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Urinary 8-Hydroxyguanine May Be a Better Marker of Oxidative Stress Than 8-Hydroxydeoxyguanosine in Relation to the Life Spans of Various Species

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ABSTRACT

Oxidative DNA damage is believed to be involved in the aging process. Species with shorter potential life spans generally have a higher specific metabolic rate (SMR), and would be expected to have increased levels of oxidative stress and DNA damage, as compared to long-lived species. An automatized HPLC method based on electrochemical detection was used to measure the levels of the oxidative DNA damage markers 8-hydroxydeoxyguanosine (8-OH-dG) and 8-hydroxyguanine (8-OH-Gua) in urinary samples from mammals with various potential life spans (mice, rats, guinea pigs, cats, chimpanzees, and humans). There was no significant linear correlation ($r = -0.71$, $p = 0.11$) between the species' potential life spans (log transformed) and the urinary levels of 8-OH-dG as normalized to creatinine (8-OH-dG/creatinine), although the species with longer life spans, such as chimpanzee and human, had among the lowest levels detected. In contrast, the negative linear correlation between the species' potential life span (log transformed) and the urinary levels of 8-OH-Gua as normalized to creatinine (8-OH-Gua/creatinine), was significant ($r = -0.97$, $p = 0.002$). In addition, there was a positive linear and significant correlation between SMR and 8-OH-dG/creatinine ($r = 0.91$, $p = 0.01$) or 8-OH-Gua/creatinine ($r = 0.90$, $p = 0.01$). These results suggest that 8-OH-Gua, rather than 8-OH-dG, may be a more general marker for oxidative damage. *Antioxid. Redox Signal.* 8, 985–992.

INTRODUCTION

THE PRODUCTION OF OXYGEN radicals in living organisms has been proposed to be linked with the deleterious changes associated with the aging process (8). In general, species with shorter potential life spans have higher metabolic rates, and would be expected to produce more oxygen radicals, leading to increased damage of cellular components. Superoxide dismutase (SOD) is an antioxidant enzyme that protects cells from the harmful effects of superoxide radicals. In various organs from different mammals, there is a positive correlation between the maximum life span potential and the ratio of SOD specific activity to specific metabolic rate (23).

An important marker for DNA damage is 8-hydroxydeoxyguanosine (8-OH-dG), which is formed by the attack of oxygen radicals (13), repaired by the process of base and nucleotide excision, and excreted through the urine in the form of the base 8-OH-guanine (8-OH-Gua) and the nucleoside 8-OH-dG (4). In mammalian nuclear DNA, there was no correlation found between the 8-OH-dG levels and the maximum life span potential, although in mitochondrial DNA there was a significant inverse correlation for the same comparison (2). In this context, it is meaningful to assess the amounts of oxidative damage produced in various species.

Previously, researchers using a method involving high performance liquid chromatography (HPLC) and GC/MS re-

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ported a significant negative correlation between potential life spans and urinary levels of 8-OH-Gua for six mammalian species, including human (6). In the same report, significant positive correlations were found between the different species' specific metabolic rates (SMR) and the urinary levels of 8-OH-Gua, 8-OH-dG, and 5-(hydroxymethyl)uracil (6).

Our goal was to use a newly developed technology, based on HPLC with electrochemical detection (EC), to analyze the oxidative damage markers 8-OH-dG and 8-OH-Gua in urine samples from various mammals with different potential life spans. The urine samples were obtained from mouse, rat, guinea pig, cat, chimpanzee, and human. Chimpanzee urine is especially important to include, since this species has a relatively long potential life span (around 50 years). The advantage of our HPLC method is that it does not require pre-purification or derivatization of urine samples, and by adding a ribonucleoside marker to each urine sample, the fraction containing 8-OH-dG or 8-OH-Gua may be precisely collected following anion exchange chromatography, by means of automatic peak detection. The collected fraction is subsequently fractionated by reversed phase chromatography for 8-OH-dG or 8-OH-Gua detection with the EC detector, as shown previously for 8-OH-dG in human, mice, and rat urine (14, 22). Thus, by using these recently developed HPLC-EC methods, we wanted to add data from new species and to compare our results with the previously published data (6) regarding the urinary excretion of 8-OH-dG or 8-OH-Gua, and to determine their possible correlation with the species' potential life span and SMR.

