

REVIEW

Endogenous DNA damage in humans: a review of quantitative data

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DNA damage plays a major role in mutagenesis, carcinogenesis and ageing. The vast majority of mutations in human tissues are certainly of endogenous origin. A thorough knowledge of the types and prevalence of endogenous DNA damage is thus essential for an understanding of the interactions of endogenous processes with exogenous agents and the influence of damage of endogenous origin on the induction of cancer and other diseases. In particular, this seems important in risk evaluation concerning exogenous agents that also occur endogenously or that, although chemically different from endogenous ones, generate the same DNA adducts. This knowledge may also be crucial to the development of rational chemopreventive strategies. A list of endogenous DNA-damaging agents, processes and DNA adduct levels is presented. For the sake of comparison, DNA adduct levels are expressed in a standardized way, including the number of adducts per 10⁶ nt. This list comprises numerous reactive oxygen species and products generated as a consequence (e.g. lipid peroxides), endogenous reactive chemicals (e.g. aldehydes and S-adenosylmethionine), and chemical DNA instability (e.g. depurination). The respective roles of endogenous versus exogenous DNA damage in carcinogenesis are discussed.

Introduction

DNA damage plays a major role in mutagenesis, carcinogenesis and ageing. The chemical events that lead to DNA damage include hydrolysis, exposure to reactive oxygen substances (ROS) and other reactive metabolites. These reactions are triggered by exposure to exogenous chemicals or they can result from metabolic, endogenous processes. The concentrations and mutagenic potentials of known carcinogens to which we are exposed in our environment are insufficient to explain the high incidence of sporadic cancer that is actually seen in our population (Epe, 2002). Innate factors can also not suffice to explain this high incidence. Epidemiology shows that, in developed societies, exogenous factors are a necessary condition in about 75–80% of cancer cases (Doll and Peto, 1981; Trichopoulos *et al.*, 1994). So, mutations due to DNA damage, caused by unidentified exogenous agents, and to an increase in endogenous damage modulated by exogenous factors must play a role in most cases of cancer, in addition to changes in gene expression due to exogenous conditions. A thorough knowledge of the types and prevalence of endogenous DNA damage can be considered essential for an understanding of the

interaction of exogenous agents and influences with endogenous processes in the induction of cancer and other diseases. In particular, this is important for risk analysis concerning low dose environmental factors. Endogenous DNA damage occurs at a high frequency compared with exogenous damage and the types of damage produced by normal cellular processes are identical or very similar to those caused by some environmental agents (Jackson and Loeb, 2001). The study of endogenous damage is also of importance to chemoprevention. It is evident that if an approach could be developed leading to a decrease in endogenous DNA damage and endogenous mutations, the incidence of cancer and other diseases might be substantially reduced, even without a reduction in exogenous mutations.

Here we present a review of quantitative data on the occurrence of endogenous DNA adducts in humans.

Oxidative DNA damage

In living cells ROS are formed continuously as a consequence of metabolic and other biochemical reactions as well as external factors. These ROS include superoxide (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radicals (OH[•]) and singlet oxygen (¹O₂) and they can oxidize DNA, which can lead to several types of DNA damage, including oxidized bases and single- and double-strand breaks. DNA damage produced by ROS is the most frequently occurring damage.

Oxidatively modified DNA is, despite extensive DNA repair, abundant in many human tissues, especially in tumours (Iida *et al.*, 2001; Li *et al.*, 2002). Many defence mechanisms within the organism have evolved to limit the levels of reactive oxidants and the damage they induce (Slupphaug *et al.*, 2003). Oxidative stress occurs when the production of ROS exceeds the body's natural antioxidant defence mechanisms, causing damage to macromolecules such as DNA, proteins and lipids. ROS also inactivate antioxidant enzymes (Kono and Fidovich, 1982; Tabatabaie and Floyd, 1994). So, as pointed out by Epe (2002), any change in the endogenous generation of ROS or cellular antioxidants or in the efficiency of DNA repair should cause a corresponding modulation of the steady-state levels of oxidative DNA modifications, which in turn should modulate the mutation rate and ultimately the cancer incidence. Epidemiological evidence from different studies points to reduced risks for cancer, particularly in the upper gastrointestinal tract and airways, associated with a diet rich in antioxidants and/or a high content of antioxidants in plasma samples (Loft and Poulsen, 1996).

Data suggest that the rate of damage decreases with age, possibly along with the decreasing rate of metabolism, whereas the steady-state levels increase due to failing repair (Loft and Poulsen, 1996).

Apart from exogenous substances, the production of ROS occurs through a variety of endogenous processes. During mitochondrial respiration 1–5% of the oxygen undergoes

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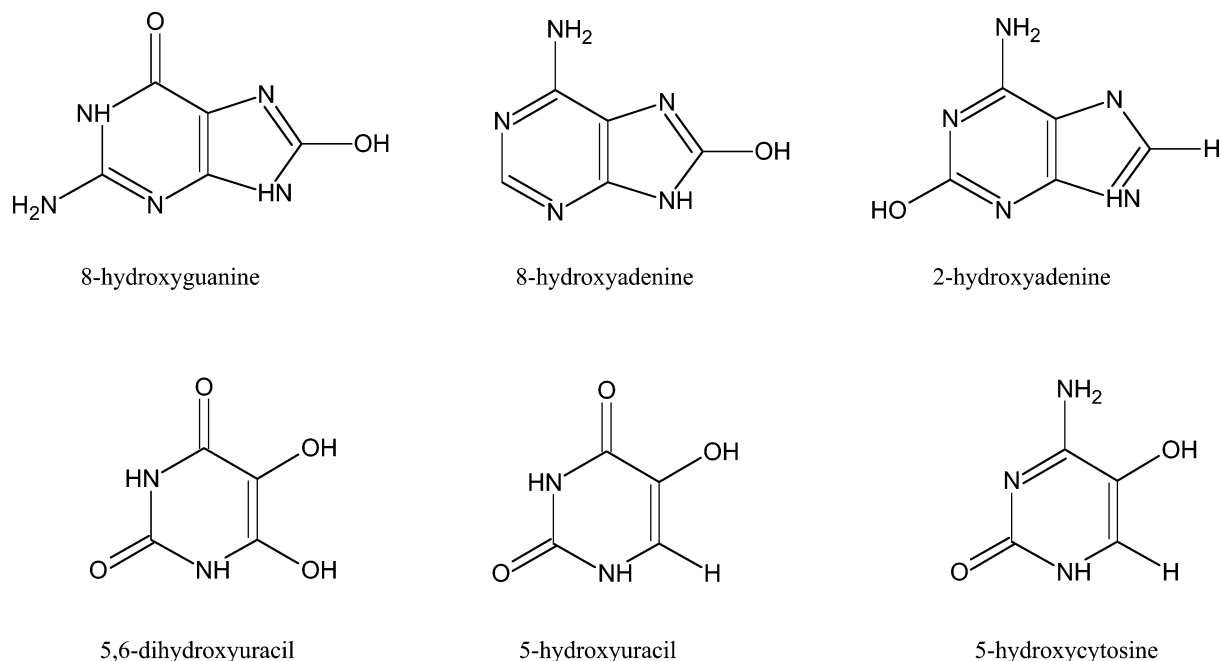
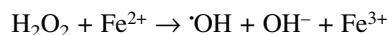
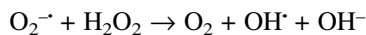


Fig. 1. Some oxidative DNA adducts.

single electron transfer, generating the superoxide anion radical (superoxide, $O_2^{\cdot-}$) (Chance *et al.*, 1979). This molecule shows limited reactivity but is converted to hydrogen peroxide by superoxide dismutase. Reduction of hydrogen peroxide to water is secured by catalase and glutathione peroxidase. However, in the presence of transition metals, such as iron and copper, hydrogen peroxide is reduced to hydroxyl radicals (HO^{\cdot}) (Loft and Poulsen, 1996). The reactivity of HO^{\cdot} is so great that it does not diffuse more than one or two molecular diameters before reacting with a cellular component (Pryor, 1986). It must be generated immediately adjacent to DNA to be able to oxidize it. It is likely that H_2O_2 serves as a diffusible, latent form of HO^{\cdot} that reacts with a metal ion in the vicinity of a DNA molecule to generate HO^{\cdot} (Marnett, 2000). This is called the Fenton reaction:



Chronic infections that elicit an inflammatory response are potent generators of ROS. Neutrophils produce oxygen bursts of superoxide radical and hydrogen peroxide, which can subsequently interact via the Haber–Weiss reaction to form the potent hydroxyl radical (Jackson and Loeb, 2001):



Shen *et al.* (2000) exposed DNA to activated neutrophils or eosinophils and this resulted in 8-oxo-deoxyguanosine (8-oxo-dG) formation through a pathway that was blocked by antioxidant agents and enzymes. Another DNA oxidant is peroxynitrite (ONO_2^-). It is the product of nitric oxide, a vascular relaxant and neurotransmitter, and superoxide and although it generates small quantities of HO^{\cdot} , its protonated form (peroxynitrous acid, ONO_2H) is an extremely reactive oxidant capable of oxidizing DNA independent of its ability to generate HO^{\cdot} (Richeson *et al.*, 1998). Nitric oxide and superoxide are produced simultaneously in macrophages, so it can be assumed that elevated levels of ONO_2^- would be

produced in activated inflammatory cells. Ambs *et al.* (1999) demonstrated an association between the occurrence of G→T transversions in the *p53* gene of human colorectal cancers and the level of expression of the inducible form of nitric oxide synthase in gastric precancerous and cancerous lesions. Many studies found a relationship between increased levels of oxidized bases and cancer or inflammatory diseases like hepatitis, cirrhosis or *Helicobacter pylori* infection (Marnett, 2000). Exposure of DNA to ONO_2^- can form strand breaks and base oxidation products and can cause, in some systems, deamination of G and A, but the major product of reaction between DNA and ONO_2^- is 8-nitro-deoxyguanosine (Burcham, 1999). This adduct has a tendency to depurinate. It has also been shown that nitrogen oxide inhibits various DNA repair enzymes, such as FAPY glycosylase, which removes 8-oxo-guanine (8-oxo-G). This suggests that there may exist a synergism between the ability of nitrogen oxide to stimulate DNA damage by the generation of peroxynitrite and the ability to inhibit the repair of this damage (Laval *et al.*, 1997; Jaiswal *et al.*, 2001).

