Mutation Research, 214 (1989) 41–46
Elsevier

MUT 02556

Endogenous DNA damage as related to cancer and aging

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(Received 18 July 1988)
(Revision received 3 August 1988)
(Accepted 22 February 1989)

Keywords: Aging; Spontaneous mutation; Oxidation; Methylation; Endogenous DNA damage

Summary

The endogenous background level of oxidant-induced DNA damage in vivo has been assayed by measuring 8-hydroxydeoxyguanosine (oh^{8}dG), thymine glycol and thymidine glycol in urine and oh^{8}dG in DNA. The level of oxidative DNA damage as measured by oh^{8}dG in normal rat liver is shown to be extensive (1/130 000 bases in nuclear DNA and 1/8000 bases in mitochondrial DNA), especially in mtDNA. The methylation adduct 7-methylguanine (m^{7}G) has also been found. m^{7}G is one of about 5 adducts found on methylating DNA, and oh^{8}dG is one of about 20 adducts found on oxidizing DNA, e.g., by radiation. We also discuss 3 hitherto unrecognized antioxidants in man.

Cancer, aging, and endogenous sources of DNA damage

The marked increase in life span that has occurred in 60 million years of primate evolution has been accompanied by a marked decrease in age-specific cancer rates. Cumulative cancer risk increases with approximately the fourth power of age (Fig. 1), both in short-lived species such as rats and mice (about 30% have cancer by the end of their 2–3-year life span) and in long-lived species such as humans (about 30% have cancer by the end of their 85-year life span).

One important factor in longevity appears to be basal metabolic rate (Cutler, 1984), which is much lower in man than in rodents and could markedly affect the level of endogenous mutagens produced by normal metabolism. We assume that DNA damage is likely to be critical for both cancer and

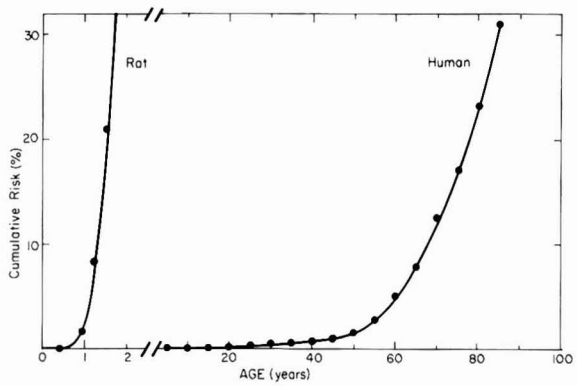

Fig. 1. Cumulative net risk of death from cancer for rats and humans. From Ames et al. (1985), with permission.

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aging. One view of the somatic damage theory of aging is that the amount of maintenance and repair of somatic tissues is always less than that required for indefinite survival. Thus, some DNA damage in somatic cells induced by endogenous mutagens will accumulate with time.

4 important endogenous processes leading to significant DNA damage are likely to be oxidation (Totter, 1980; Ames, 1983; Cutler, 1984), methylation, deamination and depurination (Saul and Ames, 1986). In support of this are the existence of specific DNA-repair glycosylases for oxidative, methylated and deaminated adducts, and a repair system for apurinic sites produced by spontaneous depurination (Lindahl, 1982). The measurement of DNA adducts by new methods shows that oxidation is a major type of DNA damage (see below).

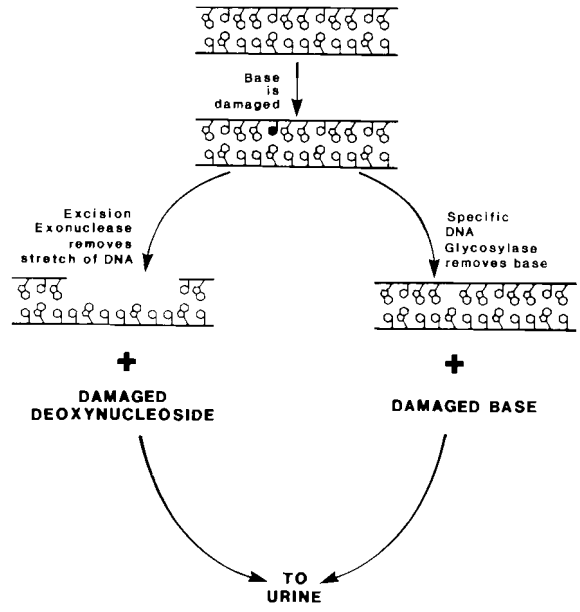

Fig. 2. Repair of a damaged DNA base by excision repair or a specific glycosylase. From Ames et al. (1985), with permission.