MATERIALS AND METHODS

Materials

The 8-OH-dG and 8-OH-Gua (2-amino-6,8-dihydroxy-purine) used for standards were obtained from Sigma Chemical Co. (St. Louis, MO) and Aldrich (Milwaukee, WI), respectively. For the first HPLC separation step, the anion exchange resin MCI GEL CA08F (7 μ m, Cl⁻ form), from Mitsubishi Chemical Corp., Tokyo, Japan, was converted to the sulfate form, as previously described (14), and was manually packed in a guard column (1.5 \times 50 mm) and a main column (1.5 \times 150 mm). For the analysis of 8-OH-dG in the second HPLC separation, a reversed phase column (CAPCELL PAK C18, 5 μ m, 4.6 \times 250 mm) from Shiseido Fine Chemicals, Tokyo, Japan, was used. 8-Hydroxyguanosine (8-OH-G), used as a marker for fraction collection, was prepared as previously described (12, 14). HPLC grade methanol and acetonitrile were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Kanto Chemical Co., Inc., Tokyo, Japan, respectively.

Collection of urine samples

For the analysis of 8-OH-dG, urine was collected from female C3H/He mice and male Wistar rats (24 h, metabolic cages), male Hartley guinea pigs (2 h, Japan SLC Corp., Shizuoka, Japan), female cats (spot samples), and male chimpanzees (spot samples, Kumamoto Primates Research Park, Kumamoto, Japan). Rats and mice were furnished with a standard diet (CE-2) and drinking water *ad libitum*. The other

animals were not given any food during collection, although drinking water was *ad libitum*. Human urine was obtained as spot samples during the winter season from 19 healthy male nonsmokers living in Finland, with ages between 25 and 60 years. The subjects' data, method of urine collection, and results, as determined in this laboratory, were presented previously (9). At the end of the collection period, the urine was frozen (-20°C or -80°C).

For the analysis of 8-OH-Gua, urine samples were collected from female C3H/He mice (spot samples) and male Wistar rats (24 h, metabolic cages) and for the other animals as described above. Human urine was collected as spot samples from five males and two females, all healthy and non-smokers, 29–58 years of age. At the end of the collection period, the samples were frozen (-80°C).

Measurement of urinary creatinine levels

The creatinine in the urine samples that were collected in Japan was measured by a commercial laboratory (BML Corp., Kitakyushu, Japan), using a colorimetric method (22). The creatinine concentrations of the human urine samples that were collected in Finland were also determined with a standard automated colorimetric method (1, 9).

Due to the low volume of mouse urine collected as spot samples, the creatinine concentration was calculated indirectly from the amount of 7-methylguanine, as detected at 305 nm during the HPLC analysis. The correlation between the concentrations [g/L] of 7-methylguanine and creatinine, in the C3H/He mouse urine, is described by the linear relationship $[\text{m}^7\text{Gua}] = 0.0284 \times [\text{creatinine}] + 0.0065$ (22).

Analyses of urinary 8-OH-dG

Mouse, rat, and human urine samples were analyzed as described in detail previously (9, 14, 22). Urine from guinea pigs, cats, and chimpanzees was analyzed by the same method as used for rat urine previously (22). However, in these experiments, the HPLC equipment used for analysis was mainly composed of Gilson components, kindly provided by M & S Instruments Trading Inc., Fukuoka, Japan. In the first anion exchange step, the ribonucleoside marker (8-hydroxyguanosine), added to each sample before analysis, was detected by UV, which allowed the subsequently eluting 8-OH-dG containing fraction to be collected in a sample loop. The 8-OH-dG fraction was then separated on a reversed-phase column kept at 48°C , with a flow rate of 0.67 ml/min, connected to an EC detector [ESA Coulochem II (ESA, Chelmsford, MA), applied potentials: guard cell = 400 mV, E1 = 240 mV, and E2 = 350 mV].

Before the analysis, the urine samples (70 μ l) were manually mixed with an equal volume of a 4% acetonitrile, sodium acetate buffer containing the ribonucleoside marker (120 μ g/ml), and were stored at $+5^{\circ}\text{C}$ overnight. After centrifugation at 13,000 rpm for 5 min, a 20 μ l aliquot of the supernatant was injected. The total time between the analyses of consecutive samples was 80 min.

