8-HYDROXYDEOXYGUANOSINE AS A URINARY BIOMARKER OF OXIDATIVE DNA DAMAGE

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Living organisms are continuously exposed to reactive oxygen species as a consequence of biochemical reactions as well as external factors. Oxidative DNA damage has been implicated in aging, carcinogenesis and other degenerative diseases. The urinary excretion of the DNA repair product 8-hydroxydeoxyguanosine (8OHdG) has been proposed as a noninvasive biomarker of oxidative DNA damage in humans in vivo. We have developed a three-dimensional HPLC analysis with electrochemical detection for the analysis of 8OHdG in urine and studied factors affecting the excretion of this biomarker in 83 healthy humans and in various laboratory animals, including dog, pig, and rat. Previously, other groups have used comparable HPLC methods or gas chromatography-mass spectrometry with selective ion monitoring for measuring the excretion of 8OHdG in humans, rats, mice, and monkeys. In the 169 humans studied so far, the average 8OHdG excretion was 200–300 pmol/kg per 24 h with a sevenfold range, and the coefficient of variation was 30–40%. This excretion corresponds 140–200 oxidative modification of guanine bases per cell per day. Thirty-two smokers from our study population excreted 50% (31–69%; 95% confidence interval) more 8OHdG than 53 nonsmokers. This indicates a 50% increased rate of oxidative DNA damage from smoking, adding to the other well-known health hazards of smoking. The biochemical-physiological basis is unknown but may be related to smoke constituents including or generating reactive oxygen species and/or consuming antioxidants and/or the well-known enhancing effect of smoking on the metabolic rate. In our 83 healthy subjects the 8OHdG excretion correlated with body composition. Thus, lean and/or male subjects excreted more than obese and/or female subjects, possibly related to differences in metabolic rate. In accordance, the excretion of 8OHdG decreased after calorie restriction, which will cause a decline in the metabolic rate. Across the investigated species, humans, dogs, pigs, and rats, the excretion of 8OHdG correlated with the specific metabolic rate, confirming data from other groups on humans, monkeys, rats, and mice. The excretion of 8OHdG decreased with age in rats in parallel with the decline in metabolic rate with advancing age. The excretion of 8OHdG reflects the formation and repair of only one out of approximately 20 described oxidative DNA modifications. So far, methods are not available for the determination of the corresponding repair products, except 8OHdG and thymidine glycol, in urine. Moreover, the importance in terms of mutagenicity, particularly regarding tumour supressor genes and oncogenes, is mainly documented for 8OHdG in DNA. In mammalian cells an excission repair enzyme complex for 8OHdG has been demonstrated, but whether the main product is 8OHdG or the base is yet unknown. In addition, 8OHdG may derive from DNA during turnover of mitochondria and cells as well as from oxida-

The present study was supported by the Danish Medical Research Council, grant 12-9374.

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Journal of Toxicology and Environmental Health, 40:391–404, 1993

0098-4108/93 $10.00 + .00

tion in the deoxynucleotide and deoxynucleoside pools that provide building blocks for new DNA. Thus, the excretion of 8OHdG will reflect the general average risk of promutagenic oxidative adducts in DNA of all tissues and organs. We suggest that the individual variation in the apparent massive extent of oxidative DNA-damage in humans predicts the rate of aging and the risk of cancer as well as other degenerative diseases. The use of 8OHdG and similar urinary biomarker of oxidative DNA damage offers a valuable tool for testing such hypotheses in humans.

INTRODUCTION

In the living organism, reactive oxygen species (ROS) are formed continuously as a consequence of biochemical reactions as well as external factors. Inadequate physiological antioxidant defense systems may thus lead to oxidative damage (Halliwell and Gutteridge, 1989). ROS are proposed as important in aging as well as a number of age-related degenerative diseases, including cancer, heart disease, arthritis and other inflammatory conditions, and cataract formation (Ames and Saul, 1986; Spector and William, 1981; Ames, 1989; Fraga et al., 1990; Lunec, 1990; Reilly et al., 1991; Greenwald, 1991; Ames and Gold, 1991).