Figure 1 shows some oxidative DNA adducts. Among the oxidatively modified bases, 8-oxo-dG is apparently the most abundant but certainly the most investigated. Steady-state levels of this adduct determined by the various techniques in human cells ranged over several orders of magnitude: between 0.07 and 145.25 adducts/ 10^6 nt (Higuchi and Lin, 1995; Spencer *et al.*, 1996). It base pairs preferentially with adenine rather than cytosine and thus generates GC→TA transversion mutations after replication. Oxidative DNA damage is predominantly repaired by base excision repair (BER) enzymes. The rate of repair of purine lesions is slower than that of pyrimidine lesions (Jaruga and Dizdaroglu, 1996). Most of the damage is removed before the cell reaches replication, the stage at which damage can be fixed as a mutation. It is evident that variations in repair capability would affect the amount of oxidative DNA damage, and indeed some rare human diseases

characterized by aberrant DNA repair (xeroderma pigmentosum, ataxia telangiectasia, Bloom's syndrome and Fanconi's anaemia) are also characterized by an elevated amount of 8-oxo-dG (Degan *et al.*, 1995; Evans *et al.*, 2000). NO, an inflammatory mediator, directly inhibits a key BER enzyme (hOgg1) responsible for BER of 8-oxo-G. NO-mediated inhibition of DNA repair could thus contribute to the mutagenic environment of chronic inflammation (Jaiswal *et al.*, 2001).

Other abundant oxidatively modified bases are the formamidopyrimidine adducts of adenine and guanine: 4,6-diamino-5-formamidopyrimidine (FapyAdenine) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGuanine) (Burgdorf and Carell, 2002). The latter is especially formed in the absence of oxygen by ionizing radiation and other ROS-producing agents. This lesion is efficiently repaired by a special formamidopyrimidine glycosylase but it can lead to GC→CG transversions (Ono *et al.*, 1995). Other modified adenine bases include 8-oxo-adenine (7,8-dihydro-8-oxoadenine) and 2-hydroxy-2'-deoxyadenosine. Oxidation of 2'-deoxycytidine can lead to the formation of 5-hydroxy-2'-deoxycytosine, 5-hydroxy-2'-deoxyuridine and 5,6-dihydroxy-5,6-dihydro-2'-deoxyuridine (Wagner *et al.*, 1992).

Other oxidized bases are 5-hydroxymethylthymidine, 5-hydroxymethyluracil, uracil glycol, cytosine glycol and thymine glycol. 5-Hydroxyuracil and uracil glycol are products of the oxidative deamination of deoxycytosine (dC). They are detected in comparable amounts to 8-oxo-dG in human DNA (Burcham, 1999). Oxygen radicals react with 5-methylcytosine to oxidize the 5,6-double bond; the intermediate product, 5-methylcytosine glycol, then deaminates to form thymine glycol. Thymine glycol base pairs with A and results in a C→T transition (Marnett and Plataras, 2001). It is a weak mutagen (Basu *et al.*, 1989) and it blocks transcription and replication (Dianov *et al.*, 2000). This lesion is primarily repaired by the BER pathway (Dianov *et al.*, 2000). Of the pyrimidine-derived lesions, 5-hydroxy-2'-deoxycytosine (5-OH-Cyt) and 5-hydroxyuracil (5-OH-Ura) have been shown to be potentially premutagenic lesions leading to GC→AT transitions and GC→CG transversions (Purmal *et al.*, 1994). 5-OH-Cyt appears to be more mutagenic than any other product of oxidative DNA damage (Feig *et al.*, 1994). 5-Hydroxymethyluracil is generated through oxidation of the methyl group of thymine (Rogstad *et al.*, 2002). This methyl group plays a major role in various DNA-protein interactions. This adduct thus interferes with the binding of transcription factors to DNA (Rogstad *et al.*, 2002). This is a non-mutagenic lesion but it is removed by higher organisms. This removal can trigger apoptosis as a consequence of chromosomal breakage and it can also result in deletion mutations (Rogstad *et al.*, 2002).

Table I summarizes measurements of oxidative DNA adducts in human tissues and Table VI shows the types of mutations that can be induced by different oxidative DNA lesions.

Lipid peroxidation

The polyunsaturated fatty acid residues of phospholipids are extremely sensitive to oxidation. Lipid hydroperoxides are the initial products of unsaturated fatty acid oxidation, but they are relatively short lived. They are either reduced by glutathione peroxidases to unreactive fatty acid alcohols or they react with

metals to produce epoxides, aldehydes, etc. The major aldehyde products of lipid peroxidation are crotonaldehyde, acrolein, 4-hydroxynonenal (HNE) and malondialdehyde (MDA). These reactive substances damage DNA by the formation of exocyclic adducts. They block the Watson-Crick base pairing region and most of them are anticipated to be highly mutagenic. MDA appears to be the most mutagenic product of lipid peroxidation, whereas HNE is the most toxic (Esterbauer *et al.*, 1990).

The levels of DNA adducts in rodent and human tissues and leucocytes were found to be highly variable and to be affected by lifestyle, the dietary intake of antioxidants and the type and amount of fatty acids and persistent chronic infections or inflammation, in which nitric oxide is often overproduced (Bartsch, 1999). Consumption of a diet high in polyunsaturated fatty acids (PUFAs) has been shown to cause an increase in propano-, etheno- and malondialdehyde-derived adducts in human leucocyte DNA in women but not in men (Fang *et al.*, 1996; Nair *et al.*, 1997). It could be suggested that etheno and propano adducts are involved in carcinogenesis because they have been detected in target tissues of rodents treated with carcinogens such as vinyl chloride, ethyl carbamate and *N*-nitrosopyrrolidine (Chung *et al.*, 1996). Table II summarizes measurements of lipid peroxidation induced DNA adducts in human tissues.

Propano adducts

These adducts appear to be derived by reaction of DNA with acrolein, crotonaldehyde and HNE, all generated by lipid peroxidation. Crotonaldehyde and acrolein are also widespread in the environment: they are generated by burning fats and by cigarette smoking. Crotonaldehyde can also react with H₂O to form 3-hydroxybutanal, which reacts with DNA to produce the Schiff base *N*²-(3-hydroxybut-1-ylidene)dG as well as several diastereomers of *N*²-paraldol-dG (Hecht *et al.*, 2001) (Figure 2). HNE is weakly mutagenic but appears to be the major toxic product of lipid peroxidation (Esterbauer *et al.*, 1991). It modulates the expression of genes that are involved in cell cycle control and apoptosis.

Pan and Chung (2002) showed that the rate of acrolein adduct formation is ~5- to 10-fold that of crotonaldehyde adducts, depending on the type of PUFA, and the rate of formation of HNE adducts from arachidonic acid is also considerably slower than that of acrolein adducts. The reactivity of enals toward dG decreases with the increasing chain length acrolein > crotonaldehyde > HNE (Pan and Chung, 2002). The numbers of propano adducts measured in humans ranged between 0.0006 and 0.40 adducts/10⁶ nt (Nath and Chung, 1994; Chung, 2000).

Some rodent studies suggest that a higher level of 1,*N*²-propano-dG adducts occurs in tissues with a high fat content, such as brain, liver and colon (Nath *et al.*, 1996). Studies in humans showed that intake of dietary fats increases the levels of these adducts in lymphocyte DNA of women (Fang *et al.*, 1996; Nair *et al.*, 1997).

Etheno adducts

The former aldehydes (acrolein, croton aldehyde and HNE) can be converted to epoxyaldehydes by different oxidative processes (Chung *et al.*, 1996). The resulting epoxyaldehydes could modify DNA bases yielding etheno adducts. Epoxyaldehydes are more reactive towards DNA than the parent enals, especially the long-chain enals (Chung *et al.*, 1996). The reaction of epoxyaldehydes of acrolein (glycidaldehyde),