Analyses of urinary 8-OH-Gua

For the analysis of urinary 8-OH-Gua in samples from animals and humans, the same Gilson equipment as described

above for the analysis of 8-OH-dG was used. The 8-OH-Gua analysis method is also based on the automatic peak recognition of the added ribonucleoside marker and the collection of the fraction containing 8-OH-Gua. With the above described columns and settings for the anion exchange in the first step, 8-OH-Gua elutes at around 31 min, a few minutes earlier than the ribonucleoside marker (around 35 min) and 8-OH-dG (around 40 min). Since the 8-OH-Gua fraction elutes before the ribonucleoside marker peak, which was collected in the loop at the switch valve, it had to be delayed by adding an additional length of PEEK tubing between the UV-detector and the loop connected to the switch valve. To obtain good separation conditions on the reversed-phase column in the second step, the column temperature was changed to +38°C, with a flow rate of 0.33 ml/min. Also, the ESA Coulochem II settings were adjusted to the following applied potentials: guard cell = 400 mV, E1 = 190 mV, and E2 = 300 mV.

The ribonucleoside (8-OH-G) used for automatic peak detection was purified by HPLC (column, CAPCELL PAK C18, 5 µm, 10 × 250 mm, Shiseido Fine Chemicals; elution, 5% acetonitrile in water) and fraction collection to obtain a pure marker solution. Before analysis, due to the high content of 8-OH-Gua, the urine samples from guinea pig, chimpanzee, and human were diluted fivefold, and those from mouse, rat, and cat were diluted 50-fold. The diluted urine samples were then centrifuged at 13,000 rpm for 5 min, and a 70 µl aliquot of the supernatant was manually mixed with an equal volume of the 4% acetonitrile, sodium acetate buffer containing the purified ribonucleoside marker (120 µg/ml). From the mixed sample, a 20 µl aliquot was then directly injected with the sampling injector (around 1 min after mixing). All of the samples analyzed were retested to check for any artifactual increase in 8-OH-Gua with time. It was noted that after 12 h at room temperature, there was an increase (between 1% to 41%) in the background content of 8-OH-Gua in the urine-marker mix for all samples (data not presented). However, this long-term increase was not considered relevant for our results, since all of our samples were injected within 2 min after the marker solution was added. The total time between analyses of consecutive samples was increased to 90 min.

Verification of urinary 8-OH-dG and 8-OH-Gua by spiking with standards

The specificity of the detected 8-OH-dG and 8-OH-Gua peaks was verified by adding a known concentration of an 8-OH-dG standard (4 ng/ml) to a pooled urine sample composed of three samples from the same species, and by adding a known concentration of an 8-OH-Gua standard (4 ng/ml) to individual urine samples from each species. For the spiked samples, the recovery of 8-OH-dG and 8-OH-Gua in HPLC-2 was calculated.

Calculation of results and statistics

The chromatograms were recorded using a Gilson 506C interface, and were integrated with computer software (Gilson Unipoint, Gilson Inc., Middleton, WI). The HPLC chromatograms for 8-OH-dG and 8-OH-Gua were quantified by comparing the peak areas with those obtained from external standards (5 ng/ml) analyzed on a daily basis. The yields

were recalculated to 8-OH-dG/creatinine (µg/g creatinine) and 8-OH-Gua/creatinine (µg/g creatinine). The specific metabolic rate (SMR) of each individual animal and human was calculated using Kleiber's equation: $SMR (\text{calories/gram/day}) = 393 \times (\text{gram body weight})^{-0.25}$ (15). The correlations of the linear regressions presented in Figs. 2–5 were considered as significantly different at $p < 0.05$. The correlation coefficients (r) and p -values were calculated using the Microcal Origin software (OriginLab Corp., Northampton, MA).

RESULTS

HPLC-EC method for the analysis of urinary and 8-OH-Gua in different species

In the EC-chromatograms for the 8-OH-dG standard and the animal and human urine, the 8-OH-dG peak eluted at around 42 min and was clearly separated from the neighboring peaks (chromatograms not shown). Even when the absolute amounts of 8-OH-dG were different between samples, as detected in the main channel E2 (higher potential), the peak area of the smaller peak in channel E1 (lower potential), could be used to monitor the specificity of the analysis. This is due to the fact that the ratio between the peak areas of channels E1 and E2 should roughly correspond to the same ratio as calculated for the pure standard. This was previously explained in detail, and the chromatograms for the 8-OH-dG analyses of mouse, rat, and human urine have been reported (14, 22).