During mitochondrial respiration a substantial part (1–5%) of the consumed oxygen escapes the simultaneous transfer of four electrons and undergoes stepwise single-electron transfer, generating ROS, the superoxide anion radical, hydrogen peroxide, and the hydroxyl radical (Turrens and Boveris, 1980; Reilly et al., 1991). The first line of defense against the superoxide radical is superoxide dismutase, which forms hydrogen peroxide. This may in turn be reduced to water by glutathione peroxidase or catalase. Hydrogen peroxide has limited reactivity and crosses membranes easily. In the presence of transition metals hydrogen peroxide is reduced to hydroxyl radicals, the most reactive ROS. Superoxide radicals and hydrogen peroxide, which in turn may give rise to hydroxyl radicals, are generated by many other mechanisms, for example, via hypoxanthine metabolism by the xanthine oxidoreductase system during postischemic reperfusion, by cooxidation during the metabolism of xenobiotics and arachidonic acid, and in phagocytes (Reilly et al., 1991). Neutrophils also generate hypochlorous acid, a very reactive ROS known from household bleach, during their respiratory burst by means of myeloperoxidase and chloride ions (Weiss, 1989). Ionizing radiation generates superoxide and hydroxyl radicals (Davies, 1987). Singlet oxygen, also an important ROS, is generated by illumination of photosensitizers and in minor amounts by cooxidation and other enzymatic reactions (Epe, 1991).

ROS can attack any cellular structure or molecule. However, with respect to aging and cancer, DNA is considered an important target (Ames and Gold, 1991). Thus, ROS may cause DNA-protein cross-links and sugar moiety damage as well as specific chemical modifications of the purine and pyrimidine bases (Dizdaroglu, 1991). Oxidation of the sugar moiety induces base release and strand breaks whereas oxidative base modifications result in mutations (Kuchino et al., 1987; Shibutani et al., 1991; Cheng et al., 1992). One of the most abundant oxidative damages to DNA bases is the C8-hydroxylation of guanine (Shigenaga et al., 1989; Floyd et al., 1986; Reilly et al., 1991; Dizdaroglu, 1991; Floyd, 1990). Whereas hydroxyl radicals generate multiple products from all four bases, singlet oxygen preferentially modifies guanine, particularly by 8-hydroxylation (Dizdaroglu, 1991; Epe, 1991). The occurrence of 8-hydroxyguanine (8OHdG) in DNA, usually measured as a ratio to deoxyguanosine after enzymatic digestion of DNA, has been used extensively to study damaging effects on DNA of ROS generated by all the mechanisms mentioned above as well as the protective or prooxidants effects of antioxidants (Kasai and Nishimura, 1984; Kasai et al., 1986; Floyd et al., 1986; Leanderson and Tagesson, 1990, 1992; Kiyosawa et al., 1990; Faga et al., 1991; Fischer-Nielsen et al., 1992).

In vivo, damaged DNA is repaired by exonucleases or glycosylases liberating deoxynucleosides or bases, respectively. These repair products are water-soluble and readily excreted into the urine without further metabolism (Bergtold et al., 1988; Shigenaga et al., 1989; Fraga et al., 1990). Exogenous DNA from the diet contributes only to the excretion of oxidized bases but not to that of oxidized deoxynucleosides (Shigenaga et al., 1989; Park et al., 1992; Bergtold and Simic, 1991). Thus, the urinary excretion of oxidized deoxynucleosides, such as 8OHdG and/or thymidine glycol, has been proposed as biomarkers of the current oxidative DNA-damage and repair (Cathcart et al., 1984; Shigenaga et al., 1989; Bergtold et al., 1988; Shigenaga and Ames, 1991). At present most data are available regarding 8OHdG.

METHODS FOR ANALYSIS OF 8OHdG IN URINE

A number of analytical methods have been developed for the measurement of 8OHdG in urine. The group of Ames and co-workers used multiple solid-phase extractions on mini columns before HPLC gradient separation, but more recently the extractions have been accomplished by immunoaffinity columns with specific antibodies to 8OHG and 8OHdG, requiring only isocratic separation (Shigenaga et al., 1989; Degan et al., 1991; Park et al., 1992). In both methods detection is electrochemically and radiolabeled 8OHdG is used as internal standard. A Swedish group has developed an isocratic coupled column HPLC method also with electrochemical detection (Tagesson et al., 1992).

