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Genetics

Oxidative damage to DNA: Relation to species metabolic rate and life span

(aging/thymidine glycol/evolution)

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ABSTRACT **Oxidative damage to DNA is caused by reactive by-products of normal metabolism, as well as by radiation. Oxidized DNA bases excised by DNA repair enzymes and excreted in urine were measured in four different species to determine the relation between specific metabolic rate (ml of O_2 consumed per gram of body weight per hr) and oxidative DNA damage. An average of 6.04 nmol of thymine glycol per kg/day and 2.58 nmol of thymidine glycol per kg/day were found in mouse urine and 1.12 nmol of thymine glycol per kg/day and 0.95 nmol of thymidine glycol per kg/day were found in monkey urine. On a body weight basis, mice excrete 18 times more thymine glycol plus thymidine glycol than do humans, and monkeys excrete 4 times more thymine glycol plus thymidine glycol than do humans. When results among mice, rats, monkeys, and humans are compared, specific metabolic rate correlates highly with oxidative DNA damage. These findings are consistent with the theory that free radical-induced DNA damage may play a central role in the aging process.**

Free radicals that are formed in the body as a consequence of aerobic metabolism can produce oxidative damage to macromolecules in somatic cells (1–5). This type of damage may be an important factor in aging and age-dependent diseases such as cancer and heart disease (6). Circumstantial evidence implicating free radicals in aging includes the impressive inverse correlation between the specific metabolic rate (ml of O_2 consumed per gram of body weight per hr of a given species and the life span of that species (for review, see ref. 7). One explanation for this inverse correlation is that smaller animals, with higher metabolic rates, consume greater quantities of oxygen (on a body-weight basis) and so produce harmful free-radical by-products at a higher rate, leading to a higher rate of damage to critical cellular targets. The higher rate of damage to cells might then cause a higher rate of cellular aging.

Many uncertainties remain about the role of free radicals in aging (20). One major obstacle to analytical tests of the theory is that the critical targets that might be damaged are not known; the critical targets involved in cellular aging could include informational macromolecules such as DNA, RNA, and enzymes, as well as structural components, such as certain proteins and lipids. DNA seems particularly plausible as a critical target in aging because of the central role of DNA in information transfer between generations of somatic cells. Our strategy was to measure oxidative damage to cellular DNA in whole live mammals to detect possible damage from intracellular free radicals. The specific approach was to analyze mammalian urine for altered deoxyribosides and bases that could have been removed from cellular DNA by enzymatic repair mechanisms. Most of our efforts focused on one DNA oxidation product, the thymine glycol lesion (5,6-dihydro-5,6-dihydroxythymine) because this lesion has suitable properties of repair, product excretion, stability (for recovery and measurement of its repair products), and traceability of the origin of the repair products to cellular DNA. Background levels of thymine glycol and thymidine glycol in the human were determined, and a total oxidative damage rate to DNA was estimated as ≈300 hits per cell per day in human for just these two oxidized bases (8, 9). Hydroxymethyluracil, another known oxidative DNA damage product, was also assayed and accounted for another 700 hits per cell per day (8, 9). Because many known oxidative DNA damage products have been described, we suspect that the total damage rate is thousands of hits per cell per day. The rat has a damage rate about 15 times that of the human (8, 9). In this paper, we report the background levels of thymine glycol and thymidine glycol in mouse and monkey and compare the levels in all four species to specific metabolic rate and life span.

MATERIALS AND METHODS

Collection of Mouse and Monkey Urine. Four male C3H mice (26–31 grams; 28 days old) were obtained from Charles River Breeding Laboratories. Because a single mouse does not produce enough urine per day for use in our assay, all four mice were housed together in one metabolic cage (Nalgene). The mice were given laboratory chow (5015C, Ralston Purina, Richmond, IN) and water ad libitum. The 24-hr urine outputs of the four mice were collected for each of 6 consecutive days. Immediately after collection, each sample was diluted to 25 ml with water and frozen.

Twenty-four-hour urine samples from three male monkeys (*Macaca fascicularis*; 2.4–2.6 kg; 2.5–3.0 yr old) were obtained from Charles River Research Primates (Port Washington, NY). These monkeys had been individually housed in appropriate metabolic cages and fed a standard monkey chow and water ad libitum. Samples were kept frozen during shipment to our laboratory.

Analysis of Thymine Glycol and Thymidine Glycol in Urines. Urines from mice and monkeys were analyzed for thymine glycol and thymidine glycol as previously described (8). In brief, ^{3}H-labeled standards of thymine glycol and thymidine glycol were added to the urines, and the samples were purified first by phenylboronate affinity chromatography to select compounds that contained *cis*-glycol function; these samples were then purified by semipreparative reversed-phase HPLC. Samples were next derivatized to thymine by reduction with hydroiodic acid, and the thymine was purified and measured by reversed-phase HPLC with UV detection at 265 nm. Final recovery of radiolabeled thymine was used to calculate the original thymine glycol or

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Table 1. Summary of thymine glycol and thymidine glycol levels in urine

