative stress to DNA of all body cells. In most experimental situations in vivo, it is reasonable to argue that a given person is in a steady state, i.e. a constant 8-oxodG level in DNA and a constant repair. Mass conservation will be applicable and consequently the amount of excreted 8-oxodG will equal newly formed 8-oxodG. The urinary measurement is therefore equal to the rate of oxidative stress to DNA. If an experimental or other form of change happens (say smoking cessation, antioxidant intervention), a new steady state will soon be reached and a change in the rate of oxidation of DNA can be identified. It is important to stress that this measure is independent of DNA repair, a point often not recognized.

Tissue levels of 8-oxodG in DNA reflect a balance between newly formed 8-oxodG's and removal by repair. An increased level can consequently reflect either an increased formation (increased oxidative stress) or a decrease in repair or a combination. It is important to note that a distinction cannot be made solely from measurement of the concentration in DNA of modified bases. In conclusion urinary excretion of 8-oxodG is a measure of the rate of whole body DNA oxidation, and tissue levels reflect the concentration resulting from balance between formation and repair.

Biological consequences of oxidative stress

Oxidative stress can result in damage to important macromolecules such as lipids, proteins and DNA, and have been related to arteriosclerosis (Salonen, 1988,

Table 1. Oxidative DNA damage and exposure to polluted air in humans

Chemical exposure	Increased biomarkers	No increase
Tobacco smoking	8-oxodG in urine, WBC and sperm, comet SSB	Comet assay 8-oxodG in WBC and sperm
Urban air pollution	8-oxodG and 8-oxoGua excretion, comet SSB	
Benzene	8-oxodG excretion	8-oxodG in WBC
	Comet SSB	Comet SSB, Fpg
Styrene, solvents,	Comet SSB	
Vinyl chloride	Comet SSB	Fpg/Endonuclease III sites
Waste matter	Comet SSB	
Wood dust	Comet SSB	Comet SSB

Adapted from Loft and Poulsen (1996, 1998a, b, 1999); Loft et al. (1998, 1999); Moller et al. (2000).
SSB: single strand breaks; Fpg: fapyguanine glycosylase.

2000; Steinberg et al., 1989; Steinberg and Witztum, 1990), cancer (Ames, 2001; Ames et al., 1993, 1995; Halliwell, 1998; Hoeijmakers, 2001) and cataract (Shichi, 2004; Ramana et al., 2004; Varma et al., 1984).

Cellular processes lead to generation of oxidants, including ROS. However, also a large number of environmental factors can lead to increased DNA oxidation. Some relevant environmental factors are listed in Table 1.

The theory of oxidative stress and disease development builds on a rather simplistic view that oxidation was damaged and if prevented, disease could be prevented or the onset protracted. A number of trials have been initiated; however, particularly in the prevention of cancer the results have been negative. The ATBC study (Albanes et al., 1996; The Alpha-Tocopherol and Beta-Carotene Cancer Prevention Group, 1994) showed that smokers who ingested large amounts of beta-carotene had an increased risk of developing lung cancer and no protection against arteriosclerosis, which was also confirmed in other subsequent large controlled trials (Hennekens et al., 1996). Most recently, a controlled trial with prevention of secondary primary cancers in head and neck reported higher rate after high vitamin E intake (Bairati et al., 2005). All these trials used single or few antioxidants in high doses.

The recent SU.VI.MAX study (Hercberg et al., 2004) pointed at a possible benefit in men but not in women from low doses of multiple antioxidant vitamins.

Presently, it must be concluded that large-dose antioxidant vitamins do not prevent cancer or arteriosclerosis.

The negative findings in clinical trials give rise to reconsiderations about the underlying mechanisms. The trials indicate that antioxidant vitamins have biological effects, although they do not appear on the beneficial side, and probably the simplistic idea about quenching of damaging ROS by antioxidants is not sufficient, if valid at all.

It is becoming increasingly clear that the redox status and oxidants per se induce a large number of changes in cells. In Table 2, some important genes that are regulated by oxidants are compiled. Table 3 provides examples of changes in intracellular signalling brought about by oxidants and antioxidants. The exact meaning and consequences of such changes are presently not sufficiently clear to understand the relation to disease development.