In our laboratory an automated three-dimensional HPLC method with isocratic separation and electrochemical detection has been developed for the analysis of 8OHdG in urine (Loft et al., 1992). A flow path diagram is shown in Figure 1. By this method, urine is chromatographed under alkaline (pH 7.9) conditions on a 15-cm C18 5-μm column. A 1-ml fraction of the effluent containing the 8OHdG is brought on a 2-cm cation-exchange column via a 6-port automatic valve. With a switch of the valve this column is then flushed with an acidic eluent (pH 2.2), bringing the content

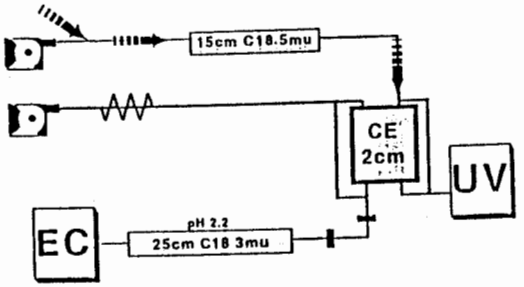

FIGURE 1. Diagram of a three-dimensional HPLC method for analysis of 8-hydroxydeoxyguanosine. A six-port valve directs the effluent (pH 7.9) from the first column (15 cm) to waste or on a 2-cm cation exchange (CE) column monitored by ultraviolet detection. The valve also directs the second mobile phase (pH 2.2) to either flush or bypass the CE column before eluting the separation column (25 cm). The effluent of this is monitored by an ESA Colouchem II electrochemical detector set at 120 mV (electrode 1) and 300 mV (eletrode 2).

onto a 25-cm C18 3-μm column. The effluent of this column is monitored by an ESA Columchem II electrochemical detector in the oxidation mode set at 120 mV for electrode 1 and 300 mV for electrode 2. For quantification, samples are run with and without addition of genuine 8OHdG and the concentrations calculated from the peak heights. The performance of the method includes an interassay coefficient of variation of 10% and a limit of detection of 0.2 n*M* as compared to concentrations ranging from 1 to 70 n*M* in human urine. Moreover, the method is reasonably simple and automated, allowing the handling of larger number of samples.

By means of gas chromatography-mass spectrometry with selected-ion monitoring, several oxidatively modified deoxynucleosides, including 8OHdG and thymidine glycol, may be simultaneously measured in urine (Bergtold et al., 1990). Indeed, this method may potentially allow determination of urinary excretion of repair products of the many oxidative DNA modifications demonstrated in human tissue, as well as in many in vitro model systems (Dizdaroglu, 1991; Malins and Haimanot, 1991; Halliwell and Dizdaroglu, 1992; Olinski et al., 1992). So far, however, limited data have emerged from this highly specific and sensitive method.

FACTORS DETERMINING 8OHdG EXCRETION

In humans the excretion of 8OHdG is normally distributed with a 7- to 10-fold range and the coefficient of variation is 30–40% (Table 1) (Park et al., 1992; Bergtold and Simic, 1991; Loft et al., 1992). The intraindividual variation appears to be somewhat less. Thus, the urinary excretion of 8OHdG measured on two occasions 130 d apart in 20 individuals correlated closely (r^2 = .53) (Park et al., 1992). This is in agreement with data obtained

in one subject studied repetitively for 1 yr (Simic and Bergtold, 1991) and our own unpublished experience. The excretion of 8OHdG has been shown to be associated with a number of factors which by inference are thought to modify oxidative DNA damage.