Table I. Oxidative DNA damage

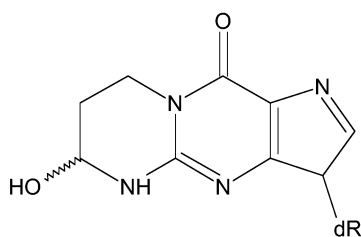
Adduct	Method	Tissue	Quantity		Reference
				Adducts/10 ⁶ nt	
5-Hydroxy-2'-deoxycytidine	HPLC/EC	Leucocytes	3.1 ± 1.6 fmol/μg DNA	1.02	Wagner <i>et al.</i> (1992)
	HPLC/EC ^a	Lymphocytes	2.9 ± 1.4 adducts/10 ⁶ dG	0.58	Lenton <i>et al.</i> (1999)
5-Hydroxy-2'-deoxyuridine	GC/MS/SIM	Bronchial epithelium	0.161 ± 0.005 nmol/mg DNA	52.79	Spencer <i>et al.</i> (1996)
	HPLC/EC	Leucocytes	2.1 ± 1.8 fmol/μg DNA	0.69	Wagner <i>et al.</i> (1992)
5,6-Dihydroxy-5,6-dihydro-2'-deoxyuridine	HPLC/EC	Leucocytes	6.2 ± 4.6 fmol/μg DNA	2.03	Wagner <i>et al.</i> (1992)
8-Oxo-7,8-dihydro-2'-deoxyguanosine	HPLC/EC	Human leucocytes	3.5 ± 2.1 fmol/μg DNA	1.15	Wagner <i>et al.</i> (1992)
	³² P/HPLC	Human leucocytes	8 adducts/10 ⁵ dG	15.92	Wilson <i>et al.</i> (1993)
	HPLC/EC and HPLC/EC	Leucocytes before versus after smoking	3.3 ± 0.8 versus 5.1 ± 2.5 adducts/10 ⁶ dG	0.66 versus 1.01	Kiyosawa <i>et al.</i> (1990)
	HPLC/EC	Leucocytes	3.9 ± 0.26 adducts/10 ⁵ dG	7.76	Degan <i>et al.</i> (1995)
	HPLC/EC	PMN leukocytes	118 mol/10 ⁶ mol dG	23.48	Bashir <i>et al.</i> (1993)
	GC/MS	Human peripheral lymphocytes	30 adducts/10 ⁵ dG	59.70	Podmore <i>et al.</i> (1998)
	HPLC/EC	Lymphocytes	68 mol adducts/10 ⁶ mol dG	13.52	Bashir <i>et al.</i> (1993)
	HPLC/EC ^a	Lymphocytes	4.5 ± 1.8 adducts/10 ⁶ dG	0.90	Lenton <i>et al.</i> (1999)
	HPLC/EC ^a	Lymphocytes	1.19–2.17 adducts/10 ⁶ dG	0.24–0.43	Bianchini <i>et al.</i> (2001)
	HPLC/EC	HeLa cells (mtDNA)	0.35 adducts/10 ⁶ dG	0.07	Higuchi and Lin (1995)
	HPLC/EC	Brain (mtDNA)	15.78–27.34 adducts/10 ⁵ dG	31.40–54.41	Mecocci <i>et al.</i> (1993)
	HPLC/EC	Brain (nDNA)	1.25–2.70 adducts/10 ⁵ dG	2.49–5.37	Mecocci <i>et al.</i> (1993)
	HPLC/EC	Brain (mtDNA)	15.3–35.7 adducts/10 ⁵ dG	30.45–71.04	Mecocci <i>et al.</i> (1994)
	HPLC/EC	Brain (nDNA)	1.20–2.60 adducts/10 ⁵ dG	2.39–5.17	Mecocci <i>et al.</i> (1994)
	GC/MS/SIM	Bronchial epithelium	0.443 ± 0.095 nmol/mg DNA	145.25	Spencer <i>et al.</i> (1996)
	Fpg/Southern blot	mtDNA	5 adducts/10 ⁶ bases	5.00	Anson <i>et al.</i> (2000)
	HPLC/EC	Sperm cells	13 fmol/μg DNA	4.26	Fraga <i>et al.</i> (1991)
	HPLC/EC	Pancreas	19 ± 6.1 add adducts/10 ⁸ nt	0.19	Kadlubar <i>et al.</i> (1998)
	HPLC/EC	Peripheral blood mononuclear cells from controls	11.1 ± 6.4 add./10 ⁵ dG	22.09	Evans <i>et al.</i> (2000)

Molecular weight of DNA 327.6 g/mol; human genome = 3.2×10^9 bp; 19.9% guanine, 30.2% adenine, 19.6% cytosine, 30.3% thymine (Snustad *et al.*, 1997). HPLC, high performance liquid chromatography; EC, electrochemical detection; GC/MS, gas chromatography/mass spectrometry; SIM, selective ion monitoring; Fpg, formamidopyrimidine glycosylase; ³²P, ³²P-post-labelling.

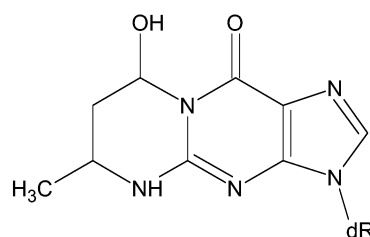
^aSpecial care was taken to avoid artefactual oxidation.

Table II. Epoxyaldehydes and their etheno adducts

Aldehyde	Epoxyaldehyde	Adduct	Reference
Acrolein Crotonaldehyde	Glycidaldehyde 2,3-Epoxybutanal	1,N ² -ethenoguanine	Golding <i>et al.</i> (1996)
		1,N ² -ethenoguanosine	Nair and Offerman (1985)
		1,N ⁶ -ethenoadenosine	
HNE	2,3-Epoxy-4-hydroxynonanal	3,N ⁴ -ethenocytidine	
		1,N ² -ethenodeoxyguanosine	Sodum and Chung (1988)
		1,N ⁶ -ethenoadenine	Sodum and Chung (1991)
		3,N ⁴ -ethenocytidine	Nair <i>et al.</i> (1999)
		N ² ,3-ethenodeoxyguanosine	



Acrolein-dG



Crotonaldehyde-dG

Fig. 2. Propano adducts.

Table III. Lipid peroxidation derived DNA adducts

Adduct	Method	Tissue	Quantity		Reference
				Adducts/10 ⁶ nt	
M1G	³² P/reversed phase HPLC	White blood cells	2.6 ± 1.2 adducts/10 ⁷ nt	0.26	Vaca <i>et al.</i> (1995)
	Imafin/GC/EC NCI/MS	Leucocytes	6.2 ± 1.2 adducts/10 ⁸ bases	0.062	Rouzer <i>et al.</i> (1997)
	³² P/HPLC	Lung	1.00 ± 0.40 adducts/10 ⁷ nt	0.1	Yi <i>et al.</i> (1998)
	GC/EC NCI/MS	Pancreas	32 ± 23 adducts/10 ⁸ nt	0.32	Kadlubar <i>et al.</i> (1998)
	³² P/HPLC	Pancreas	3.58 ± 1.46 adducts/10 ⁷ nt	0.36	Yi <i>et al.</i> (1998)
	³² P/HPLC	Liver	1.40 adducts/10 ⁷ nt	0.14	Yi <i>et al.</i> (1998)
	GC/EC NCI/MS	Hepatic DNA	9 adducts/10 ⁷ bases or 5400 adducts/cell	0.90	Chaudhary <i>et al.</i> (1994)
	³² P/reversed phase HPLC	Breast tissue	3.0 ± 1.3 adducts/10 ⁷ nt	0.30	Vaca <i>et al.</i> (1995)
	Imafin/ ³² P/TLC	Liver	0–27 adducts/10 ⁹ parent bases	0–0.027	Nair <i>et al.</i> (1995)
EdA, EdC edA	GC/NCI/MS/SIM	Placental DNA	2.32 ± 0.02 adducts/10 ⁶ adenine	0.70	Chen <i>et al.</i> (1999)
	LC/ESI/MS/MS	Placental DNA	2.48 ± 0.25 εA/10 ⁶ adenine	0.75	Chen <i>et al.</i> (1999)
	HPLC/fluorescence assay	Placental DNA	2.77 ± 0.24 εA/10 ⁶ adenine	0.84	Chen <i>et al.</i> (1999)
	LC/MS	Placental DNA	1.1 adducts/10 ⁸ normal bases	0.011	Doerge <i>et al.</i> (2000)
	Imafin/ ³² P/TLC	Pancreas	1.8 ± 1.3 adducts/10 ⁸ nt	0.018	Kadlubar <i>et al.</i> (1998)
	Imafin/ ³² P/TLC	Pancreas	1.2 ± 0.92 adducts/10 ⁸ nt	0.012	Kadlubar <i>et al.</i> (1998)
edC	LC/ESI/MS	Liver	0.06 ± 0.01 pmol/mg DNA	0.020	Yen <i>et al.</i> (1996)
	HPLC/ ³² P/TLC/HPLC ^a	Liver, colon	3–9 nmol/mol guanine	0.0006–0.0018	Chung (2000)
N ² ,3-ethenoguanine HNE-dG Acrolein-dG (AdG)	³² P/HPLC	Gingival tissue non-smokers	0.46 ± 0.26 μmol/mol guanine	0.092	Nath <i>et al.</i> (1998)
	³² P/HPLC	Gingival tissue smokers	1.36 ± 0.90 μmol/mol guanine	0.27	Nath <i>et al.</i> (1998)
	HPLC/ ³² P/TLC/HPLC ^a	Liver	0.3–2.0 adducts/10 ⁶ guanine	0.06–0.40	Nath and Chung (1994)
Crotonaldehyde-dG1	³² P/HPLC	Gingival tissue non-smokers	0.06 ± 0.07 μmol/mol guanine	0.012	Nath <i>et al.</i> (1998)
	³² P/HPLC	Gingival tissue smokers	0.53 ± 0.44 μmol/mol guanine	0.11	Nath <i>et al.</i> (1998)
Crotonaldehyde-dG2	³² P/HPLC	Gingival tissue non-smokers	0.31 ± 0.40 μmol/mol guanine	0.062	Nath <i>et al.</i> (1998)
	³² P/HPLC	Gingival tissue smokers	1.72 ± 1.26 μmol/mol guanine	0.34	Nath <i>et al.</i> (1998)

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^a ³²P post-labelling followed by TLC and several HPLC steps.

crotonaldehyde (2,3-epoxybutanal) and HNE (2,3-epoxy-4-hydroxynonanal) give rise to four etheno adducts: 3,N⁴-ethenodeoxycytidine (εdC), N²,3-ethenodeoxyguanosine (N²,3-εdG), 1,N²-ethenodeoxyguanosine (1,N²-εdG) and 1,N⁶-ethenodeoxyadenosine (εdA) (Table III and Figure 3). These adducts are also formed from the carcinogens vinyl chloride and urethane (Bartsch and Nair, 2000). N²,3-εdG levels in liver DNA were similar to levels of propano adducts, ~2 orders of magnitude higher than εdA and εdC (Svenberg *et al.*, 1995). Background ε adduct levels in tissues from humans and rodents were found to be highly variable, which in part may be associated with differences in dietary intake of antioxidants and/or ω-6 PUFAs (Nair *et al.*, 1999).

The ε bases mainly produce base pair substitution mutations. εdA is a highly mutagenic DNA lesion (Palejwala *et al.*, 1993) and can lead to AT→GC transitions, to AT→TA and AT→CG transversions (Pandya and Moriya, 1996; Basu *et al.*, 1999). εdC can lead to CG→AT transversions and CG→TA transitions (Palejwala *et al.*, 1993; Moriya *et al.*, 1994), N²,3-εdG can lead to GC→AT transitions (Cheng *et al.*, 1991) and 1,N²-εdG to GC→TA and GC→CG transversions (Langouët *et al.*, 1997). Pandya and Moriya (1996) indicated that the intrinsic mutagenic potency of εdA is comparable to that of εdC in mammalian cells. *In vitro* studies found that 1,N²-ethenogua-

nine tends to strongly block replication and generate deletions, rearrangements, double mutants and base pair substitutions (Langouët *et al.*, 1997; Akasaka and Guengerich, 1999). Table VI shows the types of mutations induced by etheno adducts.