Presently, there is no epidemiological evidence available that high levels of oxidative DNA modification in tissue or high urinary excretion of oxidatively modified nucleic acid products are predictive for cancer development in man. Such data are mandatory to establish that DNA oxidation is relevant for cancer development, and also a requirement in other diseases and ageing.

Conclusions

Oxidative stress is well documented as a biological phenomenon. It has been demonstrated that oxidative stress leads to oxidation of important macromolecules, particularly DNA and it has been hypothesized as an important pathogenic factor in the development of cancer. Controlled trials with antioxidant supplementation did show biological effects of such supplementation; however, the results consistently point at increased cancer rates. Oxidants as well as antioxidants have been shown to modulate gene regulation and intracellular signalling. The hypothesis of antioxidant function as quenches of free oxygen radicals is most probably much too simple; rather, is should be appreciated that a large number of cellular processes including gene regulation and intracellular signalling are modulated by oxidants and antioxidants resulting in a complex cellular change. If this has relation to the oxidation of DNA is not clear. Presently, it is clear that the complex result of large-dose antioxidants can result in increased cancer rates. It is

Table 2. Oxidative stress and gene regulation/activity

Gene/enzyme/protein	Regulation by oxidants: (O) = oxidants; (A) = antioxidants	Regulation by anti-oxidants	Reference
JUN kinase (JNK)	Induced (O)	Inhibited (GSH)	Choi and Moore, 1993; Karin and Smeal, 1992; Karin, 1995; Lander et al., 1995
c-FOS	Increased transcription (O)	Inhibited (phenolics)	Choi and Moore, 1993; Sachsenmaier et al., 1994; Stein et al., 1989
MAP kinase	Stimulation (O)		Whisler et al., 1995
Tyrosine phosphatase	Inhibition (O)		Whisler et al., 1995
KAM-1, IL-1alfa, IL6, IL8, heme oxygenase	Induced (O)		Grether-Beck et al., 1997
Metal binding proteins (MT-genes)	Induced (O)		Bauman et al., 1991, 1992a, b; Dalton et al., 1994, 1996; De et al., 1990; Min et al., 1991
Heme oxygenase-1	Induced (O)		Inamdar et al., 1996
Caspases	Induced (O)		Yang et al., 1997; Kluck et al., 1997; Zamzami et al., 1995, 1996; Dypbukt et al., 1994
Caspases	Inhibited by severe oxidative stress (O)		Kim et al., 1997
PKC	Induced (O)		Whisler et al., 1995; Larsson and Cerutti, 1989; Brawn et al., 1995; Kass et al., 1989
PKC	Inactivated (O)		Gopalakrishna and Anderson, 1987, 1991
Ras	Activated (O)		Lander et al., 1995, 1996, 1997
Phospholipase A and D	Activated		Ito et al., 1997; Natarajan et al., 1996

Table 3. Oxidative stress and intracellular signalling

Elements	Effect of oxidants (O) and anti-oxidants (A)	Reference
NF-kb	Activation (O) blocking (A) controversial	Devary et al., 1993; Mohan and Meltz, 1994; Schulze-Osthoff et al., 1993, 1995, 1997; Staal et al., 1990 Toledano and Leonard, 1991; Droge et al., 1994; Galter et al., 1994
AP-1	Activated (O,A) blocking (O,A)	Abate et al., 1990, 1991; Edwards and Mahadevan, 1992; Hunter and Karin, 1992; Karin, 1995; Yang-Yen et al., 1990
Ca^{2+}	Intracellular increase (O)	Hoyal et al., 1996; Suzuki et al., 1997
AP-1	Activated (O)	Abate et al., 1990; Okuno et al., 1993
p53	Complex regulation	Hainaut and Milner, 1993; Hainaut et al., 1995; Hupp et al., 1993

unclear if it is possible to devise a means based on such principles that will be able to reduce cancer rates. The effects of attempts to modulate disease outcome by dietary antioxidants clearly point very complicated and complex mechanisms.

References

Abate C, Patel L, Rauscher III FJ, Curran T. Redox regulation of fos and jun DNA-binding activity in vitro. Science 1990;249:1157–61.

Abate C, Luk D, Curran T. Transcriptional regulation by Fos and Jun in vitro: interaction among multiple activator and regulatory domains. Mol Cell Biol 1991;11:3624–32.