Species

Across species, including humans, monkeys, rats, and mice, linear correlations have been reported between the specific metabolic rate and the excretion of 8OHdG and thymine and thymidine glycol, other repair products of oxidative DNA damage (Adelman et al., 1988; Shigenaga et al., 1989; Bergtold and Simic, 1991). Supposedly, the high mitochondrial oxygen metabolism in small animals, such as rodents, allows generation of more ROS as the most important source of oxidative DNA damage. We have investigated the correlation between 8OHdG excretion and table values (Spector, 1956) of the specific metabolic rate among humans (Loft et al., 1992), pigs, dogs and rats. Urine was collected for 4 h under anesthesia by means of suprapubic bladder catheters in 7 pigs aged 8 wk, for 3 h by means of bladder catherization in 1 beagle dog aged 24 mo and for 24 h by means of metabolic cages in 12 male rats aged 7 wk and 10 rats aged 15 wk (Fig. 2; unpublished data). Within these species we found a linear correlation although the slope was steeper than in the study by Ames and co-workers (Shigenaga et al., 1989). As shown in Table 1, the data on human 8OHdG excretion agree between the laboratories, whereas the excretion in our rats was more than twice the value reported by Ames and co-workers (Fraga et al., 1990; Park et al., 1992). Moreover, in mice a 15 times higher

TABLE 1. Reported Values of 8OHdG Excretion in Various Species

Species	Age	Smoking Status	8OHdG excretion (pmol/kg per 24 h, mean ± SD)	Reference
Human (n = 63)	Unknown	Unknown	172 ± 79	Park et al. (1992)
Human (n = 23)	Unknown	Unknown	300 ± 100	Bergtold and Simic (1991)
Human (n = 53)	40–64 yr	Nonsmokers	213 ± 84	Loft et al. (1992)
Human (n = 30)	40–64 yr	Smokers	320 ± 9	Loft et al. (1992)
Rat (n = 6)	8 wk	—	480 ± 160[a]	Fraga et al. (1990)
Rat (n = 6)	104 wk	—	160 ± 65[a]	Fraga et al. (1990)
Rat (n = 18)	7 wk	—	1298 ± 417	S. Loft, H. E. Poulsen, E. Thorling, and I. B. Jeding (unpublished)
Rat (n = 20)	15 wk	—	943 ± 298	S. Loft, H. E. Poulsen, E. Thorling, and I. B. Jeding (unpublished)
Rat (n = 30)	Unknown	—	370 ± 63	Park et al. (1992)
Mice (n = 6)	Unknown	—	680 ± 110[a]	Shigenaga et al. (1989)
Mice (n = 16)	Unknown	—	11,000 ± 2000	Bergtold and Simic (1991)

[a]Read from graphs.

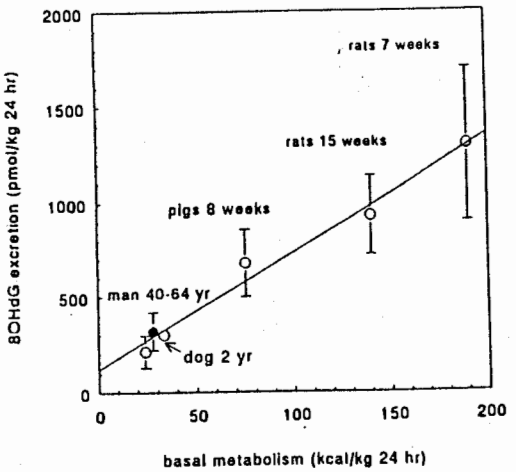

FIGURE 2. Interspecies correlation of the excretion of 8-hydroxydeoxyguanosine (8OHdG) and the specific metabolic rate. In humans, nonsmokers ($n = 53$) are shown with an open symbol and smokers ($n = 30$) with a closed symbol. Values are mean with SD; dog, $n = 1$; pigs, $n = 7$; rats, $n = 18$ (7 wk) and $n = 20$ (15 wk).

rate of 8OHdG excretion has been reported by Bergtold and Simic (1991) as compared to the value published by Ames and co-workers (Table 1) (Shigenaga et al., 1989). The reason for these substantial interlaboratory differences with respect to rodent data is unknown and interlaboratory exchange of samples has not been reported. However, they are less (i.e., two-fold) with respect to thymidine glycol excretion in mice (Adelman et al., 1988; Bergtold and Simic, 1991).

