MEASUREMENT OF OXIDATIVE DAMAGE TO DNA NUCLEOBASES IN VIVO

Interpretation of Nuclear Levels and Urinary Excretion of Repair Products

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1. ABSTRACT

Oxidatively modified nucleobases can be measured in cells although with large inter-methodological variation. The excretion rates of corresponding repair products correspond to 10^4 oxidative DNA modifications per cell per day indicating >99% repair. The urine and nuclear measurements represent two fundamentally different estimates, i.e. the average rate of damage and the local balance between damage and repair, respectively. The important determinants of oxidative damage in humans include smoking, air pollution, oxygen consumption, cancer therapy, inflammation and neurodegenerative diseases, whereas diet and antioxidant supplements have minimal influence. The oxidative DNA damage biomarkers may provide proof of causal relationships with cancer and aging and improve prevention.

2. BIOMARKERS OF OXIDATIVE DNA DAMAGE

Oxidative damage to DNA has been proposed to be an important factor in carcinogenesis, supported by experimental studies in animals and in vitro (Ames et al. 1995, Loft and Poulsen 1996, Wiseman and Halliwell 1996). With respect to aging, mainly the oxidative modifications of DNA in both nucleus and mitochondria are thought to be involved (Ames et al. 1993). Indeed cells are constantly exposed to oxidants from both physiological processes, such as mitochondrial respiration (Chance et al. 1979), and pathophysi-

Advances in DNA Damage and Repair, edited by Dizdaroglu and Karakaya. Kluwer Academic / Plenum Publishers, New York, 1999.

ological conditions such as inflammation, ischemia/reperfusion, foreign compound metabolism and radiation (Ames et al. 1995). The continously ongoing damage to DNA is constantly repaired with high efficiency in the cells in the body (Demple and Harrison 1994). Many of the repair products are excreted in quantifiable form in the urine.

The use of biomarkers of oxidative DNA damage may provide further proof of a causal relationship with cancer and aging as well as serve as intermediate endpoints in human intervention studies which may target the optimum intervention strategy for the large scale intervention (Schulte and Perera 1993). Moreover, the biomarker approach is applicable in mechanistic animal experiments and cancer bioassays as well as in vitro.

In DNA more than 100 different oxidative modifications have been observed (Dizdaroglu 1991, Cadet et al. 1994). However, so far only a few of the base modifications have been used as biomarkers and of these the oxidative C-8 adduct of guanine is by far the most studied as either the nucleoside or base. In principle, the level in nuclear or mitochondrial DNA from target or surrogate tissues or cells or the excretion of repair products into the urine can be measured. Under the usual steady state conditions the latter will reflect the rate of damage whereas the former will reflect the balance between damage and repair.

3. ANALYSIS OF OXIDATIVELY MODIFIED NUCLEOBASES AND NUCLEOSIDES

3.1. Cellular DNA

In tissue or cell samples the level of oxidatively modified nucleobases can be measured by various techniques, including HPLC-EC (or MS or UV), GC/MS-SIM, TLC with ^{32}P-postlabelling and various immunoassays. Except in the slot blot technique and immunohistochemistry (Musarrat and Wani 1994, Yarborough et al. 1996), DNA or chromatin is isolated and hydrolyzed by enzymes or acid at high temperature. In all the assays the abundant unmodified nucleobases may be oxidized and thus cause artificially high values. Particularly, the derivatisation with silyl-groups for the GC/MS is prone to give rise oxidation and could be carried out after removal of the unmodified base (Ravanat et al. 1995, Douki et al. 1996) or under controlled temperature and other conditions (Hamberg and Zhang 1995). Similarly, the gamma-radiation from the ^{32}P-phosphate used for post-labeling could oxidize guanine and thus explain the rather high values measured in human lymphocytes and rat organs by that method (Wilson et al. 1993, Devanaboyina and Gupta 1996, Collins et al. 1997a). Even with the HPLC-EC method the reported values for 8-oxodG in leukocyte DNA varies from 0.3 to 13 per 10^5 dG (Loft and Poulsen 1996). There is no doubt that oxidation may occur during DNA extraction, particularly with the use of impure phenol and during drying the DNA after ethanol precipitation (Floyd et al. 1990, Claycamp 1992, Adachi et al. 1995). The lowest values have been obtained with anaerobic DNA extraction (Collins et al. 1996, Nakajima et al. 1996a). Some of the immunoassays are calibrated by HPLC-EC values (Degan et al. 1991, Musarrat and Wani 1994) whereas others appear to yield much higher 8-oxodG values than the HPLC-EC assays (Yin et al. 1995) but whether that is due to insufficient specificity of the antibodies is unknown.