	Thymine glycol, nmol per kg/day	Thymidine glycol, nmol per kg/day
Mouse		
Day 1	6.00	2.75
Day 2	5.12	2.30
Day 3	7.00	2.68
Average	6.04	2.58
Monkey		
Day 1	1.17	1.64
Day 2	0.87	0.76
Day 3	1.31	0.44
Average	1.12	0.95

Pooled urine of four mice was collected daily and analyzed for thymine glycol and thymidine glycol on 3 different days; 24-hr urines for three monkeys were each analyzed for thymine glycol and thymidine glycol.

thymidine glycol level in the urine corresponding to the final thymine measurement for each sample. For mouse urines, 10 ml of the diluted urines was combined with 10 ml of 2.0 M ammonium acetate buffer, pH 8.8, and applied to the phenylboronate column as previously described for rat urine (8). For monkey urines, 20 ml of undiluted urine was used as previously described for human urine (8). A minor procedural improvement was to eliminate the water-wash step used just before glycol elution from the phenylboronate column because this wash causes the column pH to reach >10, which promotes glycol decomposition. Instead, the column was eluted six times with 5-ml portions of 0.1 M acetic acid; the eluate was collected in six tubes, and the contents of the tubes containing most radioactivity (usually tubes 4 and 5) were combined, lyophilized, and analyzed as described previously (8).

RESULTS

Thymine Glycol and Thymidine Glycol Levels in Mouse Urine. The urines of four C3H mice collected on days 3, 4, and 6 were analyzed for thymine glycol and thymidine glycol (Table 1). Average daily output for mice, expressed on a body weight basis, for thymine glycol was 6.04 nmol per kg/day and for thymidine glycol was 2.58 nmol per kg/day.

Thymine Glycol and Thymidine Glycol Levels in Monkey Urine. A 24-hr urine sample from each of three monkeys (*M. fascicularis*) was analyzed for thymine glycol and thymidine glycol (Table 1). The average level of thymine glycol was 1.12 nmol per kg/day, and the average level of thymidine glycol was 0.95 nmol per kg/day.

Comparison of Thymine Glycol and Thymidine Glycol Levels Among Four Species. The average urinary outputs of thymine glycol and thymidine glycol by mice, rats, monkeys, and humans are shown in Fig. 1 as a function of the specific metabolic rate of each species. Urinary glycol levels for mice and monkeys are from this work, and those levels for rats and humans are from the report of Cathcart *et al.* (8). Specific metabolic rates used for this comparison were 0.25, 0.65, 1.40, and 2.65 ml of O_2 per g/hr for man, monkey, rat, and mouse, respectively. These values were estimated from values in the literature (10–13).

DISCUSSION

The daily urinary output of the DNA oxidation products thymine glycol and thymidine glycol is an indication of the rate at which the thymine glycol adduct is formed in cellular DNA in whole live mammals. Rate of formation of this particular oxidation product is likely to be a general indication of the extent to which reactive oxygen species produce genetic damage in mammals.

Our experimental approach has several advantages over the more direct approach of detecting DNA adducts that remain within the DNA. Analysis of urine is a much more