Age

In a human study population with a narrow age range there was no correlation between age and 8OHdG excretion (Loft et al., 1992). However, in rats the excretion of 8OHdG has been shown to decrease with age while the modified base accumulates in tissue DNA (Fraga et al., 1990). Similarly, 8OHdG has been reported to accumulate in the mitochondrial DNA of the human diaphragm muscle cells (Hayakawa et al., 1991). The accumulation may be a result of slight reduction of repair efficiency (Fraga et al., 1990; Hayakawa et al., 1991). From the rate of 8OHdG excretion and accumulation it has been estimated that less than 1 in 50 damaged bases are left unrepaired in the kidney and even less in other organs (Fraga et al., 1990). If the age-related decrease in 8OHdG excretion resulted from failing repair, a much more massive accumulation of oxidatively modified bases would be expected. Moreover, as shown in Figure 2, the decrease in 8OHdG excretion may be explained by the decrease in metabolic rate with age.

Smoking

In 83 healthy subjects the most important factor determining 8OHdG excretion was smoking. Thus, smokers excreted 50% (31–69%; 95% confidence interval) more 8OHdG than nonsmokers (Fig. 2 and Table 1) (Loft et al., 1992). In agreement, a 30% higher ratio between 8OHdG and creatinine was found in spot urine collected from 10 smokers as compared with 24 nonsmokers (Tagesson et al., 1992).

In vitro, tobacco smoke and several of its constituents, such as hydroquinone and cathecol, have been shown to generate ROS and to induce oxidative damage to isolated DNA as well as to produce 8OHdG in cell cultures (Leanderson and Tagesson, 1990, 1992). The volatile phase of tobacco smoke induces lipid peroxidation and lipoprotein oxidation in human plasma in vitro (Frei et al., 1991). In the plasma of smokers low concentrations of the important antioxidants, ascorbic acid and β-carotene, have been found, suggesting increased consumption and reduced antioxidant capacity (Ziegler, 1989; Riemersma et al., 1991; Frei et al., 1991). Moreover, in smokers leukocyte DNA has been found to contain approximately 50% more 8OHdG than in nonsmokers (Kiyosawa et al., 1990). The finding of increased urinary 8OHdG excretion in smokers is the first verification of these suggestions in vivo in humans. The apparent increasing effect of tobacco smoking on oxidative DNA damage may also be related to its well-documented enhancing effect on the basic metabolic rate (Moffatt and Owens, 1991).

Body Composition

In 83 healthy subjects the significant predictors of the 8OHdG excretion were, except smoking, either the body mass index (BMI: body weight divided by the square of the height) and gender or the fat-free body fraction measured by body impedance (Loft et al., 1992). There was an inverse relationship between relative 8OHdG excretion and BMI that may relate to the fact that lean persons have a higher metabolic rate than obese (Shah et al., 1988). By the same token, men have a higher basic metabolic rate than women (Meijer et al., 1992), which may provide an explanation of a higher 8OHdG excretion in men (Loft et al., 1992). Moreover, the sedentary daily energy expenditure has been shown to correlate closely with fat-free body mass and BMI (Webb and Sangal, 1991). Accordingly, concurrent measurements of 8OHdG excretion and metabolic rate in subjects with different body compositions are warranted. Nevertheless, the data suggest that, ideally, the 8OHdG excretion should be corrected for lean body mass for comparison of individuals with respect to external factors, such as smoking.

Diet

Reduction of the calorie intake by 40–50% reduced the excretion of 8OHdG and thymidine glycol by an even larger fraction in two subjects

(Simic and Bergtold, 1991). Although not measured, this effect may have been related to a decrease in the metabolic rate due to the calorie restriction. In rats calorie restriction decreased the content of 8OHG in tissue DNA (Chung et al., 1992).

In two subjects a high intake of fruit and vegetables as compared to an isocaloric diet with none of these components reduced the excretion of thymidine glycol substantially, supposedly due to differences in antioxidant content (Simic and Bergtold, 1991). Moreover, a high intake of vitamin C has been reported to protect against 8OHdG formation in human seminal DNA (Fraga et al., 1991). By contrast, in a study population of 83 healthy subjects the intakes of vitamins A, C, and E were not correlated to the 8OHdG excretion (Loft et al., 1992), although as antioxidants they could have been expected to have a protective effect. However, the generally modest intake, which may not be accurately recorded from the diaries, may have less or no effect on 8OHdG formation. In fact, the antioxidant effect of vitamin C may be outbalanced by a prooxidant effect if hydroxyl radicals are generated by transition metals in Fenton-like reactions (Borg and Schaich, 1989; Fischer-Nielsen et al., 1992).