Oxidative damage to nucleobases can be assessed indirectly as strand breaks, DNA unwinding or relaxation of supercoiling induced by treatment of nuclear material by the relevant repair enzymes, i.e. Fpg and endonuclease III for purines, inluding 8-oxodG, and pyrimidine lesions, respectively (Epe 1995, Collins et al. 1997b). This approach has been

applied in alkaline elution and alkaline unwinding of DNA as well as in alkaline single cell gel electrophoresis (Epe 1995, Hartwig et al. 1996, Collins et al. 1997b). However, the 8-oxodG values estimated by those assays are around 0.3 per 10^6 dG, i.e. 10 times lower than the lowest values obtained by HPLC-EC. So far, it is unknown whether the HPLC-EC assay still gives artificially high values or the enzyme based assays are missing 8-oxodG in some of the DNA as suggested by a completeness of only 50% of FPG repair of damaged DNA in vitro (Karakaya et al. 1997). Nevertheless, there was a strong correlation ($r=0.89$, $p<0.01$) between 8-oxodG concentration and comet tail length after 2-nitropropane induced DNA damage in rat bone marrow cells (Deng et al. 1997).

3.2. Urine

The repair products from oxidative DNA damage, i.e. oxidized bases and nucleosides, are poor substrates for the enzymes involved in nucleotide synthesis, fairly water soluble, and generally excreted into the urine without further metabolism (Shigenaga et al. 1989, Loft et al. 1995a). Indeed, animal experiments have shown that injected 8-oxodG is readily excreted unchanged into the urine whereas 8-oxodG in the diet or oxidation of dG during excretion does not contribute (Shigenaga et al. 1989, Park et al. 1992, Loft et al. 1995a). Among the possible repair products from oxidative DNA modifications 8-oxodG, 8-oxoGua, Tg, dTg and 5-OHmU have so far been identified in urine (Fig. 1, (Cathcart et al. 1984, Shigenaga et al. 1989, Simic and Bergtold 1991, Faure et al. 1993, Suzuki et al. 1995, Teixeira et al. 1995, Loft and Poulsen 1996). Of these 8-oxodG and the thymine derivatives are the most intensively studied ones. The levels of concentration and excretion of the oxidized bases and nucleosides obtained in different laboratories are in the same range (Loft and Poulsen 1996).

The assays for the urinary DNA repair products include HPLC with detection by electrochemistry for 8-oxodG and 8-oxoGua and by UV absorbance for dTg and Tg, whereas all the repair products can potentially be measured by GC/MS (Simic and Ber-

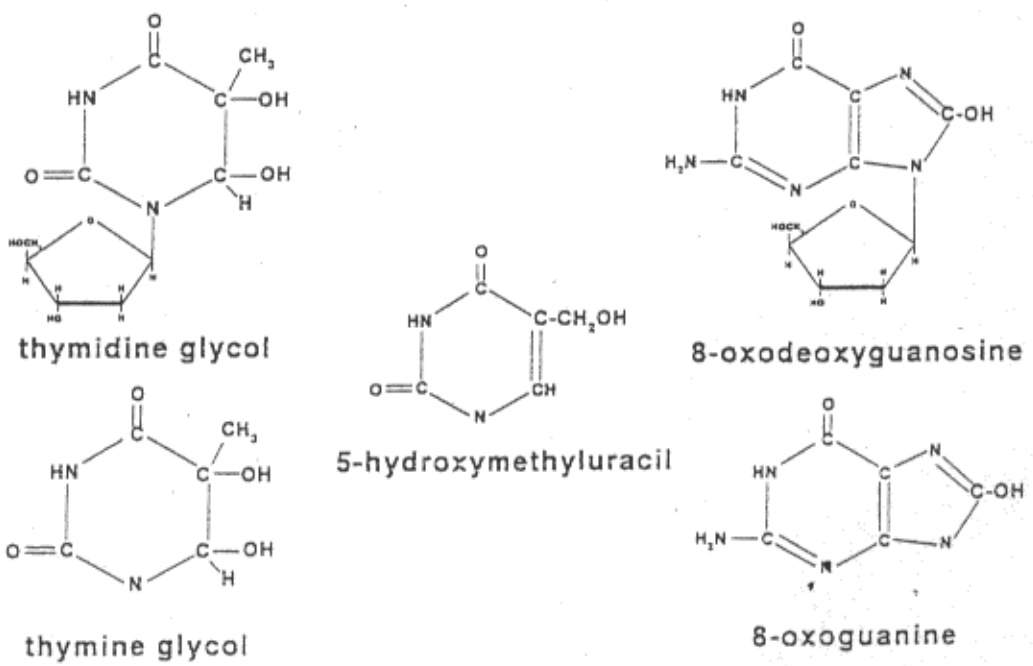

Figure 1. Repair products of oxidative DNA damage identified in urine and used as biomarkers.