Bartsch and Nair (2000) showed that the level of these DNA lesions was increased in conditions resulting in oxidative stress and known to be associated with an increased risk of cancer. Oxygen/nitrogen intermediates generated during inflammatory processes led to the formation of ε adducts, likely through peroxynitrite-mediated lipid peroxidation and/or increased oxidative arachidonic acid metabolism. A high ω-6 PUFA diet increased ε-DNA adducts in white blood cells, particularly in female subjects. Nair *et al.* (1998) showed that NO overproduction *in vivo* is associated with the formation of etheno adducts.

Etheno bases are repaired efficiently by mammalian DNA glycosylases *in vitro* (Bartsch, 1999). εdC is removed by a human mismatch-specific thymine-DNA glycosylase (Hang *et al.*, 1998; Sapparbaev and Laval, 1998). 1,N²-ethenoguanine is a primary substrate of human alkylpurine-DNA-N-glycosylase (Sapparbaev *et al.*, 2002). It has been shown that the etheno adducts of adenine and cytosine appear to be repaired much more efficiently than the guanine adduct (Dosanjh *et al.*, 1994).

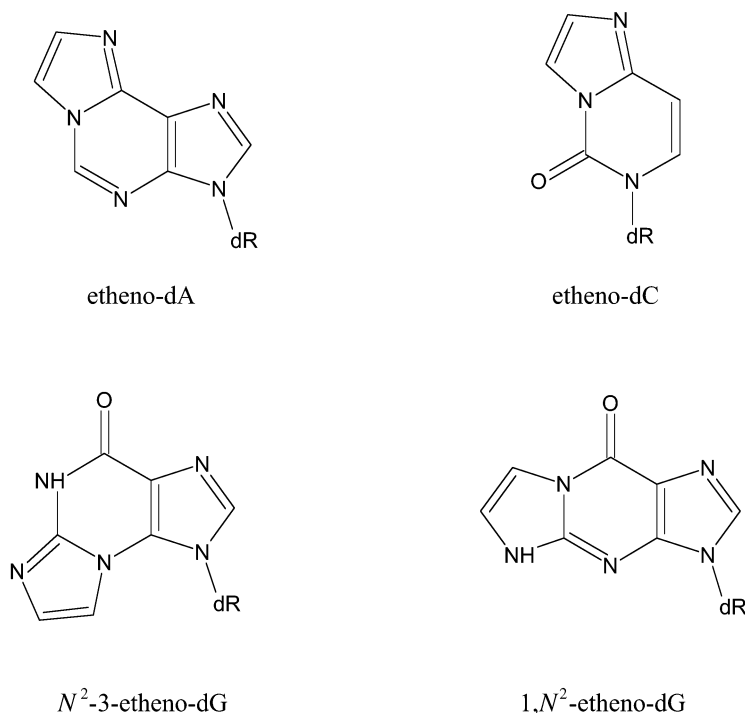


Fig. 3. Etheno adducts.

MDA-induced damage

MDA is a toxic and mutagenic metabolite produced by lipid peroxidation and in the biosynthesis of prostaglandin. It is highly mutagenic in bacterial and mammalian cells and carcinogenic in rats (Basu and Marnett, 1983; Spalding, 1988). MDA reacts with DNA to form adducts of dG [pyrimido[1,2- α]purin-10(3*H*)-one (M1G)] (Figure 4), dA [*N*⁶-(3-oxopropenyl)deoxyadenosine (M1A)] and dC [*N*⁴-(3-oxopropenyl)deoxycytidine (M1C)]. The deoxyguanosine adduct (M1G) has been detected in liver, white blood cells, colon, pancreas and breast from healthy human beings (Marnett, 2002) and levels ranged between 0.062 and 0.9 adducts/10⁶ nt (Chaudhary *et al.*, 1994; Rouzer *et al.*, 1997). Comparison of the yields of the various adducts produced in the reaction of MDA with DNA *in vitro* indicates that M1G is produced at roughly five times the amount of M1A while M1C is formed in trace amounts (Marnett, 2002). There is also considerable tissue-to-tissue variation observed in levels of M1G. M1G levels appear to increase with age and the unsaturated fatty acid content of the diet (Marnett, 1999).

Genotoxic substances derived from DNA oxidation: base propenals

Oxidation of DNA and other macromolecules leads to electrophilic mutagenic substances, such as base propenals (Dedon *et al.*, 1998). Direct oxidation of DNA by agents that abstract the 4'-hydrogen atom of the sugar backbone initiates a cascade of reactions that lead to the formation of these base propenals. Kadlubar *et al.* (1998) found a correlation between M1G and 8-oxo-dG, which seems consistent with the hypothesis that M1G is formed primarily by reaction of DNA with a base propenal (Dedon *et al.*, 1998). According to Dedon *et al.* (1998), several lines of evidence suggest that base propenals derived from DNA are a more important source of M1G than

lipid peroxide-derived MDA. Indeed, these base propenals arise in immediate proximity to DNA. Plastaras *et al.* (2000) showed that base propenals are 30–150 times more potent than MDA in M1G formation and are 30–60 times more mutagenic than MDA. The order of reactivity is as follows: adenine propenal > cytosine propenal > thymine propenal > MDA (Plastaras *et al.*, 2000). The most common sequence changes induced by MDA were base pair substitutions (see Table VI). Of these, 43% were transversions, most of which were G→T, and 57% were transitions and consisted exclusively of C→T and A→G (Marnett, 1999). These are mutations frequently detected in oncogenes or tumour suppressor genes from human tumours (Marnett, 2002).

Endogenous oestrogens

Several oestrogen metabolites can cause DNA damage directly or indirectly, through redox cycling processes that generate reactive radical species (Yager and Liehr, 1996). In oestrogen-induced carcinogenesis, oestrogen metabolites, particularly catechol oestrogens (CEs), are involved in the initiation process through oxidative DNA damage and oestrogens themselves enhance cell proliferation, leading to tumour promotion (Hiraku *et al.*, 2001). Oestrogen-induced direct or indirect DNA damage *in vitro* or in rodents include single-strand breaks, 8-hydroxylation of guanine bases, bulky DNA adducts (unknown structure), estradiol-induced MDA adducts and oestrogen–DNA adducts (Liehr, 2000).

Oxidation of estradiol (E2) by specific cytochrome P-450 isoforms includes formation of 2,3- and 3,4-catechols. The catechols are inactivated by *O*-methylation mediated by catechol *O*-methyltransferase as well as by glucuronidation and sulphation (Martucci and Fishman, 1993). The metabolic clearance of 4-hydroxy-E2 is slower than that of 2-hydroxy-E2 (Roy *et al.*, 1990).

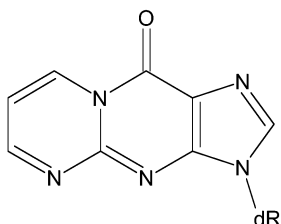


Fig. 4. M1G.

If this inactivation is incomplete, CEs may be oxidized to semiquinones (CE-SQ) and quinones (CE-Q) by redox cycling. This process consists of organic hydroperoxide-dependent oxidation of the CE to the quinone and NADPH-dependent cytochrome P450 reductase-catalysed reduction of the quinone intermediate back to the CE. The semiquinone free radical is an intermediate in each of these metabolic conversions. It may react with molecular oxygen and form quinone and superoxide radicals. Alternatively, non-enzymatic redox couples between copper ions and CEs also generate reactive oxygen radicals. Thus, metal ion-catalysed or enzyme-mediated redox cycling results in the continuous formation of free radicals (Liehr, 2000).

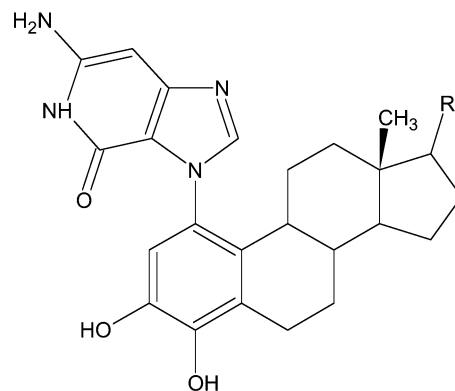
Direct damage

CE-Q is inactivated by conjugation with glutathione or by reduction to CE. If these two inactivating processes are incomplete, CE-2,3-Q reacts with DNA to form stable adducts [*N*²-(2-hydroxyestron-6-yl)deoxyguanosine (2-OHE1-6-N²dG) and *N*⁶-(2-hydroxyestron-6-yl)deoxyadenosine (2-OHE1-6-N⁶dA)] that remain in DNA unless repaired (Dwivedy *et al.*, 1992; Stack *et al.*, 1996). The other quinone, estradiol-3,4-quinone and estrone-3,4-quinone (CE-3,4-Q) can react with DNA to form depurinating adducts. These adducts are lost from DNA by cleavage of the glycosidic bond, leaving apurinic sites. CE-Q leads to the formation of following adducts (Li *et al.*, 1998; Stack *et al.*, 1996): 7-[4-hydroxyestron-1(α,β)-yl]guanine [4-OHE1-1(α,β)-N7Gua], 7-[4-hydroxyestradiol-1(α,β)-yl]guanine [4-OHE2-1(α,β)-N7Gua] (Figure 5), 3-[4-hydroxyestron-1(α,β)-yl]adenine [4-OHE1-1(α,β)-N3Ade], 3-[4-hydroxyestradiol-1(α,β)-yl]adenine [4-OHE2-1(α,β)-N3Ade], 2-OHE1-6-N²dG and 2-OHE1-6-N⁶dA. Exposure of DNA *in vitro* to CE-3,4-Q leads to the depurinating adduct 7-[4-hydroxyestron-1(α,β)-yl]guanine or 7-[4-hydroxyestradiol-1(α,β)-yl]guanine [4-OHE1(E2)-1(α,β)-N7Gua] (Cavalieri *et al.*, 1997). The 4-CE that form predominantly depurinating adducts are carcinogenic, whereas the non-carcinogenic 2-CE exclusively form stable DNA adducts (Cavalieri *et al.*, 2000).