In women at high risk of breast cancer a low-fat diet decreased oxidative DNA damage in nucleated peripheral blood cells as measured by the levels of oxidized thymine (Djuric et al., 1991). In another human study population, however, the relationship between dietary fat and the excretion of 8OHdG was negative—if any (Loft et al., 1992).

Ionizing Radiation

Ionizing radiation generates hydroxyl as well as superoxide radicals, resulting in oxidative DNA damage. Thus, the content of 8OHdG increases in DNA from tissues and cell cultures after radiation (Kasai et al., 1984, 1986). In two patients receiving 1.8 Gy of radiation therapy for breast and colon cancer, respectively, (i.e., a limited radiation field), the excretion of 8OHdG and thymidine glycol was fourfold increased (Bergtold et al., 1990). Moreover, in a single subject total body irradiation with 10 Gy was followed by a short 20-fold increase in 8OHdG excretion, which returned to almost normal within 16 h (Blount et al., 1991). In a comparison of irradiated mice and humans the relative increase in thymidine glycol excretion was similar in the two species and directly proportional with the radiation dose (Simic and Bergtold, 1991). This is in agreement with linear dose-response curves concerning radiation-induced oxidative damage in isolated DNA, including the formation of 8OHdG (Dizdaroglu, 1991).

Conclusion

Except for the effects of ionizing radiation, the variation in 8OHdG excretion within and between subjects and species may at least partly be explained by differences in metabolic rates. In support, mitochondrial DNA, which is closer to the source of ROS generated from the electron transport chain, has a 16-fold higher content of 8OHdG than nuclear DNA, although some of this difference may be related to a more efficient repair in the latter (Richter et al., 1988). A protective effect of dietary antioxidants towards oxidative DNA damage has been suggested by observations in two subjects but needs to be confirmed in large-scale intervention studies, preferably including smokers.

LIMITATIONS OF 8OHdG AS A URINARY BIOMARKER

The excretion of 8OHdG reflects only the (potential) formation and repair of one oxidative DNA modification. Thus, in several in vitro systems with ROS generation as well as in human tissue a whole series of DNA modifications, including ring-opened base products, has been described (Dizdaroglu, 1991; Malins and Haimanot, 1991; Olinski et al., 1992). However, the determination of most of these in DNA requires gas chromatography-mass spectrometry. So far, methods are not available for the determination of the corresponding repair products, except 8OHdG and thymidine glycol, in urine, and factors possibly associated with or affecting their excretion have not been studied. Moreover, the consequences of oxidative DNA modifications other than 8OHdG (see later discussion) and pyrimidine dimers have not been as thoroughly investigated.

The exact sources of urinary 8OHdG have yet to be determined. So far, the FPG (formiamidopyrimidine-DNA glycosylase) enzyme of *Escherichia coli* has been shown to repair 8OHdG by liberating the base initially (Tchou et al., 1991; Boiteux et al., 1992). In mammalian cells a similar enzyme complex has been shown to repair by excission at a different site, the proximal and distal phosphodiester bond at the 3′ and 5′ side of lesion, respectively (Yamamoto et al., 1992). However, whether the base is liberated initially or the end product is 8OHdG is yet unknown. In addition, 8OHdG will be liberated from DNA during turnover of mitochondria and from nuclear DNA at apoptosis and other types of cell turnover. Mitochondrial DNA contain relatively more 8OHdG due to its proximity to the respiratory chain, but the amounts are small in absolute terms compared with nuclear DNA (Richter et al., 1988). Other potential sources of urinary 8OHdG include oxidation in the deoxynucleotide, and deoxynuleoside pools that provide building blocks for DNA. No matter which of these are most important, the excretion of 8OHdG will reflect the general average risk of promutagenic oxidative adducts in DNA of all tissues and organs.

CONSEQUENCES OF 8OHdG FORMATION IN DNA

The available data regarding the urinary excretion of 8OHdG and thymine and thymidine glycol suggest that oxidative damage to DNA is extensive in humans, approximately 10^4 base oxidations per cell per day (Cathcart et al., 1984; Adelman et al., 1988; Bergtold et al., 1988;

Shigenaga et al., 1989; Loft et al., 1992). In agreement, oxidative modifications, including 8OHdG, are found in large quantities in human DNA (Malins and Haimanot, 1991; Olinski et al., 1992). The possible health implications of the variation in the excretion 8OHdG and similar potential biomarkers and by inference oxidative DNA damage are undetermined.