gtold 1991, Faure et al. 1993, Dizdaroglu 1994, Teixeira et al. 1995). The major problem with all these assays involves separation of the very small amounts of analyte from urine that is a very complicated matrix. Thus, although several of the products are electrochemically active and high sensitivity is achievable the HPLC methods require extensive clean up procedures such as multiple solid phase extractions, HPLC column switching techniques or immunoaffinity columns (Cathcart et al. 1984, Shigenaga et al. 1989, 1994, Loft et al. 1992, Park et al. 1992, Tagesson et al. 1992, 1995, Brown et al. 1995, Germadnik et al. 1997). The complicated extraction procedures cause recovery problems in both HPLC and GC/MS-SIM methods and may require labeled internal standards. Moreover, the complicated procedures limit the analytical capacity. In the future HPLC/MS-MS is likely to solve many of the problems. An ELISA assay based on monoclonal antibodies has been developed for estimation of 8-oxodG in urine samples (Osawa et al. 1995). However, the values obtained in rat urine were 3–5 times higher than other published values. Similarly, in 4 smokers studied before and after smoking cessation the urinary 8-oxodG excretion values estimated by the ELISA method were 8 times higher than and showed only a weak correlation (r=0.42) with the values obtained by HPLC (Prieme et al. 1996). In another study the ELISA method yielded an 8-oxodG to creatinine ratio of around 8 nmol/mmol in healthy subjects (Erhola et al. 1997), as compared with 1 to 2 nmol/mmol in a large number of reports using HPLC-EC and GC/MS assays (Loft and Poulsen 1996).

Collection of urine for 24 hours or longer is quite straight forward, however, it may present some practical problems. For convenience the use of spot urine samples corrected for creatinine would be simpler than 24-h collection of urine. However, in 74 healthy subjects we collected urine for 24 h and a spot urine sample from the subsequent voiding (unpublished data). The correlation between the 8-oxodG to creatinine ratio in the spot samples and the 24 h excretion of 8-oxodG per kg lean body mass was rather poor (r=0.50). The insufficiency of creatinine corrected spot urine samples for estimation of the 8-oxodG excretion rates may partly explain some of the apparent differing data obtained in various human studies as discussed below.

4. INTERPRETATION OF NUCLEAR DNA LEVELS AND URINARY EXCRETION OF OXIDIZED NUCLEOBASES AND NUCLEOSIDES

The level of oxidized bases in DNA from tissues or cells is in a steady state determined by a simple balance of the influx and efflux of oxidized bases as outlined for 8-oxodG in Fig. 2. The major part of the oxidized bases in DNA arises from oxidation of bases within the DNA whereas incorporation of oxidized nucleotides for the cellular pool is probably of minor quantitative importance although highly mutagenic and thus of large qualitative importance (Tajiri et al. 1995). The efflux is determined mainly by the repair of the modified bases that for 8-oxodG in DNA results in 8-oxodG or 8-oxoGua by nucleotide excision and base excision, respectively (Bessho et al. 1993). Recently, the human 8-oxoGua glycosylase was cloned by several groups (Radicella et al. 1997, Roldan-Arjona et al. 1997) whereas nucleotide excision repair was shown to contribute to the repair of 8-oxodG in DNA. (Reardon et al. 1997). 8-OxodG will also come from the highly specific 8-oxodGTP phosphatase (MutT) and 8-oxodGMP nucleotidase enzymes sanitizing the nucleotide pool (Mo et al. 1992, Hayakawa, H. et al. 1995). Digestion of DNA from apoptotic cells and turnover of mitochondria could also be a source of 8-oxodG. A few of the

