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ENDOGENOUS OXIDATIVE DNA DAMAGE, AGING, AND CANCER

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Progress in identifying the important endogenous processes damaging DNA and developing methods to assay this damage in individuals is presented. This approach may aid studies on modulation of cancer and aging.

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

KEY WORDS: DNA, free radicals, mitochondria, 8-hydroxydeoxyguanosine.

ENDOGENOUS SOURCES OF DNA DAMAGE, AGING AND CANCER

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 (Figure 1), both in short-lived species such as rats and mice (about 30% have cancer by the end of their 2- to 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). Thus, cancer is fundamentally a degenerative disease of old age, though it can be increased (e.g., cigarette smoking in humans) or decreased (e.g., calorie restriction in rodents) by exogenous factors.

One important factor in longevity appears to be basal metabolic rate,[2] 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 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.

Four important endogenous processes leading to significant DNA damage are likely to be oxidation,[2-4] methylation, deamination, and depurination.[5] 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.[6] The measurement of DNA adducts by new methods shows that oxidation is a major type of DNA damage (see below).

OXIDATIVE DNA DAMAGE PRODUCTS IN URINE

Oxidants are produced by by-products of normal metabolism and of lipid peroxida-

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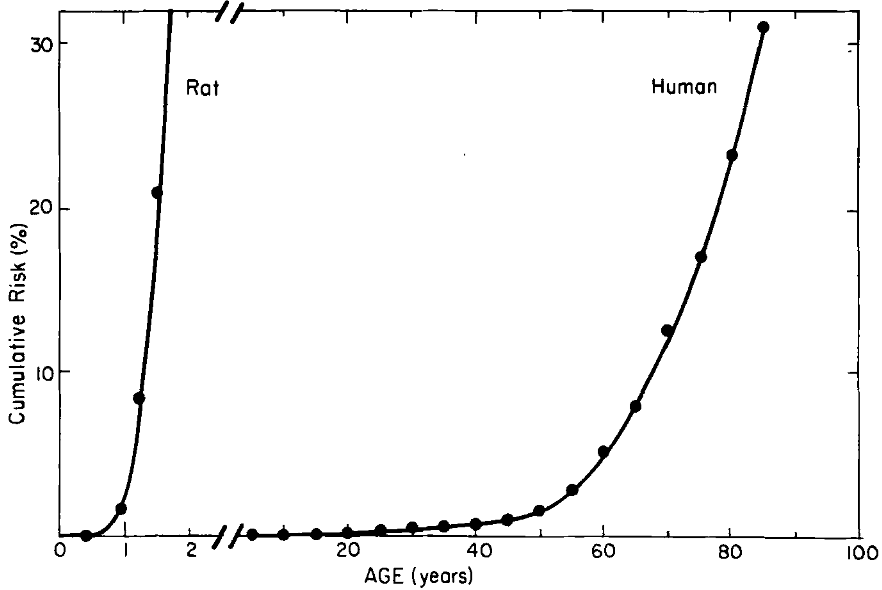

FIGURE 1 Cumulative net risk of death from cancer for rats and humans.[1]

tion (Figures 2 and 3). 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 Figure 4. 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

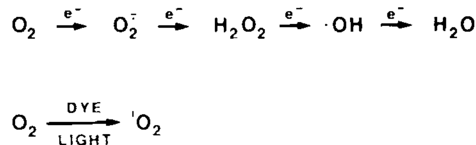

FIGURE 2 The formulation of superoxide, hydrogen peroxide, and hydroxyl radicals by successive additions of electrons to oxygen.[1] Cytochrome oxidase adds 4 electrons fairly efficiently during energy generation in mitochondria, but some of these toxic intermediates leak out. Singlet oxygen is generated from oxygen by the absorption of energy from a dye activated by light.

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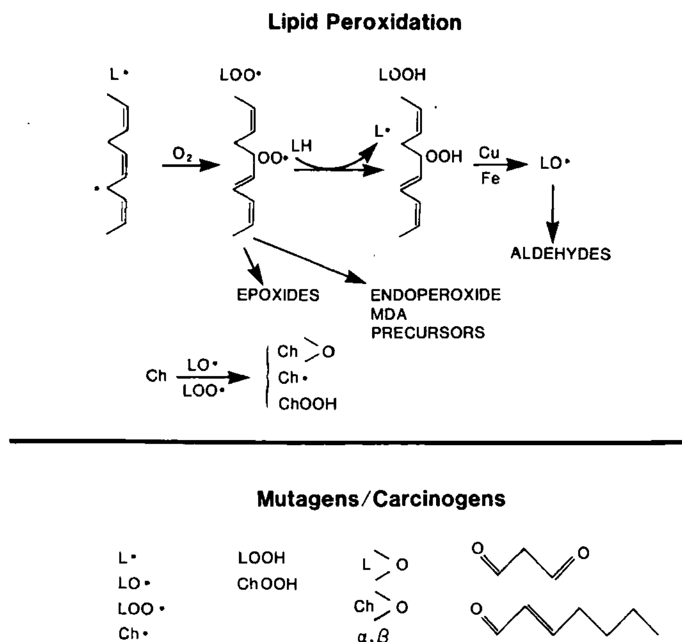

FIGURE 3 Lipid peroxidation.[7] L• = lipid radical; LO• = alkoxy lipid radical; LOO• = hydroperoxy lipid radical; LOOH = lipid hydroperoxide; Ch = cholesterol; Ch>O = cholesterol epoxide; L>O = lipid epoxide; MDA = malondialdehyde.

glycosylase from mouse cells, which repairs 5-hydroxymethyluracil and differs from the specific DNA glycosylase repair enzyme for thymine glycol in mouse cells.[8] 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 the known radiation damage products thymine glycol, thymidine glycol, hydroxymethyluracil, and hydroxymethyldeoxyuridine in urine.[9-11] Our results indicate that normal humans excrete a total of about 100 nmol/day of the first three 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.[9,10,12] 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 three of about 20 products of oxidative damage of DNA,[13,14] the total number of all types of oxidative hits of DNA per cell per day in man may be more than 10^4.