Indirect damage

Redox cycling generated by enzymatic reduction of CE-Q to CE-SQ and subsequent autooxidation back to CE-Q by oxygen forms superoxide radicals and hydroxy radicals (Figure 6).

Secondarily, oestrogens can cause macrophage proliferation and activation (Cavalieri *et al.*, 2000). On stimulation, macrophages produce oxidants such as O₂⁻ and H₂O₂. Furthermore, they produce nitric oxide, which can interact with O₂⁻ to form peroxynitrite, a very potent oxidant (Cavalieri *et al.*, 2000). Yoshie and Ohshima (1998) have demonstrated that DNA strand breakage is induced synergistically in the presence of both a NO-releasing compound [2-(*N,N*-diethylamino)-diazeneolate-2-oxide.diethylammonium salt] and a CE.

Fig. 5. 4-OHE1(E2)-1-(α,β)-N7Gua (E1, R is =O; E2, R is -OH).

Oestrogens can also affect the function of polymorphonuclear leucocytes (PMNs) resulting in the release of oxidants, including hypochlorite/hypochlorous acid (HOCl/OCl⁻) (Jansson, 1991; Frenkel, 1992). 2-Hydroxylated oestrogens, however, act as powerful inhibitors of PMN activity, possibly one of the protective properties of the 2-hydroxylated CE.

Endogenous alkylating agents

Besides oxygen, living cells contain several other small reactive molecules that might cause DNA damage. The most important of these is *S*-adenosylmethionine (SAM). It is a reactive methyl group donor contributing to physiological enzymatic DNA methylation, which plays a role in regulation of gene expression (Holliday and Ho, 1998). However, mutagenic adducts can also be formed (Stern *et al.*, 2000). Rydberg and Lindahl (1982) showed that purified DNA can be non-enzymatically methylated by SAM and, as this reaction probably occurs to the same extent *in vivo*, they expect that an intracellular concentration of 4×10^{-5} M SAM generates 4000 7-methylguanine, 600 3-methyladenine and 10–30 *O*⁶-methylguanine residues per day in a mammalian cell. Other endogenous methylation agents are betaine and choline and simple alkylating agents. The latter may be formed endogenously from cellular precursors, but they may also originate from exogenous sources such as diet, tobacco smoke or environmental pollution. Some of these sources may be so common that most humans are exposed to small amounts and it is difficult to apportion the exogenous and endogenous contributions (Zhao *et al.*, 1999). Examples are *N*-nitroso compounds.

Table IV summarizes measurements of alkylated DNA adducts in human tissues. The most frequent effect of DNA methylation is the generation of 7-methylguanine and 3-methyladenine. 7-Methylguanine is relatively harmless, because this modification does not alter the coding specificity of the base. However, destabilization of the glycosyl bond due to *N*-7 substitution on guanine results in generation of mutagenic apurinic (AP) sites and imidazole ring opening of 7-methylguanine, which results in the stoppage of DNA replication (Barbarella *et al.*, 1991; Tudek *et al.*, 1992). 3-Methyladenine is a cytotoxic DNA lesion that blocks replication. All living cells have an efficient DNA glycosylase that removes 3-methyladenine from DNA, generating an AP site. Atamna *et al.* (2000) showed that the activity of this enzyme decreases with age. 7-Methylguanine is poorly repaired and

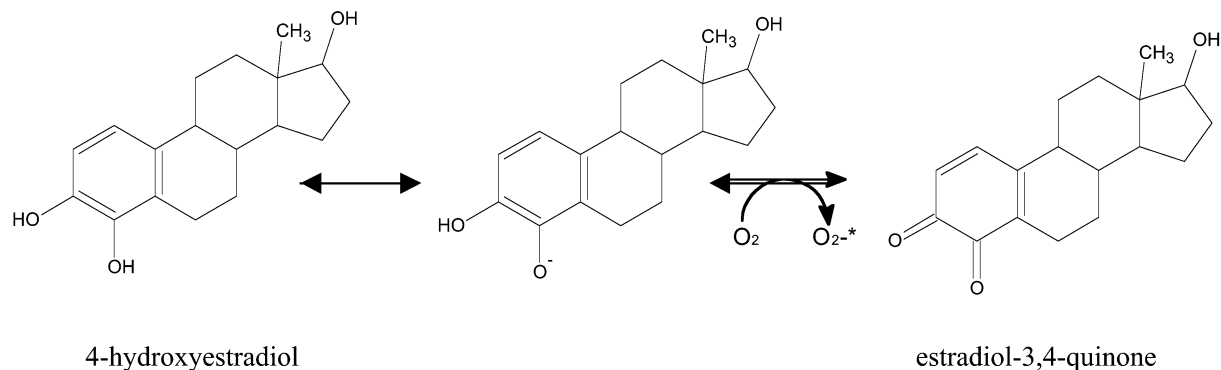


Fig. 6. Metabolic redox cycling of catechol oestrogens.

might be expected to accumulate to a measurable degree in the DNA of mammalian cells, although the chemical lability of the 7-methylguanine–deoxyribose bond ensures that a steady-state of base modification and loss would be achieved within a few days. Measurements in humans ranged between 0.028 and 7.2 7-methylguanine/ 10^6 nt (Shields *et al.*, 1990; Kato *et al.*, 1993) but stayed generally below 1 adduct/ 10^6 nt. 3-Methyladenine was found in human cells at 0.094 adducts/ 10^6 nt/day (Rydberg and Lindahl, 1982). SAM also generates the minor pyrimidine lesions 3-methylthymine and 3-methylcytosine. 3-Methylthymine blocks DNA replication *in vivo* (Huff and Topal, 1987) and 3-methylcytosine is a strong inhibitor of DNA synthesis and could be responsible for the mutagenesis observed after cell treatment with alkylating agents (Boiteux and Laval, 1982; Saffhill, 1984).

Some other alkylated and highly mutagenic DNA lesions of endogenous origin are O^6 -methylguanine (Figure 7), O^4 -methylthymine and O^4 -ethylthymine. They can cause GC→AT and TA→CG transitions during DNA replication (Gerchman and Ludlum, 1973; Abbot and Saffhill, 1979; Saffhill, 1985; Singer *et al.*, 1986) (see Table VI). Compared with *N*-alkylation products, however, the detection of *O*-alkylated adducts in human tissues is difficult, because of their low yield and rapid rate of repair (Lindahl, 1982; Pegg, 1983). O^6 -methylguanine is repaired by ubiquitous O^6 -methylguanine-DNA methyltransferases which demethylate O^6 -methylguanine in cellular DNA and transfer the methyl group to one of their active site cysteine residues (Pegg *et al.*, 1995). The mean value of the ratio of O^6 -methylguanine to O^4 -methylthymine is about six (Kang *et al.*, 1995). Measurements of O^6 -methylguanine in humans ranged between 0.0014 and 0.22 adducts/ 10^6 nt (Shields *et al.*, 1990; Kang *et al.*, 1995) and measurements of O^4 -methylthymine ranged between 0.003 and 0.42 adducts/ 10^6 nt (Kang *et al.*, 1995). They are both formed by *N*-nitroso compounds, potential human carcinogens. Humans are exposed to *N*-nitroso compounds (e.g. *N*-nitrosodimethylamine) through nitrite-treated meat and other nitrate- or nitrite-containing food, in the workplace, from cigarette smoke (Blömeke *et al.*, 1996) and through endogenous formation in the oral cavity, stomach, lungs and by bacteria and macrophages in infected or inflamed organs (Bartsch *et al.*, 1990).

Nitrosated bile salts are carboxymethylating agents which predominantly form N^7 -carboxymethylguanine when they react with DNA. N^3 -Carboxymethyladenine and O^6 -carboxymethylguanine are formed as minor products (O'Driscoll *et al.*, 1999).

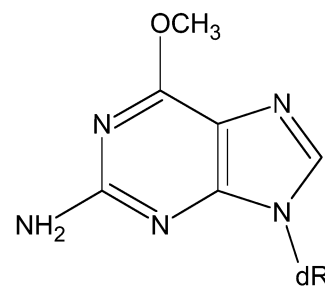


Fig. 7. O^6 -methylguanine.

Cadet *et al.* (1999) described the formation of cyclic adducts of purine DNA bases after reaction with two aldehyde compounds, 4,5-dioxovaleric acid and 2,4-decadienal, which are involved in 5-aminolaevulinic acid accumulation and lipid peroxidation.

7-(2-Hydroxyethyl)-guanine adducts are formed by exogenous exposure to ethylene oxide, a known carcinogen, which besides being an industrial chemical is also a component of cigarette smoke. Ethene, which can be metabolically converted to ethylene oxide, is also formed endogenously from several possible sources, including lipid peroxidation of unsaturated fats, oxidation of free methionine, oxidation of hemin in haemoglobin and metabolism by intestinal bacteria (Törnqvist *et al.*, 1989). Measurements in humans ranged between 0.0095 and 1.90 7-(2-hydroxyethyl)-guanine/ 10^6 nt (Van Delft *et al.*, 1994; Bolt *et al.*, 1997) but lay mostly below 1 adduct/ 10^6 nt. Kumar and Hemminki (1996) revealed that the level of 7-(2-hydroxyethyl)-guanine was twice the level of 7-methylguanine adducts in total white blood cells, whereas in isolated lymphocytes it was at least four times more than the 7-methylguanine adduct.