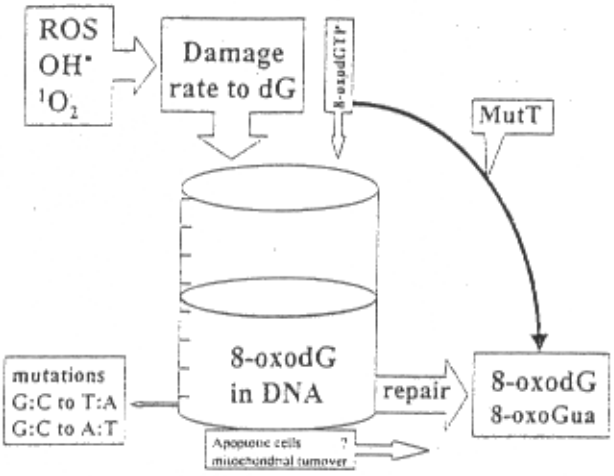

Figure 2. Mass balance of 8-oxodG formation in DNA and nucelotide pool and fates in terms of repair, cell and mitochondrial turnover and mutations. ROS are reactive oxygen species. MutT are 8-oxodGTP phosphatase and 8-oxodGMP nucleotidase sanitizing the nucleotide pool (Mo et al. 1992, Hayakawa, H. et al. 1995). Repair result in 8-oxodG or 8-oxoGua by nucleotide excision and base excision, respectively (Bessho et al. 1993).

8-oxodG lesions in DNA will after replication without repair or misrepair lead to mutations, 8-oxodG formed within DNA can lead to G to T transversions whereas incoporation of 8-oxodGTP can result in both G to T and G to A transversions (Kuchino et al. 1987, Shibutani et al. 1991, Tajiri et al. 1995).

If the rate of damage to DNA, e.g. 8-oxodG formation, is increased by oxidative stress, the nuclear level will increase until an increased repair rate matches the 8-oxodG-influx rate and a new steady state is achieved. An increased enzymatic repair rate can result just from the increased substrate availability (i.e. the 8-oxodG level) as well as from increased enzyme activity as shown in lymphocytes from smokers (Asami et al. 1996). The concept of steady state in the 8-oxodG level is supported by the limited accumulation with age shown in various human and animal cells and tissues investigated so far (Table 1) (Loft and Poulsen 1996). Moreover, in cultured human cells induced levels of oxidized nucleobases return to initial values within some hour's (Jaruga and Dizdaroglu 1996). In human and experimental animals exposed to radiation the increase in oxidized bases in DNA from leukocytes or liver as well as urinary excretion of repair products is temporary (Kasai et al. 1986, Bergtold et al. 1990, Blount et al. 1991, Olinski et al. 1996). The effect of the environmental factors on the level of modified bases in DNA in humans studied so far have been in the order of a factor two or less (Loft and Poulsen 1996). Further support for the concept of a steady state is the relative low variation in individual urinary excretion of 8-oxodG in the control as well as intervention groups in controlled trials (Loft et al. 1995b, Verhagen et al. 1995, Priemé et al. 1997).

The use of urinary excretion of oxidized nucleosides and bases as biomarkers requires almost complete repair and minimal accumulation as argued above. In a study of 8-oxodG in human brain the accumulation rate in the nuclear DNA corresponded 2 lesions per cell per day and possible less in other cells (Mecocci et al. 1993). In humans the reported values of the urinary excretion of the repair products, 8-oxodG, are in the range 15–50 nmol per 24 h (Loft and Poulsen 1996) and the alternative repair product 8-oxoGua appears to be excreted in similar amounts (Suzuki et al. 1995). The sum of these products thus corresponds to an average of 300–1000 lesions per day for each of the assumed 5 x 10^{13} cells in the body per day (Loft et al. 1992, 1995b). Accordingly, the calculated repair efficiency under these assumptions ranges from 99.4% to 99.8%. Each cell contains around 2 x 10^9 dG residues and assuming that 1 per 10^5 is oxidized, the cellular burden is 20,000 8-oxodG's. If repair suddenly stopped a doubling of the number of oxidized nucleobases would take about 20 to 66 days

Table 1. Factors studied with regard to the level of oxidative modifications nucleobases in DNA from humans