The measurement of oxidative DNA damage

Oxidative damage of cellular DNA has been detected by chemical, physical, enzymatic, and immunochemical methods. The methods have been sensitive enough to detect DNA damage induced by severe stresses such as kilorad doses of radiation, but have not generally been useful for examining the background levels of damage products formed from normal aerobic metabolism. Our laboratory has overcome this problem by using an approach based on the pathways shown in Fig. 2. Non-specific DNA-repair enzymes excise DNA adducts to release deoxynucleotides, or specific DNA repair glycosylases release free bases. Deoxynucleotides are enzymatically hydrolyzed to the deoxynucleosides which are not usually further metabolized, and both these and the free bases may be recovered in the urine. Two products of oxidative damage to DNA are thymine glycol and 5-hydroxymethyluracil. We have described a specific DNA-repair enzyme, a DNA glycosylase from mouse cells, which repairs 5-hydroxymethyluracil and differs from the specific DNA glycosylase repair enzyme for thymine glycol in mouse cells (Hollstein et al., 1984). The existence of these specific repair enzymes points to the importance of this type of DNA damage in vivo.

Our method suffers from being an indirect measurement of what was in the DNA and being potentially subject to artifacts. Nevertheless, it has two very powerful advantages. It can be made extremely sensitive, in part because DNA lesions from all the cells in the body are concentrated in a relatively small volume of urine, and it is noninvasive.

In order to quantify the daily removal of these lesions from DNA, we developed a high-performance liquid chromatography (HPLC) assay for thymine glycol, thymidine glycol, hydroxymethyluracil and hydroxymethyldeoxyuridine in urine (Cathcart et al., 1984; Saul et al., 1987; Ames and Saul, 1988; unpublished observations). Our results indicate that normal humans excrete a total of about 100 nmoles/day of the first 3 compounds. We have considerable evidence that most of this total is derived from repair of oxidized DNA, rather than from alternative sources, e.g., diet or bacterial flora (Cathcart et al., 1984; Saul et al., 1987; Ames and Saul, 1988). This total may therefore represent an average of about 10^3 oxidized thymine residues per day for each of the body's 6×10^{13} cells. Because these products are only 3 of about 20 products of oxidative damage of DNA (Cadet and Berger, 1985; von Sonntag, 1987), the total number of all types of oxidative hits of DNA per cell per day in man may be more than 10^4.

Oxidant-induced DNA damage as related to metabolic rate

Although considerable speculation about the relationships among oxidants, cancer, and aging abounds, the experimental evidence is still weak. The impressive inverse interspecies correlation between the specific metabolic rate and the rate of aging, i.e., species with high metabolic rates also have a high age-specific cancer incidence (Fig. 1), is circumstantial evidence implicating oxidants in aging. The faster rate of aging and the faster accumulation of carcinogenic events for mammals with higher specific metabolic rates may be explained by assuming that these species have higher rates of production of oxidants per cell, leading to faster accumulation of somatic damage, carcinogenic events and aging. We now have data on 4 species that provide additional circumstantial evidence for this theory and are consistent with the possibility that DNA is a critical target in aging. Rats, which have a higher specific metabolic rate and a shorter life span than humans, excrete about 15 times more thymine glycol and thymidine glycol per kg of body weight than do humans (Fig. 3) (Cathcart et al., 1984; Adelman et al., 1988). Mice have an even higher metabolic rate and a shorter life span, and they have higher levels of thymine glycol and thymidine glycol than rats. Data obtained with monkeys are consistent with this relationship (Adelman et al., 1988).