In vivo many (but not all) methylated adducts are removed from DNA via the BER pathway, in which the modified bases are recognized by DNA glycosylases, leaving an abasic site (Ye *et al.*, 1998). The resulting abasic sites are potentially mutagenic if left unrepaired. Atamna *et al.* (2000) showed that human leucocytes isolated from old donors possess a reduced activity of glycosylases that remove methylated bases, suggesting a decline in the activity of BER. Zhao and Hemminki (2002), on the other hand, showed that at steady-state the levels of DNA alkylation products are independent of age, suggesting that the ratio between endogenous DNA damage, through

Table IV. Adducts as a consequence of DNA alkylation

Adduct	Method	Tissue	Quantity	Reference	
				Adducts/ 10 ⁶ nt	
7-Methylguanine	³² P/TLC/HPLC	White blood cells	2.9 adducts/10 ⁷ nt	0.29	Zhao <i>et al.</i> (1999)
	³² P/TLC/HPLC	White blood cells smokers with lung cancer	3.3 ± 0.9 adducts/10 ⁷ nt	0.33	Zhao <i>et al.</i> (1999)
	³² P/AEC	White blood cells smokers	6.9 adducts/10 ⁷ nt	0.69	Mustonen and Hemminki (1992)
	³² P/AEC	White blood cells non-smokers	3.4 adducts/10 ⁷ nt	0.34	Mustonen and Hemminki (1992)
	³² P/HPLC	White blood cells non-smokers	2.5 adducts/10 ⁷ nt	0.25	Mustonen <i>et al.</i> (1991)
	³² P/HPLC	Lymphocytes (age ≤ 50)	1.0 ± 0.9 adducts/10 ⁷ nt	0.10	Zhao and Hemminki (2002)
	³² P/HPLC	Lymphocytes (age ≥ 80)	0.8 ± 0.4 adducts/10 ⁷ nt	0.080	Zhao and Hemminki (2002)
	HPLC/ ³² P	Lymphocytes	5.0–8.3 adducts/10 ⁷ dGp	0.10–0.17	Kato <i>et al.</i> (1993)
	³² P/AEC	Lymphocytes non-smokers	2.3 adducts/10 ⁷ nt	0.23	Mustonen <i>et al.</i> (1993)
	³² P/AEC	Lymphocytes smokers	11.5 adducts/10 ⁷ nt	1.15	Mustonen <i>et al.</i> (1993)
	³² P/AEC	Lymphocytes smokers	23.6 adducts/10 ⁷ nt	2.36	Mustonen and Hemminki (1992)
	³² P/AEC	Lymphocytes non-smokers	13.5 adducts/10 ⁷ nt	1.35	Mustonen and Hemminki (1992)
	³² P/AEC	Granulocytes smokers	4.7 adducts/10 ⁷ nt	0.47	Mustonen and Hemminki (1992)
	³² P/AEC	Granulocytes non-smokers	2.8 adducts/10 ⁷ nt	0.28	Mustonen and Hemminki (1992)
	³² P/TLC/HPLC	Lung tissue	4.0 adducts/10 ⁷ nt	0.40	Zhao <i>et al.</i> (1999)
	³² P/TLC/HPLC	Lung tissue smokers with lung cancer	6.3 ± 1.9 adducts/10 ⁷ nt	0.63	Zhao <i>et al.</i> (1999)
	HPLC/ ³² P	Lung tissue	1.4–5.4 adducts/10 ⁷ dGp	0.028–0.11	Kato <i>et al.</i> (1993)
	HPLC/ ³² P	Lung tissue smokers	14–72 adducts/10 ⁷ nt	1.40–7.20	Shields <i>et al.</i> (1990)
	HPLC/ ³² Pa	Lung tissue	2.5 ± 2.3 adducts/10 ⁷ dGp	0.05	Blömeke <i>et al.</i> (1996)
	³² P/AEC	Bronchus smokers	17.3 adducts/10 ⁷ nt	1.73	Mustonen <i>et al.</i> (1993)
³² P/AEC	Bronchus non-smokers	4.7 adducts/10 ⁷ nt	0.47	Mustonen <i>et al.</i> (1993)	
³² P/TLC/HPLC	Bronchus smokers with lung cancer	6.1 ± 1.5 adducts/10 ⁷ nt	0.61	Zhao <i>et al.</i> (1999)	
Imafin/HPLC/EC	Pancreas	2–7 pmol/μmol guanosine	0.40–1.39	Bianchini <i>et al.</i> (1993)	
Immunoslot blot	Colon mucosa	0.33–1.34 μmol/mol dG	0.066	Harrison <i>et al.</i> (2001)	
Immunoslot blot	Rectal mucosa	0.30 μmol/mol dG	0.060	Harrison <i>et al.</i> (2001)	
O ⁶ -methylguanine	PREPI ^b	Leucocytes	0.7–4.6 adducts/10 ⁸ guanine	0.0014–0.0092	Kang <i>et al.</i> (1995)
	PREPI ^b	Liver	1.1–6.7 adducts/10 ⁷ guanine	0.022–0.13	Kang <i>et al.</i> (1995)
O ⁴ -methylthymine	HPLC/ ³² P	Lung tissue smokers	1–11 adducts/10 ⁷ dGp	0.020–0.22	Shields <i>et al.</i> (1990)
	PREPI ^b	Liver	0.1–14 adducts/10 ⁷ thymine	0.003–0.42	Kang <i>et al.</i> (1995)
O ⁴ -ethylthymine	PREPI ^b	Liver	0.5–140 adducts/10 ⁷ thymine	0.015–4.24	Kang <i>et al.</i> (1995)
7-(2-Hydroxyethyl)guanine	HPLC/fluorescence assay	Blood cells	2.1–5.8 pmol/mg DNA	0.69–1.90	Bolt <i>et al.</i> (1997)
	³² P/TLC/HPLC	White blood cells smokers with lung cancer	0.6 ± 0.2 adducts/10 ⁷ nt	0.06	Zhao <i>et al.</i> (1999)
	Immunoslot blot	White blood cells	0.65 adducts/10 ⁷ nt	0.065	Van Delft <i>et al.</i> (1994)
	Immunoslot blot	WBC smokers	0.11 adducts/10 ⁷ nt	0.011	Van Delft <i>et al.</i> (1994)
	Immunoslot blot	WBC non-smokers	0.095 adducts/10 ⁷ nt	0.0095	Van Delft <i>et al.</i> (1994)
	³² P/HPLC	Lymphocytes (age ≤ 50)	3.8 ± 3.4 adducts/10 ⁷ nt	0.38	Zhao and Hemminki (2002)
	³² P/HPLC	Lymphocytes (age ≥ 80)	3.0 ± 2.7 adducts/10 ⁷ nt	0.30	Zhao and Hemminki (2002)
	³² P/TLC/HPLC	Lung samples smokers with lung cancer	0.8 ± 0.3 adducts/10 ⁷ nt	0.08	Zhao <i>et al.</i> (1999)
	³² P/TLC/HPLC	Bronchus samples smokers with lung cancer	1.0 ± 0.8 adducts/10 ⁷ nt	0.10	Zhao <i>et al.</i> (1999)
	7-Alkylguanine	³² P/AEC	Leucocytes	13.1 adducts/10 ⁷ nt	1.31
³² P/AEC		Larynx	22.7 adducts/10 ⁷ nt	2.27	Szyfter <i>et al.</i> (1996)
7-Ethyl-dGp	HPLC/ ³² P	Lymphocytes	0.3–1.4 adducts/10 ⁷ dGp	0.006–0.028	Kato <i>et al.</i> (1993)
	HPLC/ ³² P	Lung	0.6–3.1 adducts/10 ⁷ dGp	0.012–0.062	Kato <i>et al.</i> (1993)
	HPLC/ ³² Pa	Lung	1.6 ± 1.7 adducts/10 ⁷ dGp	0.032	Blömeke <i>et al.</i> (1996)
7-Methylguanine and 7-(2-hydroxyethyl)-guanine	³² P/TLC/HPLC	White blood cells	0.7–1.5 adducts/10 ⁷ nt	0.07–0.15	Kumar and Hemminki (1996)
	³² P/TLC/HPLC	Lymphocytes non-smokers	1.1–8.4 adducts/10 ⁷ nt	0.11–0.84	Kumar and Hemminki (1996)
	³² P/TLC/HPLC	Lymphocytes smokers	5.6–12 adducts/10 ⁷ nt	0.56–1.20	Kumar and Hemminki (1996)

Molecular weight of DNA 327.6 g/mol; human genome = 3.2 × 10⁹ bp; 19.9% guanine, 30.2% adenine, 19.6% cytosine, 30.3% thymine (Snustad *et al.*, 1997). HPLC, high performance liquid chromatography; EC, electrochemical detection; GC/MS, gas chromatography/mass spectrometry; SIM, selective ion monitoring; Fpg, formamidopyrimidine glycosylase; ³²P, ³²P-post-labelling. Imafin, immunoaffinity purification; NCI, negative chemical ionization; ESI, electrospray ionization; LC, liquid chromatography; TLC, thin layer chromatography; AEC, anion exchange chromatography.

^aAdducts were isolated through two sequential HPLC steps.

^b³²P-post-labelling, followed by HPLC and immunoprecipitation.

Table V. Abasic sites

Adduct	Method	Tissue	Quantity	Reference	
				AP sites/10 ⁶ nt	
Abasic site	Radioactive labelling of purines ASB ^a	Cells	2000–10 000 lesions/cell generation	0.31–1.56 per day	Lindahl and Nyberg (1972)
		Liver	8–9 lesions/10 ⁶ nt, 50 000–200 000 lesions/cell	8–9	Nakamura and Swenberg (1999)
	ARP/ELISA	Cultured cells, leucocytes	<0.67 lesions/10 ⁶ nt or 4000 lesions/genome	< 0.67	Atamna <i>et al.</i> (2000)

Molecular weight of DNA 327.6 g/mol; human genome = 3.2×10^9 bp; 19.9% guanine, 30.2% adenine, 19.6% cytosine, 30.3% thymine (Snustad *et al.*, 1997). ARP, biotin-containing aldehyde-reactive probe; ELISA, enzyme-linked immunosorbent assay.

^aA combination of an ARP and slot blot techniques.

methylation or lipid peroxidation, and the repair of such damage may not be altered in lymphocytes of older individuals.

DNA hydrolysis

The glycosidic bond between bases and deoxyribose in DNA is labile under certain conditions, such as heating, alkylation of bases or the action of *N*-glycosylases (Lindahl, 1982). Cleavage of the glycosidic bond in DNA leads to an abasic site. AP sites are not only produced by spontaneous depurination but to a large extent also by ROS (Nakamura *et al.*, 2000). Abasic sites are among the most frequent endogenous lesions found in DNA, with an estimated 10 000 lesions/human cell/day (Lindahl, 1993). Nakamura and Swenberg (1999) found levels corresponding to 50 000–200 000 AP sites/genome in many human and rodent tissues. Table V summarizes measurements of AP sites in human tissues. In DNA purines are lost at a 20 times higher rate than pyrimidines (Lindahl and Karlström, 1973).