Factor	lesion(s)	Assay(s)	Cells or tissues showing a significant increase (or decrease) related to factor	Cells or tissues not showing a significant effect
Sex	8-oxodG	HPLC-EC		leukocytes (Degan et al. 1995)
Age	8-oxodG	HPLC-EC	mtDNA: brain (Mecocci et al. 1993), diaphragm (Hayakawa, M. et al. 1991), heart (Hayakawa, M. et al. 1992), nuDNA: brain (Mecocci et al. 1993), leukocytes (Degan et al. 1995)	leukocytes (Takeuchi et al. 1994), MN+PMN (Bashir et al. 1993, Nakajima et al. 1996b)
Smoking	8-oxodG	HPLC-EC Immunoassay	leukocytes (Kiyosawa et al. 1990, Degan et al. 1995, Asami et al. 1996), sperm (Fraga et al. 1996) oral mucosa cells (Yarborough et al. 1996), placenta (Yin et al. 1995)	leukocytes (Hanaoka et al. 1993, Takeuchi et al. 1994), MN+PMN (Nakajima et al. 1996b), placenta (Daube et al. 1997)
Exercise	8-oxodG	HPLC-EC	lymphocytes (decrease) (Inoue et al. 1993)	
Vitamin C deficiency	8-oxodG	HPLC-EC	sperm cells (Fraga et al. 1991)	lymphocytes (Jacob et al. 1991)
Energy restriction	8-oxodG	HPLC-EC		lymphocytes (Velthuis-te Wierik et al. 1995)
Low fat diet	5-OHmU	GC/MS	leukocytes (decrease) (Djuric et al. 1991)	
Asbestos exposure	8-oxodG	HPLC-EC		leukocytes (Hanaoka et al. 1993)
Radiation	8-oxodG	HPLC-EC+TLC-^{32}P	leukocytes (Wilson et al. 1993)	
	7 bases[a]	GC/MS-SIM	lymphocytes (Olinski et al. 1996)	
Autoimmune diseases[a]	8-oxodG	HPLC-EC	lymphocytes+PMN (Bashir et al. 1993)	
Liver diseases (liver from)	8-oxodG	HPLC-EC	chronic hepatitis (Shimoda et al. 1994), Wilson's disease (decrease) (Carmichael et al. 1995),	cirrhosis (Shimoda et al. 1994), haemochromatosis (Carmichael et al. 1995)
Helicobacter pylori infection	8-oxodG	HPLC-EC	infected gastric mucosa (Baik et al. 1996)	
Cancers of the	8-oxodG	HPLC-EC	kidney (Okamoto et al. 1994), colon (Oliva et al. 1997)	breast (Nagashima et al. 1995), liver (Shimoda et al. 1994)
	8-oxodG	immunoassay	breast (Musarrat et al. 1996)	
	up to 12 bases[b]	GC/MS-SIM	breast (Malins and Haimanot 1991, Malins et al. 1993), lung (Olinski et al. 1992, Jaruga et al. 1994), other sites (Olinski et al. 1992)	
	5-OHmdUridine	GC/MS	whole blood from breast cancer patients (Djuric et al. 1996)	
Neurodegenerative diseases of (various regions of the brain)	8-oxodG [b]	HPLC-EC	Huntington (Browne et al. 1997), Alzheimer (Lyras et al. 1997), Parkinson (Sanchez-Ramon et al. 1994)	
	6 bases	GC/MS-SIM	Alzheimer (Lyras et al. 1997)	
Diabetes mellitus	8-oxodG	HPLC-EC	MN (Dandona et al. 1996)	
Fanconi's anaemia	8-oxodG	HPLC-EC	leukocytes (Degan et al. 1995)	
Prostatic hyperplasia	7 bases[b]	GC/MS-SIM	hyperplastic tissue (Olinski et al. 1995)	

mt: mitochondrial; nu: nuclear; PMN: polymorphonuclear granulocytes; MN: mononuclear leukocytes; [a]SLE, RA, vasculitis and Behçet's disease; [b]of 8-oxoGua, 8-oxoAde, 5-OHmU, Tg, 5-OH-Cyt, 2-OH-Ade, 5,6-diOH-Ura, 5-OH-Ura, 5-OH-Hyd, 5-OH-5-MeHyd, FapyGua and/or FapyAde; not all were increased related to factors.

Several points can be inferred from the concept of a steady state outlined in Fig. 2. As efflux must equal influx, the rate of damage is estimated from the rate of excretion of the repair product, i.e. 8-oxodG in this case. The unknowns in that equation are the contribution of the glycosylase pathway to the repair and the contribution from cellular and mitochondrial turnover to 8-oxodG formation. However, these contributions would also be expected to be in a steady state and the latter actually a part of oxidative damage to DNA. It should be noted that the urinary excretion represent the cumulated body burden and that it usually cannot be determined if this originated from an impact to all body cells or to much higher insult to one or several organs. In contrast, tissue or cellular levels represent the measure of a concentration, reflecting the balance between rate of oxidation and the rate of repair. Moreover, in human studies the cellular levels are frequently measured in surrogate cells, such as lymphocytes, rather than in true target tissues. The general interpretation of a change in the urinary excretion rate of oxidized nucleosides and bases is a change in the rate of damage inflicted by oxidative stress. A change in tissue concentration of oxidized nucleobases/nucleosides cannot with certainty be related to a change in oxidative stress, a change in repair or a combination. Accordingly, the two groups of biomarkers are supplementary. However, much more knowledge regarding repair pathways and kinetics are warranted for the optimum interpretation of these biomarkers.