We have now tested urines for thymine glycol and thymidine glycol from normal human volunteers aged 22–84 years (Saul et al., 1987). The preliminary evidence is that their urinary outputs

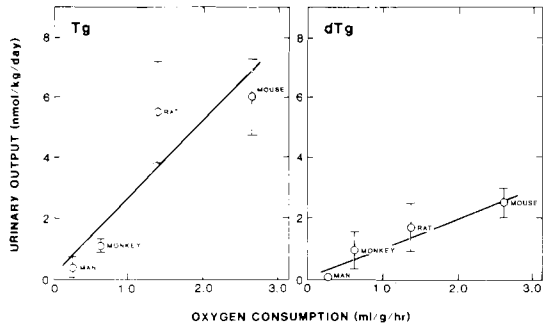

Fig. 3. Average urinary output of thymine glycol (Tg) and thymidine glycol (dTg) for 4 different species, expressed as a function of the specific metabolic rate. From Adelman et al. (1988), with permission.

are age-independent, which suggests that the rate of oxidative DNA damage in man does not change substantially with age. This is consistent with age-independent somatic damage theories of aging (Peto et al., 1985).

8-Hydroxydeoxyguanosine in urine

Our urinary thymine glycol assay is difficult and takes about 3 weeks. We are thus developing a simpler urinary assay for 8-hydroxydeoxyguanosine (oh^8dG), which has been measured in DNA by electrochemical detection at about 1000 times the sensitivity of UV detection (Floyd et al., 1986; Kasai et al., 1986; Kuchino et al., 1987). This new assay (Cundy et al., 1988; Shigenaga et al., 1988) can be done quickly and thus will enable a much more rapid and simpler assay of urine for oxidized DNA. The level in human urine is of the same order as dTg and is much lower than the level in rodents. We also have preliminary evidence for the presence of 8-hydroxyguanosine, a measure of oxidative damage to RNA. We have used this assay to measure oh^8dG in urine of humans with chronic granulomatous disease (Cundy et al., 1988). These people lack the respiratory burst from phagocytic cells, yet they still produce oh^8dG in their urine. This is evidence that the presence of oxidized DNA bases in urine is not a consequence of normal cell turnover.

8-Hydoxydeoxyguanosine in DNA

Since our work on urinary excretion of oxidized bases did not distinguish between damage to nuclear and mitochondrial DNA we have examined the level of 8-hydroxydeoxyguanosine, 1 of about 20 known radiation damage products, in both mitochondrial and nuclear DNA of normal rat liver (Richter et al., 1988). It is present at a level of 1/130000 bases in nuclear DNA and 1/8000 bases in mitochondrial DNA. The extremely high level in mitochondrial DNA may be caused by the immense oxygen metabolism, relatively inefficient DNA repair and the absence of histones in mitochondria. Thus mitochondria may be accumulating mutations with age which could compromise energy supplies to the cell.

7-Methylguanine in DNA

The 7-methylguanine (m^7G) adduct in the DNA of rat liver has been determined as an indicator of

exposure to exogenous and endogenous methylating agents (Park and Ames, 1988). A new method for the analysis of m^7G adducts has been developed by combining the selectivity of separation of reversed-phase high-performance liquid chromatography with the specificity and high sensitivity of electrochemical detection (Park and Ames, 1988). The sensitivity of the method is about 10000-fold that of optical methods and is sufficient to determine the endogenous background of DNA methylation.

DNA from the liver of normal young rats (6 months old) contains m^7G. Since m^7G is only one of about 5 deleterious methylation adducts, the true steady-state level of all adducts should be higher. The endogenous methylating agent is likely to be *S*-adenosylmethionine, which is present in the nucleus as the methyl donor in the enzymatic synthesis of the normal base 5-methylcytosine, but which can act as a non-specific alkylating agent to some extent (Barrow and Magee, 1982; Rydberg and Lindahl, 1982).