We have now tested urines for thymine glycol and thymidine glycol from normal human volunteers aged 22 to 84 years.[10] The preliminary evidence is that their urinary

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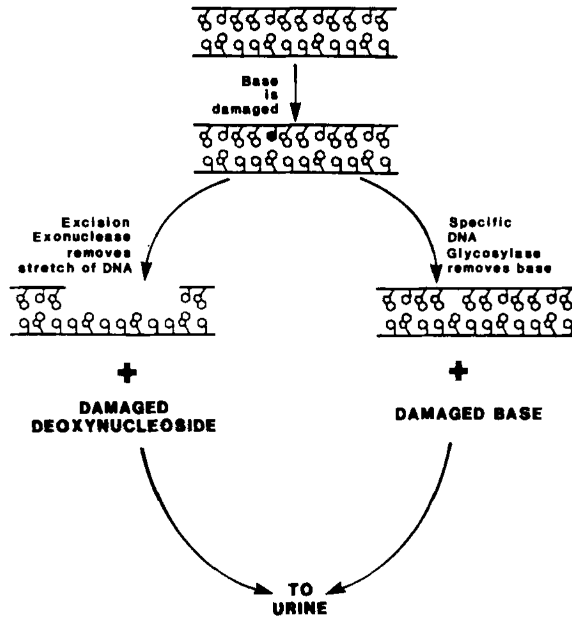

FIGURE 4 Repair of a damaged DNA base by excision repair or a specific glycosylase.[1]

outputs 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.[15]

METABOLIC RATE AND OXIDANT-INDUCED DNA DAMAGE

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 (Figure 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 four 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 (Figure 5).[9,16] 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.[15]

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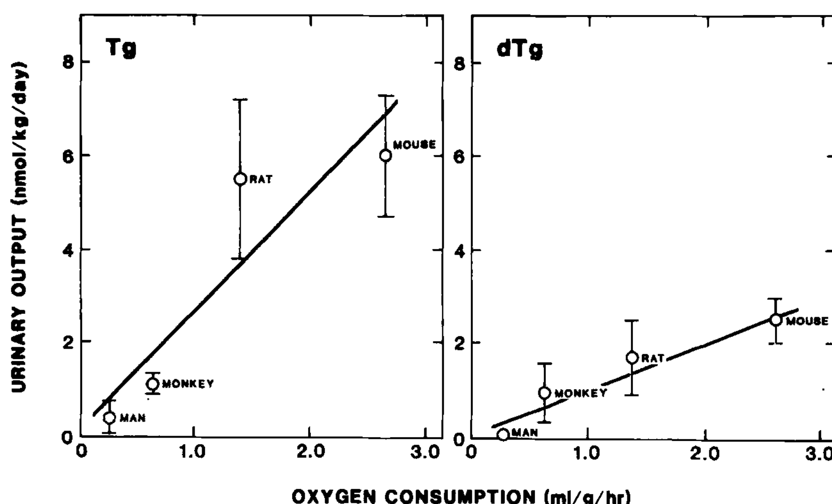

FIGURE 5 Average urinary output of thymine glycol (Tg) and thymidine glycol (dTg) for four different species, expressed as a function of the specific metabolic rate.[16]

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URINARY 8-HYDROXYDEOXYGUANOSINE

Our urinary thymine glycol assay is difficult and takes about 3 weeks. We have thus developed a simpler urinary assay for 8-hydroxydeoxyguanosine (oh^{8}dG), one of about 20 known radiation damage products, which has been measured in DNA by electrochemical detection at about 1000 times the sensitivity of UV detection.[17-19] This new assay[20,21] 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 evidence for the presence of 8-hydroxyguanosine, a measure of oxidative damage to RNA.[21] We have used this assay to measure oh^{8}dG in urine of humans with chronic granulomatous disease.[20,21] These people lack the respiratory burst from phagocytic cells, yet they still produce oh^{8}dG in their urine. This is evidence that the presence of oxidized DNA bases in urine can not be accounted for by normal cell turnover.

8-HYDROXYDEOXYGUANOSINE 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 in both mitochondrial and nuclear DNA of normal rat liver.[22] It is present at a level of 1/130,000 bases in nuclear DNA and 1/8,000 bases in mitochondrial DNA. Our preliminary evidence suggests that the level of oh^{8}dG does not

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increase much, if at all, with age. 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. Mitochondria are likely to be a major source of the oxidants damaging nuclear DNA.

DEFENSES AGAINST OXIDANTS

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 a long life span and large, metabolically active brain.[23] 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 antioxidants.[24-26] The bilirubin in human blood is bound at a specific site on albumin at a concentration of 20 μM.[24-26] 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.[24]

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.[27]

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.[28] 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.

ENDOGENOUS DNA DAMAGE COULD CAUSE AGING BY VARIOUS MECHANISMS

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 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.

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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. This would, in turn, lead to deficiencies in repair enzymes, or to cell death.

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. In addition, oxidative cell damage activates the proto-oncogenes *c-fos* and *c-myc* thus acting as a promotional carcinogen.[29]

Oxidation 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.[30,31] By analogy with 5-hydroxymethyluracil, 5-hydroxymethylcytosine could be formed by oxidative damage.[32] 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 is likely to interfere with maintenance methylation at neighboring or base-paired cytosines and cause loss of 5-methylcytosine.

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