In a recent experimental rat study we compared target tissue levels and urinary excretion of 8-oxodG. A temporary excess of around 3 8-oxodG per 10^5 dG was induced in the liver by administration of the hepatocarcinogen, 2-nitropropane. Assuming that a rat liver contains 6×10^8 cells and that each cell contains 2×10^9 dG residues this excess corresponds 3.6×10^{13} molecules or 60 pmoles of 8-oxodG. In the same study period the excess urinary excretion of 8-oxodG was 40 pmoles showing a correspondence between the target tissue level and the urinary excretion of repair product. The remaining 8-oxodG may have been excreted as 8-oxoGua that is difficult to quantify in rats because dietary purines are the major source (Park et al. 1992). The present calculations are of course subject to substantial variation and assumptions, however the consistent numbers appear to support that urinary 8-oxodG is a biomarker of 8-oxodG formation in target tissues.

5. FACTORS DETERMINING OXIDATIVE DNA DAMAGE IN HUMANS

A large number of (patho)physiological and environmental factors have been studied in humans with regard to influence on the tissue or cell level of oxidized bases/nucleosides in DNA and the urinary excretion of repair products, i.e. the damage rate (Table 1 and 2). Most of the exact data were recently summarized (Loft and Poulsen 1996).

Sex and age are probably not important determinants of the urinary excretion of 8-oxodG (Table 1 and 2). The excretion may be slightly higher in men and it may decrease some with age (Loft and Poulsen 1996). According to the steady state concept discussed above such a decrease is most likely due to a decreasing damage rate following a decreased metabolic rate (Loft et al. 1994, Loft and Poulsen 1996). The possible accumulation of nuclear 8-oxodG in brain tissue (Mecocci et al. 1993) and in leukocytes showed in one study (Degan et al. 1995) but not in other studies (Bashir et al. 1993, Takeuchi et al. 1994, Nakajima et al. 1996b) is probably caused by failing repair. However, related to neurodegenerative diseases also associated with aging the increased accumulation of oxidized nucleobases could be related to an increased rate of damage as other markers of oxidative stress are increased (Sanchez-Ramon et al. 1994, Browne et al. 1997, Lyras et al.

Table 2. Factors studied with regard to the urinary excretion of oxidatively modified nucleobases and deoxynucleosides in humans

Factor	Lesion(s)	Assay	Excretion parameters showing a significant increase (or decrease) related to factor	Excretion parameters not showing a significant effect
Sex	8-oxodG	HPLC-EC	male>female: 24 h excretion (Loft et al. 1992),	ratio to creatinine (Tagesson et al. 1995)
	dTg and Tg	HPLC-UV	concentration (Tagesson et al. 1996)	24 h excretion (Lunec et al. 1994)
Age	8-oxodG	HPLC-EC	ratio to creatinine (Lagorio et al. 1994), concentration (Tagesson et al. 1996)	24 h excretion (Loft et al. 1992), ratio to creatinine (Lunec et al. 1994)
Smoking	8-oxodG	HPLC-EC	24-h excretion (Loft et al. 1992, 1994, Prieme et al. 1998), ratio to creatinine or concentration (Tagesson et al. 1992, 1996)	ratio to creatinine (Lagorio et al. 1994)
	8-oxoGua	HPLC-EC	ratio to creatinine (Suzuki et al. 1995)	
Oxygen consumption	8-oxodG	HPLC-EC	24 h excretion (Loft et al. 1994)	
Exercise	8-oxodG	HPLC-EC	ratio to creatinine after extensive exercise for 30 d (Poulsen et al. 1996)	ratio to creatinine after swimming/running (Inoue et al. 1993) or rowing (Nielsen et al. 1995)
Antioxidants: beta-carotene, vitamins C and E and coenzyme Q	8-oxodG 8-oxoG	HPLC-EC HPLC-EC		24 h excretion (van Poppel et al. 1995, Priemé et al. 1997), 24 h excretion (Witt et al. 1992)
Brussels sprouts rich diet	8-oxodG	HPLC-EC	decreased 24 excretion in non-smokers (Verhagen et al. 1995)	24 h excretion (Verhagen et al. 1997)
Vegetable and fruit rich diet	8-oxodG	HPLC-EC		24 h excretion (Hertog et al. 1997)
Energy restriction	8-oxodG	HPLC-EC		24 h excretion in 16 non-smokers (Loft et al. 1995b)
	8-oxodG + dTg	GC/MS	decreased 24 h excretion in 1 subject (Simic and Bergtold 1991)	
Benzene exposure	8-oxodG	HPLC-EC	ratio to creatinine (Lagorio et al. 1994), pre-postshift (Nilsson et al. 1996)	
glass work fumes	8-oxodG	HPLC-EC	exposed smokers-unexposed non-smokers (Tagesson et al. 1996)	exposed-unexposed smokers or non-smokers (Tagesson et al. 1996)
rubber + asbestos + azo dye	8-oxodG	HPLC-EC	ratio to creatinine (Tagesson et al. 1993)	
polluted urban air	8-oxoGua	HPLC-EC	ratio to creatinine (Suzuki et al. 1995)	
Various cancer chemotherapy	8-oxodG	HPLC-EC	ratio to creatinine (Tagesson et al. 1995)	
Adriamycin therapy	5-OhmU	GC/MS	24 h excretion (Faure et al. 1996)	
Radiation	8-oxodG+dTg	GC/MS, HPLC-EC, ELISA	24 h excretion (Bergtold et al. 1990), ratio to creatinine (Blount et al. 1991, Tagesson et al. 1995, Erhola et al. 1997)	
Autoimmune diseases	8-oxodG	HPLC-EC	ratio to creatinine increased in RA, decreased in SLE (Lunec et al. 1994)	
Cystic fibrosis	8-oxodG	HPLC-EC	ratio to creatinine in children (Brown et al. 1995)	
Various cancers	8-oxodG	HPLC-EC	ratio to creatinine (Tagesson et al. 1992, 1995)	24 h excretion (Tagesson et al. 1995)
	8-oxodG	ELISA	ratio to creatinine (Erhola et al. 1997)	ratio to creatinine (Erhola et al. 1997)
	dTg and Tg	HPLC-UV		24 h excretion (Cao and Wang 1993)