Antioxidant defenses

Many defense mechanisms within the organism have evolved to limit the levels of reactive O_2 species and the damage they induce. Among the defenses are SOD, catalase and GSH peroxidase as well as the antioxidants β-carotene, tocopherols and vitamin C. We have discussed several previously unappreciated antioxidants that have appeared in evolution. *Uric acid* is a powerful antioxidant that appeared in primate evolution concomitant with the development of long life span and large, metabolically active brain (Ames et al., 1981). Uric acid is the main antioxidant in saliva and is 300 μM in human blood. It is present in much lower amounts in animals before the primates. Uric acid levels increased in primate evolution at about the same time as we lost the ability to synthesize ascorbic acid, so that these events may be related.

Heme is degraded to *biliverdin*, and in mammals biliverdin is converted to *bilirubin*, both of which are shown to be powerful anti-oxidants (Stocker et al., 1987a, b; Stocker and Ames, 1987). The bilirubin in human blood is bound at a specific site on albumin at a concentration of 20 μM (Stocker et al., 1987a, b; Stocker and Ames, 1987). This is a much higher level than in rat blood. Conjugated bilirubin also appears to be the most important antioxidant in bile, and with the copper ions present in bile forms a powerful redox system for oxidizing xenobiotics and destroying hydroperoxides (Stocker and Ames, 1987).

Carnosine, which is present in high concentrations in human muscle and brain, has been shown to have antioxidant properties and to chelate copper and iron ions so as to inhibit oxidative reactions. We have postulated that it is a physiologically significant antioxidant (Kohen et al., 1988).

Because of the finite time between generation of oxidants and their destruction by a defense mechanism, low levels can exist for sufficient time to produce damage to cellular macromolecules (Chance et al., 1979). For nuclear DNA, however, the mammalian cell has three more levels of defense. First, nuclear DNA is compartmentalized away from mitochondria and peroxisomes where most oxidants are probably generated. Second, most nonreplicating nuclear DNA is surrounded by histones and polyamines which may protect against oxidants. Finally, most of the types of DNA damage produced can be repaired by efficient enzyme systems. The net result of this multilevel defense is that nuclear DNA is very well, but not completely, protected from oxidants.

Mechanisms relating endogenous DNA damage, cancer and aging

Several models relate endogenous DNA damage, cancer and aging. One possibility is that mutagens react with nuclear DNA to produce somatic mutations, both point mutations and clastogenic events such as deletions. The sources of mutagenic oxidants could include: long-lived oxidants generated outside the nucleus in the mitochondria and cytoplasm and capable of crossing the nuclear membrane, lipid-soluble oxidants generated in the nuclear membrane itself, and oxidants generated within the nucleus. The spontaneous methylation of DNA would cause both point mutations and apurinic sites which lead to breaks and clastogenic events. Somatic mutation could disrupt the cell by altering gene products or by altering their regulation.

Another model for mutagenic damage is that

the high rate of oxidative damage to mitochondria causes an accumulation of mutations with age in mitochondrial DNA that results in energy deficiencies in old cells.

In addition to mutation, DNA damage can prevent DNA replication and lead to cell death. This can cause neighboring cells to proliferate, a promotional stimulus for carcinogenesis in cells that do not normally undergo DNA replication.

Oxidation or methylation could also cause loss of 5-methylcytosine, an epigenetic change. 5-Methylcytosine is important in turning off genes in differentiation so that its loss by DNA damage could cause de-differentiation and contribute to cancer and aging (Wilson et al., 1987; Holliday, 1987). By analogy with 5-hydroxymethyluracil, 5-hydroxymethylcytosine could be formed by oxidative damage (Cannon et al., 1988). This could lead to lack of methylation after DNA replication as the DNA maintenance methylase is not likely to recognize 5-hydroxymethylcytosine as 5-methylcytosine. Similarly, 8-hydroxyguanine and 7-methylguanine are likely to interfere with maintenance methylation at neighbouring or base-paired cytosines and cause loss of 5-methylcytosine.

Acknowledgments

This work was supported by National Cancer Institute Outstanding Investigator Grant CA39910 and by National Institute of Environmental Health Sciences Center Grant ES01896. This paper was adapted in part from (Ames, 1988a, b).

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