The number of endogenous AP sites varied widely between tissues but not within tissues (Nakamura and Swenberg, 1999). The brain is the most affected organ, followed by colon and heart, and then liver, lung and kidney (Nakamura and Swenberg, 1999). Atamna *et al.* (2000) showed that the number of AP sites in human leucocytes from old donors was about seven times that in young donors, apparently due to a decline in BER activity.

The major process in the repair of AP sites is the type II AP endonuclease- β -polymerase-dependent pathway (Dianov *et al.*, 1992). AP sites are repaired rapidly and efficiently (Lindahl, 1993, 1996). Nakamura and Swenberg (1999) found high steady-state levels of AP sites in human liver (8–9 sites/10⁶ nt). So the fraction of AP sites that escapes repair is likely to contribute to mutations, chromosome aberrations and transcription errors (Nakamura and Swenberg, 1999).

Typical AP sites induce base pair substitutions (primarily AP site \rightarrow T) (Gentil *et al.*, 1990; Lawrence *et al.*, 1990) (see Table VI). The 5'-cleaved AP sites might also induce frameshift mutations, such as those detected in microsatellite sequences following treatment of plasmids with H₂O₂ (Jackson *et al.*, 1998). Abasic sites are mutagenic due to the preferential incorporation of adenine opposite abasic sites by DNA polymerases during replication (Jackson and Loeb, 2001).

Hydrolytic deamination

DNA bases are susceptible to hydrolytic deamination, although deamination is much less frequent in double-stranded compared to single-stranded DNA (Lindahl, 1993). Cytosine and its homologue 5-methylcytosine are the main targets (Lindahl, 1993). Between 100 and 500 cytosines/cell/day are deaminated to uracil (Lindahl, 1993). DNA contains a fifth distinct base, 5-methylcytosine, which base pairs with guanine. At CpG sequences it is involved in silencing gene expression (Li *et al.*, 1992). 5-Methylcytosine is a preferred target for spontaneous mutagenesis, primarily because of the fact that 5-methylcytosine can be deaminated three to four times more rapidly than cytosines (Lindahl, 1979) and secondarily because of the different rates of DNA repair. The deaminated form of cytosine is very rapidly excised by the abundant uracil-DNA glycosylase to generate a base-free site, which is efficiently corrected. The GT base pair, formed by deamination of 5-methylcytosine, is a substrate for mismatch correction systems, but these processes are slow (Lindahl, 1993). As a consequence, GC \rightarrow AT transitions at sites of cytosine methylation account for about one-third of the single site mutations that cause inherited disease in humans (Cooper and Youssoufian, 1988). The same base change is frequent in mutated *p53* tumour suppressor genes found in many human cancers (Rideout *et al.*, 1990). Chen *et al.* (1998) found that cytosine methylation greatly enhances guanine alkylation at all CpG sites in the *p53* gene by many different carcinogens. These findings suggest that mutational hot-spots at methylated CpG sequences in the *p53* gene may in part be a consequence of preferential carcinogen binding at these sites.

In comparison with the deamination of cytosine to uracil, the deamination of DNA purines is a minor reaction. Adenine is converted to hypoxanthine at 2–3% of the rate of cytosine deamination (Karran and Lindahl, 1980). Hypoxanthine pairs with C rather than with T and can thus form a mutagenic lesion. Because of the low cellular level of hypoxanthine-DNA glycosylase, the repair reaction is less efficient than that of deaminated cytosine (Hill-Perkins *et al.*, 1986). The rate of deamination of guanine to xanthine is similar to adenine deamination (Lindahl, 1993). This lesion is less mutagenic because xanthine pairs with C. The xanthine–deoxyribose bond is also susceptible to spontaneous hydrolysis, which can generate an abasic site.

Table VI. Mutation patterns of endogenous DNA lesions

Lesion	Mutation	Reference
Apurinic sites	AT→TA	Lindahl (1996); Jackson and Loeb (2001)
	GC→TA	
	APsite→T	
	Frameshift mutations	
Cytosine deamination	CG→TA	Gentil <i>et al.</i> (1990); Lawrence <i>et al.</i> (1990)
5-Methylcytosine deamination	5MeCG→TA	Jackson <i>et al.</i> (1998)
8-Oxo-G	GC→TA	Lindahl (1996)
Thymine glycol	C→T transition	Lindahl (1996); Wagner <i>et al.</i> (1992)
<i>O</i> ⁶ -methylguanine	GC→AT	Marnett and Plataras (2001)
<i>O</i> ⁴ -methylguanine	TA→CG	Lindahl (1996)
FapyGua	GC→CG transversion	Lindahl (1996)
5-OH-Cyt	CG→AT transition, GC→CG transversion	Ono <i>et al.</i> (1995)
5-OH-Ura	CG→AT transition, GC→CG transversion	Purmal <i>et al.</i> (1994)
1, <i>N</i> ² -(1,3-propano)-2'-deoxyguanosine edC	G→T transversion	Purmal <i>et al.</i> (1994)
	CG→AT transversion	Moriya <i>et al.</i> (1994)
<i>N</i> ² ,3-εdG	CG→TA transition	Palejwala <i>et al.</i> (1993); Moriya <i>et al.</i> (1994); Pandya and Moriya (1996)
	GC→AT transition	Moriya <i>et al.</i> (1994); Pandya and Moriya (1996)
	GC→TA transversion	Cheng <i>et al.</i> (1991)
	GC→CG transversion	Langouët <i>et al.</i> (1997)
edA	AT→GC transition	Basu <i>et al.</i> (1993); Pandya and Moriya (1996)
	AT→TA transversion	
	AT→CG transversion	
M1G	G→T transversion	Marnett (1999)
	C→T transition	
	A→G transition	
Frameshift mutations	M1G→A	Fink <i>et al.</i> (1997)
	M1G→T	
	M1G→C	

DNA damage by carbonyl stress

Reactive carbonyl species (RCS) are potent mediators of cellular carbonyl stress originating from endogenous chemical processes such as lipid peroxidation and glycation (Roberts *et al.*, 2003). Glycation is a major source of RCS such as glyoxal and methylglyoxal.

Methylglyoxal is a biologically important carbonyl compound. It is a reactive aldehyde endogenously formed as a product of glucose metabolism, but is also found as a constituent of various beverages (coffee) and foodstuffs. It readily forms the cyclic 3-(2'-deoxy-β-D-erythro-pentafuransyl)-6,7-dihydro-6,7-dihydroxy-6-methylimidazo[2,3-*b*]purine-9(8*H*)one adduct (MG-3'-dGMP) in human buccal epithelial cells. Vaca *et al.* (1998) measured 0.26 MG-3'-dGMP adducts/10⁶ nt.

Decomposition products of lipid hydroperoxides include the reactive aldehyde glyoxal. It can also be formed by oxidative deoxyribose breakdown or autoxidation of sugars, such as glucose, and it plays a role in the pathophysiology of diabetes and ageing (Abordo *et al.*, 1999). It readily forms DNA adducts, generating potential carcinogenic adducts such as glyoxalated deoxycytidine (Mistry *et al.*, 2003) and it has been shown to be mutagenic in bacterial and mammalian cells (Murata-Kamiya *et al.*, 1997). Awada and Dedon (2001) reported that 2-phosphoglycolaldehyde, formed by oxidation of the 3'-position of deoxyribose, reacts with dG to form 1,*N*²-glyoxal adducts. The formation of glyoxal-dG adducts occurs almost five orders of magnitude more slowly with 2-phosphoglycolaldehyde than with glyoxal.

Elevated tissue concentrations of RCS are observed in pathological conditions such as diabetes and in skin upon solar irradiation (Mizutari *et al.*, 1997; Beisswenger *et al.*, 1999). Acute treatment of cultured human keratinocytes with glyoxal resulted in pronounced DNA strand breaks, whereas methylglyoxal treatment resulted in cross-linked DNA (Roberts *et al.*, 2003).

Conclusion

One of the major endogenous sources of DNA damage is that produced by ROS and the most studied oxidative DNA adduct is 8-oxo-dG. Aldehydes derived from lipid peroxidation form another threat to DNA. Adducts formed are etheno-, propano- and malondialdehyde-derived DNA adducts. Several oestrogen metabolites can cause DNA damage directly or indirectly, through redox cycling processes that generate reactive radical species (Yager and Liehr, 1996). In addition to oxygen and lipid peroxidation products, living cells contain several other small reactive molecules that might cause DNA damage, like methylating agents. The main adducts formed are 7-methylguanine, 3-methyladenine, *O*⁴-methylthymine, *O*⁶-methylguanine and 7-(2-hydroxyethyl)guanine, of which *O*⁶-methylguanine is the most mutagenic. Another pathway for DNA damage is attack by water, causing hydrolysis of the glycosylic bond and resulting in the formation of mutagenic abasic sites in DNA. DNA bases are also susceptible to hydrolytic deamination. A last pathway for endogenous DNA damage is that caused by carbonyl stress.