1997). Possibly the increased levels of 8-oxodG in leukocytes from patients with diabetes mellitus could be attributed to either increased oxidative stress or reduced repair if urinary excretion of 8-oxodG was measured (Dandona et al. 1996).

The urinary excretion of 8-oxodG was closely correlated with oxygen consumption or metabolic rate within a group of young women (Loft et al. 1994) and in men after energy restriction (Loft et al. 1995b). Similar correlations also including dTg excretion have been found across species (Adelman et al. 1988, Cutler 1991, Simic and Bergtold 1991, Loft et al. 1993). This relationship is in keeping with the concept of mitochondrial respiration as an important source of ROS (Chance et al. 1979). Exercise would thus be expected to increase the rate of oxidative DNA damage. Indeed, the 8-oxodG to creatinine ratio was increased after 30 days of intense exercise although short term running, swimming or rowing had no effect in this respect (Inoue et al. 1993, Nielsen et al. 1995). The decrease in the 8-oxodG level in lymphocytes seen after running could be due to recruitment of young lymphocytes as supported by increased counts after exercise (Inoue et al. 1993). In a controlled human study energy restriction had no beneficial effect on lymphocyte DNA level and urinary excretion of 8-oxodG (Loft et al. 1995b, Velthuis-te Wierik et al. 1995) although such intervention can reduce oxidative DNA damage in animal experiments (Kaneko et al. 1997).

Tobacco smoke is a major source of ROS per se and can induce endogenous production of ROS in leukocytes and via an increased metabolic rate. In accordance, the urinary excretion of 8-oxodG and 8-oxoGua was consistently elevated in smokers (Loft et al. 1992, 1994, Tagesson et al. 1992, 1996, Suzuki et al. 1995, Prieme et al. 1998) with the exception of one study with creatinine corrected spot samples (Lagorio et al. 1994). Moreover, in a recent smoking cessation study there was a dose-response relationship and the relevant decrease in 8-oxodG excretion that mirrored an increase in plasma vitamin C (unpublished observations). Similarly, exposure to air pollution from urban air and related to glasswork, benzene and other occupational exposures appear to cause the expected increases in 8-oxodG and 8-oxoGua excretion. However, half of the studies of 8-oxodG in DNA from leukocytes and placenta as well as a study of asbestos exposed workers have failed to show a difference between smoker and non-smokers (Hanaoka et al. 1993, Takeuchi et al. 1994, Nakajima et al. 1996b), whereas the other half and studies of DNA and oral mucosal cells have showed increased levels in smokers (Kiyosawa et al. 1990, Degan et al. 1995, Asami et al. 1996, Fraga et al. 1996). There is no explanation for this apparent discrepancy as several of the studies with both outcomes were reasonably sized and employed similar methods. The level of oxidized pyrimidines measured by the enzyme based comet assay in lymphocytes was increased in smokers (Duthie et al. 1996)