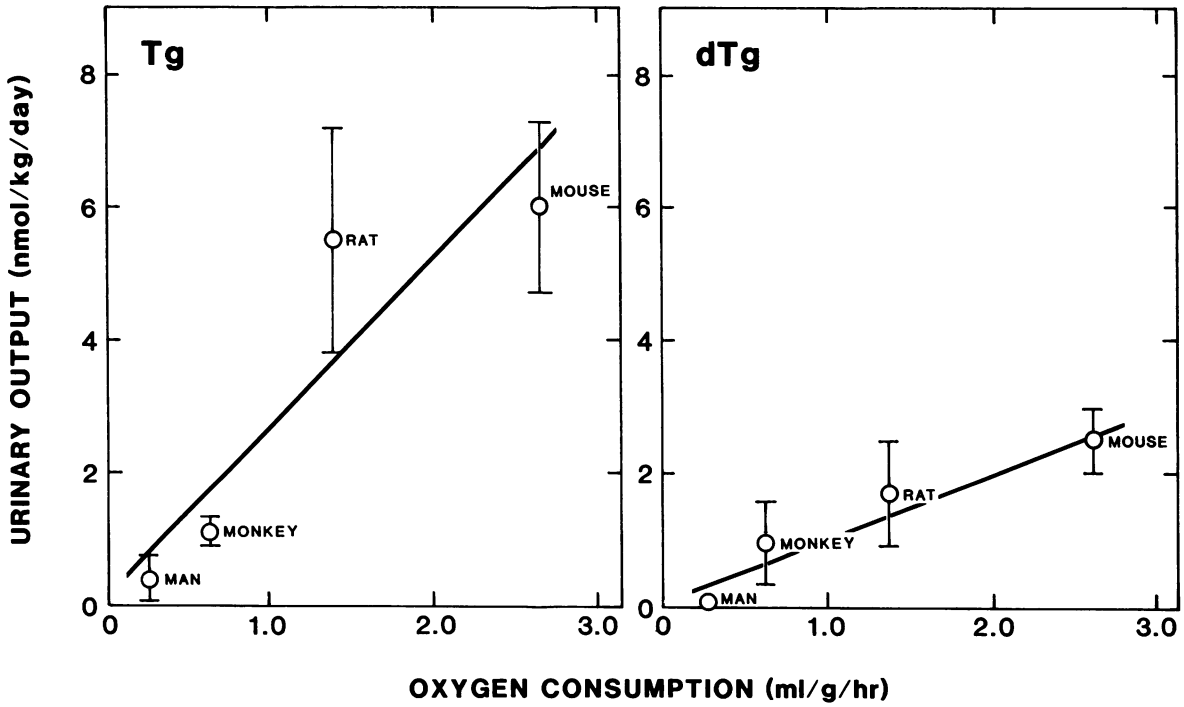

FIG. 1. Levels of thymine glycol (Tg) and thymidine glycol (dTg) in urines of four species expressed as a function of specific metabolic rate for that species (in O_2 per g/hr). Urine levels are mean values ± SEM for 10 humans (8), for nine rats (8), for four mice on 3 days, and for three monkeys.

sensitive test because cumulative output of the metabolic product over 24 hr for a 70-kg human can be measured, for example, whereas the direct approach would only allow analysis of the instantaneous, steady-state level of the product in a few grams of tissue sample. Because repair of thymine glycol is quite rapid, the steady-state background level of thymine glycol in untreated human cells might be only a few thymine glycol molecules per cell, and this amount would be too low to be seen with even the most sensitive assays currently available. Another advantage of our approach is that it is noninvasive. The major disadvantage of our approach is that it is an indirect measurement, and therefore several assumptions must be made to relate the urine measurements to actual *in vivo* events. The main assumptions are (*i*) that the thymine glycol lesion is formed in the cell, (*ii*) that the lesion is excised from DNA by repair processes, (*iii*) that the released glycols are excreted via the urine without further metabolism, and (*iv*) that no other processes contribute significantly to the observed urinary levels of these glycols. Evidence supporting the above assumptions has been presented and discussed elsewhere (8, 9, 14, 15), and we believe our assumptions to be valid.

The data in Fig. 1 show a correlation between specific metabolic rate of a species and weight-adjusted urinary output of thymine glycol and thymidine glycol for that species. The urinary output of these substances in humans and rats does not appear to increase with age (ref. 10; R.L.S. and B.N.A., unpublished data). If these glycol levels do indicate rate of oxidative damage to DNA, then these data suggest that oxidative damage to DNA increases with specific metabolic rate—consistent with the theory that DNA is a critical target in the aging process and that larger mammals owe their longer life spans to their lower specific metabolic rates and correspondingly low rates of oxidative DNA damage formation.

Note that our assay attempts to measure rate of turnover of the thymine glycol lesion and not instantaneous levels of the lesion in DNA. Because repair of this lesion is believed very efficient, in a steady state, the rate of turnover equals the rate of formation of the lesion; consequently our assay should estimate the rate of formation of oxidative DNA damage. However, biological consequences of such molecular damage will depend on two other factors, persistence of such lesions and mitotic state of the cell. Human cells generally repair DNA more efficiently than do rodent cells (14) so that the instantaneous levels of thymine glycol lesions should differ even more dramatically between human and rodents. Also, most oxidative lesions will probably be more damaging to cells that are undergoing DNA replication, so that rodents, which have higher cellular turnover, will be more affected by such molecular damage. Thus, in relation to rodents, the human appears to form oxidative lesions at a lower rate and have greater protection from such lesions through faster repair and lower cell turnover. This combination of factors may, in part, explain the 40-fold difference between the life spans of humans and rodents.

The complexity of our analytical assay for thymine glycol and thymidine glycol makes more extensive studies difficult. A much simpler assay for 8-hydroxydeoxyguanosine, another known radiation damage product that has also been found in human urine (15), should enable a more detailed analysis both within and between species.

Several models relate oxidative damage to DNA to cancer and aging. One possible mechanism is that oxidative damage to nuclear DNA produces somatic mutations. The sources of oxidants in this case could include the following: long-lived reactive species generated outside the nucleus and capable of crossing the nuclear membrane, lipid-soluble radicals and peroxides generated in the nuclear membrane itself, and oxidants generated within the nucleus. The types of DNA lesion that might lead to somatic mutation include (*i*) base changes to give point mutations, frameshift mutations, and deletions and (*ii*) strand breaks to give chromosome rearrangements. Somatic mutation could disrupt the cell by altering gene products or by altering the regulation of genes.

Other types of DNA damage could impair the cell without producing a mutagenic event. Many types of lesions, even in noncoding sequences, can prevent DNA replication and thus prevent cell proliferation. Certain unrepaired lesions in coding sequences might impair transcription and decrease protein synthesis. This type of DNA damage would be particularly important for terminally differentiated cells that do not normally undergo DNA replication. Oxidative damage to DNA is likely to cause loss of 5-methylcytosine residues by various mechanisms. Because methylation of cytosine may be important in turning off genes during differentiation, oxidative DNA damage could prevent this event to cause de-differentiation and thereby contribute to cancer and aging (16–19, 21).

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