Albanes D, Heinonen OP, Taylor PR, Virtamo J, Edwards BK, Rautalahti M, Hartman AM, Palmgren J, Freedman LS, Haapakoski J, Barrett MJ, Pietinen P, Malila N, Tala E, Liippo K, Salomaa ER, Tangrea JA, Teppo L, Askin FB, Taskinen E, Erozan Y, Greenwald P, Huttunen JK. Alpha-Tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. J Natl Cancer Inst 1996;88:1560–70.

Ames BN. DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutat Res 2001; 475:7–20.

Ames BN, Shigenaga MK, Gold LS. DNA lesions, inducible DNA repair, and cell division: three key factors in mutagenesis and carcinogenesis. Environ Health Perspect 1993;101(Suppl 5):35–44.

Ames BN, Gold LS, Willett WC. The causes and prevention of cancer. Proc Natl Acad Sci USA 1995;92:5258–65.

Bairati I, Meyer F, Gelinas M, Fortin A, Nabid A, Brochet F, Mercier jP, Tetu B, Harel F, Masse B, Vigneault E, Vass S, del Vecchio P, Roy J. A randomized trial of antioxidant vitamins to prevent second primary cancers in head and neck cancer patients. J Natl Cancer Inst 2005;97:481–8.

Bauman JW, Liu J, Liu YP, Klaassen CD. Increase in metallothionein produced by chemicals that induce oxidative stress. Toxicol Appl Pharmacol 1991;110:347–54.

Bauman JW, Madhu C, McKim Jr. JM, Liu Y, Klaassen CD. Induction of hepatic metallothionein by paraquat. Toxicol Appl Pharmacol 1992a;117:233–41.

Bauman JW, McKim Jr. JM, Liu J, Klaassen CD. Induction of metallothionein by diethyl maleate. Toxicol Appl Pharmacol 1992b;114:188–96.

Behl C. Oxidative stress in Alzheimer's disease: implications for prevention and therapy. Subcell Biochem 2005;38: 65–78.

Brawn MK, Chiou WJ, Leach KL. Oxidant-induced activation of protein kinase C in UC11MG cells. Free Radical Res 1995;22:23–37.

Carlsson PI, Laer LV, Borg E, Bondeson ML, Thys M, Fransen E, Camp GV. The influence of genetic variation in oxidative stress genes on human noise susceptibility. Hear Res 2005;202:87–96.

Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev 1979;59:527–605.

Choi HS, Moore DD. Induction of c-fos and c-jun gene expression by phenolic antioxidants. Mol Endocrinol 1993;7:1596–602.

Dalton T, Palmiter RD, Andrews GK. Transcriptional induction of the mouse metallothionein-I gene in hydrogen peroxide-treated Hepa cells involves a composite major late transcription factor/antioxidant response element and metal response promoter elements. Nucleic Acids Res 1994;22:5016–23.

Dalton TP, Li Q, Bittel D, Liang L, Andrews GK. Oxidative stress activates metal-responsive transcription factor-1 binding activity. Occupancy in vivo of metal response elements in the metallothionein-I gene promoter. J Biol Chem 1996;271:26233–41.

De SK, McMaster MT, Andrews GK. Endotoxin induction of murine metallothionein gene expression. J Biol Chem 1990;265:15267–74.

Demple B, Harrison L. Repair of oxidative damage to DNA: enzymology and biology. Annu Rev Biochem 1994;63: 915–48.

Devary Y, Rosette C, DiDonato JA, Karin M. NF-kappa B activation by ultraviolet light not dependent on a nuclear signal. Science 1993;261:1442–5.

Dizdaroglu M. Oxidative damage to DNA in mammalian chromatin. Mutat Res 1992;275:331–42.

Droge W, Schulze-Osthoff K, Mihm S, Galter D, Schenk H, Eck HP, Roth S, Gmunder H. Functions of glutathione and glutathione disulfide in immunology and immunopathology. FASEB J 1994;8:1131–8.

Drost EM, Skwarski KM, Sauleda J, Soler N, Roca J, Agusti A, Macnee W. Oxidative stress and airway inflammation in severe exacerbations of COPD. Thorax 2005;60:293–300.