Antioxidants would be expected to decrease oxidative DNA damage, however, intervention studies have generally failed to show effects of the traditional nutritional antioxidants, vitamins C and E, beta-carotene and coenzyme Q, on nuclear levels in leukocytes and urinary excretion of 8-oxodG (Jacob et al. 1991, Witt et al. 1992, van Poppel et al. 1995, Priemé et al. 1997). The only exception relates to the increase in 8-oxodG levels in sperm after depletion of vitamin C (Fraga et al. 1991). Similarly, in a comparison of subjects with a habitual diet rich and poor in fruit and vegetables there was no difference in 8-oxodG excretion (Hertog et al. 1997) whereas intervention with a diet rich in Brussels sprouts reduced the excretion (Verhagen et al. 1995), although this effect was only partly reproduced in men in a later study (Verhagen et al. 1997). In contrast, intervention with a combination of vitamins C and E and beta-carotene reduced the level of oxidized pyrimidines measured by the enzyme based comet assay in lymphocytes from smokers (Duthie et al. 1996)

In most (but not all (Shimoda et al. 1994, Nagashima et al. 1995)) of the studies of malignant (and a benign) tumors increased levels of oxidized nucleobases/nucleosides have been found in the tumor DNA as compared with the surrounding tissue (Malins and Haimanot 1991, Olinski et al. 1992, 1995, Malins et al. 1993, Jaruga et al. 1994, Okamoto et al. 1994, Musarrat et al. 1996, Oliva et al. 1997). Whether this is a causal relationship or an effect of ongoing inflammation in the tumor is not known. Indeed, inflammation, such as related to autoimmune diseases, hepatitis and Helicobacter pylori infections cause increases in the nuclear levels of 8-oxodG and the urinary excretion of 8-oxodG in rheumatoid arthritis (Bashir et al. 1993, Lunec et al. 1994, Shimoda et al. 1994, Baik et al. 1996). In some cancer patients the ratio of 8-oxodG to creatinine was reported to be increased (Tagesson et al. 1992, 1995, Erhola et al. 1997), however, the 24 h excretion was not (Tagesson et al. 1995) and the 24 excretion of dTg and Tg was not increased in cancer patients from another study (Cao and Wang 1993). Accordingly, an apparent effect of cancer in this context may be related to a low creatinine production and the use of creatinine corrected spot urine samples is probably not prudent. In contrast, an expected increasing effect on oxidative DNA damage of cancer chemotherapy and radiation appears quite consistent from all reported studies of both leukocytes and urinary excretion of repair products (Bergtold et al. 1990, Blount et al. 1991, Wilson et al. 1993, Tagesson et al. 1995, Faure et al. 1996, Olinski et al. 1996, Erhola et al. 1997).

6. CONCLUSIONS

A number of different oxidatively modified nucleobases in cellular and tissue DNA and repair products excreted in urine can be measured with a variety of methods. However, problems remain with respect to the true values and inter-method and inter-laboratory variation in particular regarding the levels in nuclear DNA.

It should be emphasized that the levels of oxidized nucleobases in tissue/cells and the excretion of the repair products represent two fundamentally different estimates that are supplementary. The urine measurement represents the number of repaired bases summed from all organs and cells during a given time period, i.e. the rate of damage. The tissue measurement is a concentration measurement in the specific tissue/cells in the moment of sampling dependent on the balance between the rates of damage and repair.

The rate and levels of oxidative DNA modifications in humans have been studied extensively. The data obtained so far indicate that the important determinants of the oxidative damage rate include tobacco smoking, air pollution, oxygen consumption, cancer therapy and some inflammatory and neurodegenerative diseases, whereas diet composition, energy restriction and antioxidant supplements have minimal influence, possibly with the exception of yet unidentified phytochemicals, e.g. from brassica vegetables. Generally these effects are in the order of a factor 2 or less. The data support that oxidative DNA damage caused by endogenous or exogenous oxidative stress is involved in the pathogenesis of cancer and degenerative diseases of aging. In the future the use of the biomarkers may provide further proof of a causal relationship in this respect as well as elucidate possible preventive measures. However, much more knowledge regarding the true sources, pathways and kinetics of repair of the oxidized nucleobases and nucleosides as well as their true levels in DNA by accurate assays is warranted.

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