Other conditions for the HPLC-EC analysis of 8-OH-Gua were used, and the resulting chromatograms for the 8-OH-Gua standard and the mouse, chimpanzee, and human urine are shown in Fig. 1A–D. The 8-OH-Gua peak in each chromatogram was clearly separated and eluted at around 23 min, as shown in the upper line (channel E2) of the figures. For the 8-OH-Gua analysis, the peak area visible in the lower lines (channel E1) of the figures may also be used to check the specificity of the analysis, by comparing the peak areas of channels E1 and E2.

To check for the urine sample matrix effects on the 8-OH-dG and 8-OH-Gua detection, pooled and individual urine samples from mouse, rat, guinea pig, cat, chimpanzee, and human, were each spiked with 4 ng/ml of an 8-OH-dG standard or with 4 ng/ml of an 8-OH-Gua standard (Table 1). All spiked samples had 8-OH-dG and 8-OH-Gua recoveries around 100% (range 98%–115%), showing that the detected peaks were not adversely affected by any urine sample matrix effects (Table 1).

Relationship between potential life spans or metabolic rates of different species and urinary 8-OH-dG and 8-OH-Gua excretion

The urinary 8-OH-dG and 8-OH-Gua excretion was compared with the different potential life spans of the mammalian species studied, as presented in Tables 2 and 3. As shown in Table 2, the lowest levels of 8-OH-dG were found in chimpanzee urine, 2.8 ± 1.3 (µg/g creatinine), which were similar to the human levels of 3.9 ± 2.0 (µg/g creatinine). The highest