Dypbukt JM, Ankarcrona M, Burkitt M, Sjoholm A, Strom K, Orrenius S, Nicotera P. Different prooxidant levels stimulate growth, trigger apoptosis, or produce necrosis of insulin-secreting RINm5F cells. The role of intracellular polyamines. J Biol Chem 1994;269:30553–60.

Edwards DR, Mahadevan LC. Protein synthesis inhibitors differentially superinduce c-fos and c-jun by three distinct mechanisms: lack of evidence for labile repressors. EMBO J 1992;11:2415–24.

Erhola M, Toyokuni S, Okada K, Tanaka T, Hiai H, Ochi H, Uchida K, Osawa T, Nieminen MM, Alho H, 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 1997;409:287–91.

Faure H, Mousseau M, Cadet J, Guimier C, Tripier M, Hida H, Favier A. Urine 8-oxo-7, 8-dihydro-2-deoxyguanosine vs. 5-(hydroxymethyl) uracil as DNA oxidation marker in adriamycin-treated patients. Free Radical Res 1998;28: 377–82.

Galter D, Mihm S, Droge W. Distinct effects of glutathione disulphide on the nuclear transcription factor kappa B and the activator protein-1. Eur J Biochem 1994;221:639–48.

Gopalakrishna R, Anderson WB. Susceptibility of protein kinase C to oxidative inactivation: loss of both phosphotransferase activity and phorbol diester binding. FEBS Lett 1987;225:233–7.

Gopalakrishna R, Anderson WB. Reversible oxidative activation and inactivation of protein kinase C by the mitogen/tumor promoter periodate. Arch Biochem Biophys 1991; 285:382–7.

Grether-Beck S, Buettner R, Krutmann J. Ultraviolet A radiation-induced expression of human genes: molecular and photobiological mechanisms. Biol Chem 1997;378: 1231–6.

Guetens G, De Boeck G, Highley M, van Oosterom AT, de Bruijn EA. Oxidative DNA damage: biological significance and methods of analysis. Crit Rev Clin Lab Sci 2002; 39:331–457.

Hainaut P, Milner J. Redox modulation of p53 conformation and sequence-specific DNA binding in vitro. Cancer Res 1993;53:4469–73.

Hainaut P, Rolley N, Davies M, Milner J. Modulation by copper of p53 conformation and sequence-specific DNA binding: role for Cu(II)/Cu(I) redox mechanism. Oncogene 1995;10:27–32.

Halliwell B. Can oxidative DNA damage be used as a biomarker of cancer risk in humans? Problems, resolutions and preliminary results from nutritional supplementation studies. Free Radical Res 1998;29:469–86.

Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano M, Ridker PM, Willett W, Peto R. Lack of long-term supplementation

with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 1996;334: 1145–9.

Hercberg S, Galan P, Preziosi P, Bertrais S, Mennen L, Malvy D, Roussel AM, Favier A, Briancon S. The SU.VI. MAX study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. Arch Intern Med 2004;164:2335–42.

Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature 2001;411:366–74.

Hoyal CR, Thomas AP, Forman HJ. Hydroperoxide-induced increases in intracellular calcium due to annexin VI translocation and inactivation of plasma membrane Ca^{2+}-ATPase. J Biol Chem 1996;271:29205–10.

Hunter T, Karin M. The regulation of transcription by phosphorylation. Cell 1992;70:375–87.

Hupp TR, Meek DW, Midgley CA, Lane DP. Activation of the cryptic DNA binding function of mutant forms of p53. Nucleic Acids Res 1993;21:3167–74.

Inamdar NM, Ahn YI, Alam J. The heme-responsive element of the mouse heme oxygenase-1 gene is an extended AP-1 binding site that resembles the recognition sequences for MAF and NF-E2 transcription factors. Biochem Biophys Res Com 1996;221:570–6.

Ito Y, Nakashima S, Nozawa Y. Hydrogen peroxide-induced phospholipase D activation in rat pheochromocytoma PC12 cells: possible involvement of Ca^{2+}-dependent protein tyrosine kinase. J Neurochem 1997;69:729–36.

Karin M. The regulation of AP-1 activity by mitogen-activated protein kinases. J Biol Chem 1995;270:16483–6.