The information of 8OHdG in nuclear DNA has been particularly implicated in carcinogenesis (Floyd, 1990). In vitro it has been shown that oxidized guanine bases in replicating DNA will lead to G-T and A-C transversions as well as other mutations (Kuchino et al., 1987; Shibutani et al., 1991; Cheng et al., 1992; Kamiya et al., 1992). Indeed, the DNA of human tumors, including pulmonary, has been shown to contain increased amounts of oxidative modifications, particularly 8OHdG (Malins and Haimanot, 1991; Olinski et al., 1992). Moreover, G-T transversions are among the most frequent hot-spot mutations in the p53 suppressor gene in human tumors (Hollstein et al., 1991). In mammalian systems 8OHdG or oxidatively induced G-T transversion in codon 12 of c-Ha-ras or K-ras oncogenes, respectively, was shown to cause activation (Higinbotham et al., 1992; Kamiya et al., 1992). ROS-mediated DNA damage frequently includes strand breaks, which will activate error-prone polymerase repair, allowing oxidized bases to cause miscoding (Floyd, 1990). Across species the extent of oxidative DNA damage is correlated to cumulative cancer risk and inversely correlated to maximum life span (Adelman et al., 1988; Ames, 1989; Simic and Bergtold, 1991). Moreover, in laboratory animals increased longevity and reduced cancer risk result from diet restriction (Masoro and McCarter, 1991), possibly related to a decreased metabolic rate and oxidative DNA damage as documented by a reduction in 8OHdG in rat tissue DNA (Chung et al., 1992). Ionizing radiation and some cancer chemotherapeutics that induce oxidative DNA damage are known to cause cancer in humans (Fry, 1991). In this context, the effect of smoking in terms of oxidative DNA damage is equivalent to a radiation dose of 0.25 Gy, assuming linearity from the increase in 8OHdG excretion in two patients treated with 1.8 Gy for cancer (Bergtold et al., 1990; Loft et al., 1992). The mutagenicity and carcinogenicity of ultraviolet radiation may, besides its link to formation of pyrimidine dimers, be related to G-T and A-C mutations in agreement with the specificity of singlet oxygen for 8OHdG formation (Epe, 1991).

The accumulation of 8OHdG in mitochondrial DNA of muscle cells suggests that oxidative DNA damage contributes to the muscle weakness acquired during aging (Hayakawa et al., 1991). The age-related declining function of other organs, such as the kidneys, has also been suggested to be related to accumulation of 8OHdG and other oxidative DNA modifications (Fraga et al., 1990; Yokozawa et al., 1992). Many other important and age-related diseases, such as atherosclerosis, are thought to involve ROS-related cellular damages in their pathogenesis (Carpenter et al., 1991; Salonen et al., 1992). In this respect, formation of 8OHdG may be a marker of risk, although not necessarily directly involved in the pathogenesis.

CONCLUSION AND PERSPECTIVES REGARDING 8OHdG AS A URINARY BIOMARKER

ROS and related DNA damage have been implicated in the pathogenesis of a number of important diseases and aging. By means of simple noninvasive measures of oxidative DNA damage, such as urinary excretion of 8OHdG, the numerous hypotheses of the importance of ROS in aging, degenerative and inflammatory diseases, and carcinogenesis may be challenged. Use of 8OHdG and similar biomarkers provides unique opportunities for investigation of the related health effects in prospective studies or in nested case-control studies with urine from biological banks. The postulated beneficial effects of antioxidant supplementation and other dietary intervention also represent unchallenged hypotheses to be tested. So far, 8OHdG and thymidine glycol are the only available noninvasive biomarkers for oxidative DNA damage. In the future, other similar repair products, such as adenine adducts and ring-opened bases, may be determined by gas chromatography-mass spectrometry and used as urinary biomarkers. Potentially, such a series of biomarkers may allow determination of the kind and source of ROS involved and the contribution from various target organs, as well as a better indication of the related health risks.

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