The adduct level data concerning some of these processes show a wide range covering several orders of magnitude. In some cases, at least part of this wide range might be due to differences in analytical methods. Generally, for the quantification of oxidative DNA adducts, GC/MS gives high values, HPLC intermediate and enzyme-based methods low values (Collins *et al.*, 1997). We compared the levels of 8-oxo-dG measured in human lymphocytes by GC/MS (59.70 adducts/10⁶ nt) with those measured by HPLC/EC (0.24–13.52 adducts/10⁶ nt) (see Table I). GC/MS clearly gives higher values. Oxidation may occur during DNA isolation and hydrolysis before HPLC or GC-MS; derivatization procedures for GC-MS can lead to the oxidation of normal unaltered guanine resulting in a spuriously high yield of 8-oxo-G (Wagner *et al.*, 1992; Collins *et al.*, 1997). However, this artificial oxidation may be prevented by, for example, prepurifying the compounds of interest or by derivatization at low temperature or by addition of antioxidants to the silylating reagents (Cadet *et al.*, 1999). HPLC, on the other hand, may underestimate the adduct levels due to incomplete enzymatic digestion of the DNA in the standard procedure (Schuler *et al.*, 1997). Enzymatic methods to convert oxidative lesions to breaks might underestimate adducts as there may be some adducts that are inaccessible to the enzyme. On the other hand, an enzyme such as Fpg might recognize additional (unknown) base modifications, which might lead to an overestimation of the number of 8-oxo-G adducts (Collins *et al.*, 1997). However, Anson *et al.* (2000) found that the FAPY glycosylase (Fpg) assay detected ~90% of the 8-oxo-dG detected by HPLC/EC. For the quantification of etheno adducts, the ³²P-post-labelling assay is probably one of the most sensitive methods available. However, quantification by this method is compromised by relatively high variability due to multiple steps and lack of an internal standard (Chung, 2000). Quantification, including immunoaffinity chromatography for adduct enrichment, can be poorly reproducible due to batch-to-batch preparation of antibody and ageing of antibody.

A number of techniques have been developed to detect alkyl DNA adducts, including immunological assays, fluorescence techniques, mass spectrometry, ³²P-post-labelling and electrochemical detection. When ³²P/HPLC was compared with GC/MS in quantifying 7-(2-hydroxyethyl)guanine from *in vivo* samples, a good qualitative agreement between methods with a correlation coefficient of 0.97 was obtained (Eide *et al.*, 1999).

It should be taken into account that over the years, the different techniques have been adjusted to avoid artefacts. Bianchini *et al.* (2001) and Lenton *et al.* (1999), for example, took measurements to avoid artefactual DNA oxidation during HPLC/EC.

Endogenous DNA damage occurs at a high frequency compared with exogenous damage (Jackson and Loeb, 2001). The high prevalence of endogenous DNA damage corresponds with highly efficient repair of such damage, necessary for cell survival (Wood, 1996; Ochs *et al.*, 1999). Most endogenous DNA damage is repaired by BER, O⁶-methylguanine DNA-methyltransferase (MGMT) and mismatch repair (Pegg *et al.*, 1995; Seeberg *et al.*, 1995; Prolla, 1998). The spontaneous dissociation of bases, a frequent form of endogenous DNA damage that can lead to AP sites, is efficiently repaired by BER (Frosina *et al.*, 1994). O⁶-methylguanine is repaired very efficiently by the direct repair enzyme MGMT. In spite of efficient repair, most mutations arise endogenously. Of particular significance for the thesis that most mutations in normal cells are of endogenous origin is the great similarity

between the mutational spectra of *HPRT* genes in the lymphocytes of normal individuals from different population groups (Burkhardt-Schultz *et al.*, 1996; Podlitsky *et al.*, 1998). Also, the mean mutation frequency of a given gene locus (e.g. *HPRT*) in a given cell type for a given age group is approximately the same in diverse Western countries (Robinson *et al.*, 1994). The same applies for different subgroups of the population in the same country (Tates *et al.*, 1991). This suggests that the mutations are mainly due either to endogenous mechanisms of mutagenesis or to ubiquitous environmental influences.

Adduct levels were reported to vary according to gender, ethnicity and individual characteristics. Thus, for gender only women have been reported to have increased levels of DNA adducts arising from lipid peroxidation products in white blood cell DNA following consumption of PUFAs (Fang *et al.*, 1996; Nair *et al.*, 1997). This also holds true for exogenous adduct levels, as female smokers have been reported to have higher bulky DNA adduct levels in lung DNA than do male smokers (Rydberg *et al.*, 1994). Mean 8-oxo-dG levels ranged from 0.1 to 40 adducts/10⁵ dG residues and lower levels were observed in the Japanese population, as compared with western populations (Povey, 2000). 8-Hydroxyguanine may be formed as a result of both endogenous and exogenous processes, so these differences may reflect environmental and/or genetic factors or variations in analytical and sampling techniques (Povey, 2000). Also, pronounced interindividual variations in the activity of enzymes that participate in the activation and inactivation pathways of carcinogen metabolism and in DNA repair pathways have been described (Autrup *et al.*, 1986; Perera, 1996). A similar variability is expected concerning the reactions of endogenous genotoxic agents with DNA and repair of the lesions. Interindividual differences in the levels of endogenous DNA damage and risk of spontaneous tumour formation could therefore be expected (Lutz, 1990).

Some endogenous genotoxic substances are formed by more than one pathway. When considering chemopreventive strategies it might be important to know which metabolic pathway predominates in generating a given substance. An example is the reactive aldehyde glyoxal. It is a decomposition product of lipid hydroperoxides, but it can also be formed by oxidative deoxyribose breakdown or autooxidation of sugars. In addition, there are specific adducts that can be generated by different genotoxic substances. The M1G adduct, for example, can be formed by MDA as well as by base propenals. That we now know that M1G is primarily formed by base propenals rather than by MDA (Dedon *et al.*, 1998) might be relevant to our thinking on chemoprevention.

Distinguishing between endogenous and exogenous DNA damage and determining the relative importance of both is a complex matter. Some exogenous mutagens also occur endogenously. Secondly, some exogenous substances, although chemically different from endogenous ones, generate the same DNA adducts. An example of adducts formed by exogenous mutagens that also occur endogenously are the exocyclic adducts formed by the reactive aldehydes acrolein and crotonaldehyde. These adducts are not only attributable to endogenous exposure, because acrolein and crotonaldehyde also occur exogenously as environmental contaminants. Methylglyoxal is another substance that is formed endogenously but can also be found in various foods and beverages. Vinyl chloride is an occupational and environmental pollutant that gives rise to 1,N⁶-ethenoadenine and 3,N⁴-ethenocytosine,

adducts that are also formed endogenously by the products of lipid peroxidation. Smoking increases the levels of DNA adducts that also arise spontaneously. The amount of 8-oxo-dG appeared higher in human leucocytes after smoking (1.01 versus 0.66 adducts/10⁶ nt) (Kiyosawa *et al.*, 1990). Nath *et al.* (1998) measured higher acrolein-dG and crotonaldehyde-dG levels in gingival tissue of smokers than in that of non-smokers (0.27 versus 0.091 and 0.11 versus 0.012 adducts/10⁶ nt, respectively). The methylated DNA adduct 7-methylguanine also occurred in higher amounts in different tissues of smokers (bronchus, lymphocytes, white blood cells and granulocytes) (Mustonen and Hemminki, 1992; Mustonen *et al.*, 1993). In assessing the risks associated with exposure to certain exogenous DNA-damaging agents the fact that they generate adducts of a type that is also generated by endogenous substances has to be taken into account. Indeed, exposure to such an agent can never take place in a 'very low dose' context as there is the background of endogenous exposure. An increase in the amount of this type of damage will probably induce a proportional biological effect.

With respect to the endogenous versus exogenous origin of mutations, it is worth noting that methylated CpG sequences, besides being involved in spontaneous mutagenesis processes, can also create preferential targets for environmental mutagens and carcinogens (Denissenko *et al.*, 1997, 1998; Shen *et al.*, 2000).

It seems likely that certain less frequent exogenous DNA lesions may have unexpectedly large biological effects due to their relatively inefficient removal by DNA repair. Exogenous DNA damage differs from the endogenous form in that it more often entails bulky DNA adducts that are removed preferentially by nucleotide excision repair (Sancar, 1995), a more complex and slower DNA repair process (Cox and Lane, 1995). This pathway also repairs dipyrimidine photoproducts, which are formed on DNA by UV light. Double-strand breaks often arise by the action of exogenous agents. Double-strand breaks are repaired in mammals by homologous recombination and to a large extent by error-prone non-homologous end-joining (Shinohara and Ogawa, 1995; Strathern *et al.*, 1995; Henle and Linn, 1997; Vamvakas *et al.*, 1997; Critchlow and Jackson, 1998).

As argued above, most mutations arise spontaneously. In tumour cells, the mutations which can be specifically ascribed to exogenous agents represent a minority of all mutations. Only in a limited number of instances was a significant correlation found between the mutational spectra of tumour cells and the type of exogenous carcinogenic agent, e.g. aflatoxin B₁ (Prieto-Alamo *et al.*, 1996; Lutz *et al.*, 1998). These were instances in which the relative risk of cancer increased significantly (typically by more than 10 times) following exposure to exogenous agents, such as skin cancer caused by UV light (Nakazawa *et al.*, 1994), lung cancer due to cigarette smoking and bladder cancer due to aromatic amines (Lutz *et al.*, 1998). It must be emphasized that the association between exposure to exogenous agents and relative cancer risk does not necessarily imply that these agents have initiated carcinogenesis. These exogenous agents might well act as a necessary condition, and thus be considered as a cause of cancer, by acting at a later stage, contributing to accumulation of mutations or to tumour promotion after initiation has taken place. Initiated cells can be assumed to be more susceptible to the mutagenic action of exogenous agents. (Deman and van Larebeke, 2001) which then contribute to the continuing accumulation of mutations

leading to malignant transformation and further progression. This is corroborated by the following observations. (i) Mutational spectra in the *HPRT* gene of normal lymphocytes from different human populations are similar. This suggests that they are predominantly due to endogenous mechanisms of mutagenesis (Podlutzky *et al.*, 1998). (ii) Mutation spectra of the *p53* oncogene in cancer cells from different types of tumours (e.g. lung versus brain) show substantial differences, reflecting the fact that they have partly been caused by different exogenous agents (Lutz *et al.*, 1998). Deman and van Larebeke (2001) hypothesized that the most probable course of events in carcinogenesis is as follows. (i) Because of the low contribution of exogenous agents to the mutation rate of normal cells, initiation is expected to be chiefly due to endogenous causes. The exception might be a situation of acute excessive exposure to a carcinogenic agent. (ii) The mutation which brings about the enhancement of the mutation rate responsible for initiation is more often one which enhances the susceptibility to exogenous mutagenic agents than the susceptibility to endogenous causes of mutations. (iii) The same applies for subsequent mutations which further enhance the mutation rate in initiated cells.

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