Karin M, Smeal T. Control of transcription factors by signal transduction pathways: the beginning of the end. Trends Biochem Sci 1992;17:418–22.

Kass GE, Duddy SK, Orrenius S. Activation of hepatocyte protein kinase C by redox-cycling quinones. Biochem J 1989;260:499–507.

Kim YM, Talanian RV, Billiar TR. Nitric oxide inhibits apoptosis by preventing increases in caspase-3-like activity via two distinct mechanisms. J Biol Chem 1997;272: 31138–48.

Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. Science 1997;275: 1132–6.

Lander HM, Ogiste JS, Teng KK, Novogrodsky A. p21ras as a common signaling target of reactive free radicals and cellular redox stress. J Biol Chem 1995;270:21195–8.

Lander HM, Milbank AJ, Tauras JM, Hajjar DP, Hempstead BL, Schwartz GD, Kraemer RT, Mirza UA, Chait BT, Burk SC, Quilliam LA. Redox regulation of cell signalling [letter]. Nature 1996;381:380–1.

Lander HM, Hajjar DP, Hempstead BL, Mirza UA, Chait BT, Campbell S, Quilliam LA. A molecular redox switch on p21(ras). Structural basis for the nitric oxide-p21(ras) interaction. J Biol Chem 1997;272:4323–6.

Larsson R, Cerutti P. Translocation and enhancement of phosphotransferase activity of protein kinase C following exposure in mouse epidermal cells to oxidants (published erratum appears in Cancer Res 1990 Jan 1;50(1):212). Cancer Res 1989;49:5627–32.

Laval F. Expression of the E-coli fpg gene in mammalian cells reduces the mutagenicity of gamma-rays. Nucleic Acids Res 1994;22:4943–6.

Leinonen J, Lehtimaki T, Toyokuni S, Okada K, Tanaka T, Hiai H, Ochi H, Laippala P, Rantalaiho V, Wirta O, Pasternack A, Alho H. New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus. FEBS Lett 1997;417:150–2.

Liu Q, Smith MA, Avila J, DeBernardis J, Kansal M, Takeda A, Zhu X, Nunomura A, Honda K, Moreira PI, Oliveira CR, Santos MS, Shimohama S, Aliev G, de la TJ, Ghanbari HA, Siedlak SL, Harris PL, Sayre LM, Perry G. Alzheimer-specific epitopes of tau represent lipid peroxidation-induced conformations. Free Radical Biol Med 2005;38:746–54.

Loft S, Poulsen HE. Cancer risk and oxidative DNA damage in man. J Mol Med 1996;74:297–312.

Loft S, Poulsen HE. Estimation of oxidative DNA damage in man from urinary excretion of repair products. Acta Biochim Pol 1998a;45:133–44.

Loft S, Poulsen HE. Measurement of oxidative damage to DNA: interpretation of nuclear levels and urinary excretion of repair products in DNA damage and repair. In: Karakaya A, Dizdaroglu M, editors. Oxygen Radical Effects, Cellular Protection and Biological Consequences. New York: Plenum Press; 1998b. p. 267–81.

Loft S, Poulsen HE. Markers of oxidative damage to DNA: antioxidants and molecular damage. Methods Enzymol 1999;300:166–84.

Loft S, Vistisen K, Ewertz M, Tjonneland A, Overvad K, Poulsen H E. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. Carcinogenesis 1992;13:2241–7.

Loft S, Larsen PN, Rasmussen A, Fischer-Nielsen A, Bondesen S, Kirkegaard P, Rasmussen LS, Ejlersen E, Torn°e K, Bergholdt R, Poulsen HE. Oxidative DNA damage after transplantation of the liver and small intestine in pigs. Transplantation 1995;59:16–20.

Loft S, Deng XS, Tuo J, Wellejus A, Sorensen M, Poulsen HE. Experimental study of oxidative DNA damage. Free Radical Res 1998;29:525–39.

Loft S, Poulsen HE, Vistisen K, Knudsen LE. Increased urinary excretion of 8-oxo-2′-deoxyguanosine, a biomaker of oxidative DNA damage, in urban bus drivers. Mutat Res 1999;441:11–9.

Maguire-Zeiss KA, Short DW, Federoff HJ. Synuclein, dopamine and oxidative stress: co-conspirators in Parkinson's disease? Brain Res Mol Brain Res 2005;134:18–23.

Masalkar PD, Abhang SA. Oxidative stress and antioxidant status in patients with alcoholic liver disease. Clin Chim Acta 2005;355:61–5.

Min KS, Terano Y, Onosaka S, Tanaka K. Induction of hepatic metallothionein by nonmetallic compounds associated with acute-phase response in inflammation. Toxicol Appl Pharmacol 1991;111:152–62.

Mohan N, Meltz ML. Induction of nuclear factor kappa B after low-dose ionizing radiation involves a reactive oxygen intermediate signaling pathway. Radiat Res 1994;140: 97–104.

Moller P, Knudsen LE, Loft S, Wallin H. The comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents and effect of confounding factors. Cancer Epidemiol Biomarkers Prev 2000;9:1005–15.

Natarajan V, Vepa S, Verma RS, Scribner WM. Role of protein tyrosine phosphorylation in H_2O_2-induced activation of endothelial cell phospholipase D. Am J Phys 1996; 271:L400–8.

Okuno H, Akahori A, Sato H, Xanthoudakis S, Curran T, Iba H. Escape from redox regulation enhances the transforming activity of Fos. Oncogene 1993;8:695–701.

Podmore ID, Griffiths H, Herbert K, Mistry N. Does vitamin C have a pro-oxidant effect? Nature 1998;395:231–2.

Poulsen HE, Prieme H, Loft S. Role of oxidative DNA damage in cancer initiation and promotion. Eur J Cancer Prev 1998a;7:9–16.

Poulsen HE, Weimann A, Salonen JT, Nyyssonen K, Loft S, Cadet J, Douki T, Ravanat JL. Does vitamin C have a pro-oxidant effect? [letter]. Nature 1998b;395:231–2.

Pourcelot S, Faure H, Firoozi F, Ducros V, Tripier M, Hee J, Cadet J, Favier A. Urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine and 5-(hydroxymethyl) uracil in smokers. Free Radical Res 1999;30:173–80.

Prieme H, Loft S, Cutler RG, Poulsen HE. Measurement of oxidative DNA injury in humans: evaluation of a commercially available ELISA assay. In: Kumpulainen JT, editor. Natural Antioxidants and Food Quality in Atherosclerosis and Cancer Prevention. The Royal Society of Chemistry; 1996. p. 78–82.

Ramana KV, Chandra D, Wills NK, Bhatnagar A, Srivastava SK. Oxidative stress-induced up-regulation of the chloride channel and Na^+/Ca^{2+} exchanger during cataractogenesis in diabetic rats. J Diabetes Complications 2004; 18:177–82.

Rodriguez H, Jurado J, Laval J, Dizdaroglu M. Comparison of the levels of 8-hydroxyguanine in DNA as measured by gas chromatography mass spectrometry following hydrolysis of DNA by *Escherichia coli* Fpg protein or formic acid. Nucleic Acids Res 2000;28:E75.

Sachsenmaier C, Radler-Pohl A, Muller A, Herrlich P, Rahmsdorf H J. Damage to DNA by UV light and activation of transcription factors. Biochem Pharmacol 1994;47:129–36.

Salonen JT. Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study. Ann Clin Res 1988;20:46–50.

Salonen JT. Markers of oxidative damage and antioxidant protection: assessment of LDL oxidation. Free Radical Res 2000;33(Suppl):S41–6.

Sato S, Mizuno Y, Hattori N. Urinary 8-hydroxydeoxyguanosine levels as a biomarker for progression of Parkinson disease. Neurology 2005;64:1081–3.

Schram KH. Urinary nucleosides. Mass Spectrom Rev 1998; 17:131–251.

Schulze PC, Lee RT. Oxidative stress and atherosclerosis. Curr Atheroscler Rep 2005;7:242–8.

Schulze-Osthoff K, Beyaert R, Vandevoorde V, Haegeman G, Fiers W. Depletion of the mitochondrial electron transport abrogates the cytotoxic and gene-inductive effects of TNF. EMBO J 1993;12:3095–104.

Schulze-Osthoff K, Los M, Baeuerle PA. Redox signalling by transcription factors NF-kappa B and AP-1 in lymphocytes. Biochem Pharmacol 1995;50:735–41.

Schulze-Osthoff K, Bauer MK, Vogt M, Wesselborg S. Oxidative stress and signal transduction. Int J Vitam Nutr Res 1997;67:336–42.

Shichi H. Cataract formation and prevention. Expert Opin Invest Drugs 2004;13:691–701.

Sivonova M, Zitnanova I, Hlincikova L, Skodacek I, Trebaticka J, Durackova Z. Oxidative stress in university students during examinations. Stress 2004;7:183–8.

Staal FJ, Roederer M, Herzenberg LA, Herzenberg LA. Intracellular thiols regulate activation of nuclear factor kappa B and transcription of human immunodeficiency virus. Proc Natl Acad Sci USA 1990;87:9943–7.

Stein B, Rahmsdorf HJ, Steffen A, Litfin M, Herrlich P. UV-induced DNA damage is an intermediate step in UV-induced expression of human immunodeficiency virus type 1, collagenase, c-fos, and metallothionein. Mol Cell Biol 1989;9:5169–81.

Steinberg D, Witztum JL. Lipoproteins and atherogenesis. Current concepts. JAMA 1990;264:3047–52.

Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-lipoprotein that increase its atherogenecity. N Engl J Med 1989; 320:915–24.

Sullivan PG, Brown MR. Mitochondrial aging and dysfunction in Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 2005;29:407–10.

Suzuki YJ, Forman HJ, Sevanian A. Oxidants as stimulators of signal transduction. Free Radical Biol Med 1997;22: 269–85.

The Alpha-Tocopherol and Beta-Carotene Cancer Prevention Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994;330:1029–35.

Thompson HJ, Heimendinger J, Haegele A, Sedlacek SM, Gillette C, O'Neill C, Wolfe P, Conry C. Effect of increased vegetable and fruit consumption on markers of oxidative cellular damage. Carcinogenesis 1999;20:2261–6.

Toledano MB, Leonard WJ. Modulation of transcription factor NF-kappa B binding activity by oxidation-reduction in vitro. Proc Natl Acad Sci USA 1991;88:4328–32.

Tsuboi H, Kouda K, Takeuchi H, Takigawa M, Masamoto Y, Takeuchi M, Ochi H. 8-hydroxydeoxyguanosine in urine as an index of oxidative damage to DNA in the evaluation of atopic dermatitis. Br J Dermatol 1998;138:1033–5.

Varma SD, Chand D, Sharma YR, Kuck Jr. JF, Richards RD. Oxidative stress on lens and cataract formation: role of light and oxygen. Curr Eye Res 1984;3:35–57.

Vasavada N, Agarwal R. Role of oxidative stress in diabetic nephropathy. Adv Chronic Kidney Dis 2005;12: 146–54.

von Sonnentag C. The Chemical Basis for Radiation Biology. London: Taylor and Francis; 1987.

Weimann A, Belling D, Poulsen HE. 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 Radical Biol Med 2001;30:757–64.

Weimann A, Belling D, Poulsen HE. 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 2002;30:E7.

Whisler RL, Goyette MA, Grants IS, Newhouse YG. Sublethal levels of oxidant stress stimulate multiple serine/threonine kinases and suppress protein phosphatases in Jurkat T cells. Arch Biochem Biophys 1995;319:23–35.

Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem J 1996;313:17–29.

Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng TI, Jones DP, Wang X. Prevention of apoptosis by Bcl-2: release of cytochrome *c* from mitochondria blocked [see comments]. Science 1997;275:1129–32.

Yang-Yen HF, Chiu R, Karin M. Elevation of AP1 activity during F9 cell differentiation is due to increased c-jun transcription. New Biol 1990;2:351–61.

Zamzami N, Marchetti P, Castedo M, Zanin C, Vayssiere JL, Petit PX, Kroemer G. Reduction in mitochondrial potential constitutes an early irreversible step of programmed lymphocyte death in vivo. J Exp Med 1995;181:1661–72.

Zamzami N, Susin S A, Marchetti P, Hirsch T, Gomez-Monterrey I, Castedo M, Kroemer G. Mitochondrial control of nuclear apoptosis [see comments]. J Exp Med 1996;183:1533–44.