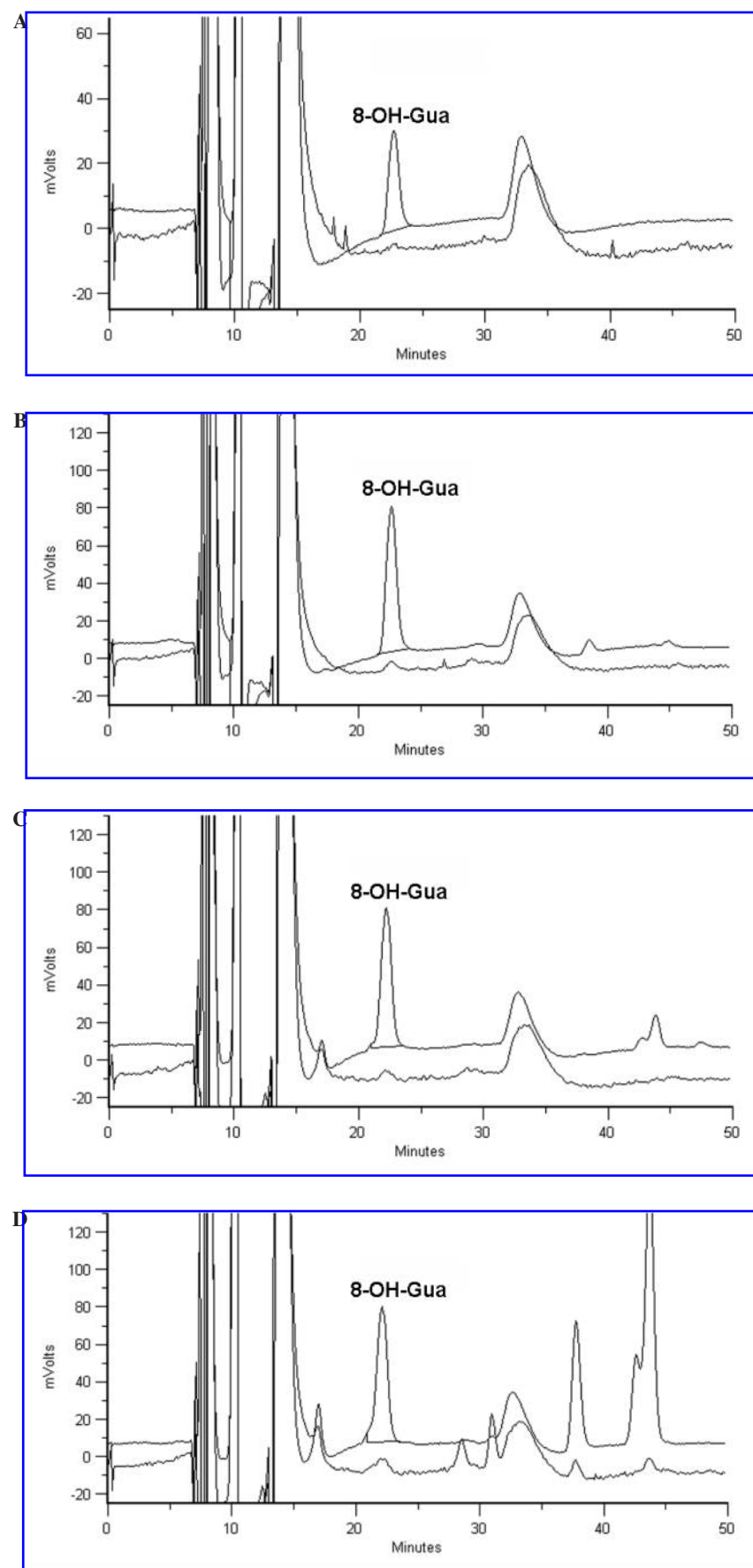


FIG. 1. Chromatograms of HPLC-2 electrochemical detection of 8-OH-Gua at applied potentials of E1 = 190 mV (*lower line*) and E2 = 300 mV (*upper line*): (A) 8-OH-Gua standard (5 ng/ml); (B) mouse (diluted $\times 50$); (C) chimpanzee (diluted $\times 5$); and (D) human (diluted $\times 5$).

TABLE 1. RECOVERY OF 8-OH-dG AND 8-OH-GUA FROM SPIKED ANIMAL AND HUMAN URINE

Species	Control 8-OH-dG ^a (ng/ml)	Spiked + 8-OH-dG (4 ng/ml)	Recovery 8-OH-dG ^a (%)	Control 8-OH-Gua ^b (ng/ml)	Spiked + 8-OH-Gua (4 ng/ml)	Recovery 8-OH-Gua ^b (%)
		8-OH-dG ^a (ng/ml)			8-OH-Gua ^b (ng/ml)	
Mouse	4.9	9.1	106	9.6	13.6	100
Rat	5.7	10.0	106	4.4	8.9	113
Guinea pig	1.9	6.0	103	7.7	12.0	108
Cat	37.5	42.0	112	7.8	12.0	105
Chimpanzee	1.8	6.0	104	6.9	11.5	115
Human	7.4	11.5	102	6.8	10.7	98
Total recovery (Mean value + SD)			106 ± 4			107 ± 7

^aFor 8-OH-dG, each mean value is based on three repeated analyses of pooled urine from three random animals ($n = 3$). Human mean values are based on four repeated analyses of pooled urine from three random subjects ($n = 3$).

^bFor 8-OH-Gua, each value is based on one analysis of urine from a randomly chosen animal/human. Before analysis, the urine was diluted 5-fold for the guinea pig, chimpanzee and human samples, and diluted 50-fold for the mouse, rat, and cat samples.

TABLE 2. DATA AND RESULTS FOR THE MAMMALS USED FOR 8-OH-dG ANALYSES

Species	Potential lifespan (years)	<i>n</i> ^a	Age during experiment (years)	Body weight (kg)	SMR (cal/g/day)	Urinary 8-OH-dG (μg/g creatinine)
Mouse	2	10	0.25 ± 0.00	0.024 ± 0.001	178 ± 2.3	7.7 ± 0.9
Rat	3	21	0.20 ± 0.00	0.372 ± 0.020	90 ± 1.2	4.3 ± 2.9
Guinea pig	10	6	1.0 ± 0.0	1.2 ± 0.1	67 ± 1.7	4.8 ± 0.6
Cat	20	3	1.1 ± 0.6	2.8 ± 1.1	55 ± 5.7	5.1 ± 1.6
Chimpanzee	50	6	22 ± 3.3	51 ± 6.1	26 ± 0.8	2.8 ± 1.3
Human ^b	110	36	43 ± 12	74 ± 15	24 ± 1.2	3.9 ± 2.0

Mean values ± standard deviations are presented.

^aNumber of individual animals/humans (n) used for urine collection.

^bData are based on a previous study at this facility (10).

levels were detected in the urine of the short-lived mouse, 7.7 ± 0.9 (μg/g creatinine). However, there was no significant correlation ($r = -0.71$, $p = 0.11$) between the potential life spans of the different species and the detected levels of uri-

nary 8-OH-dG (Fig. 2). On the other hand, the analysis of urinary 8-OH-Gua clearly revealed a significant correlation ($r = -0.97$, $p = 0.002$) with the potential life spans of the different species (Fig. 3). Thus, the lowest levels of 8-OH-Gua were

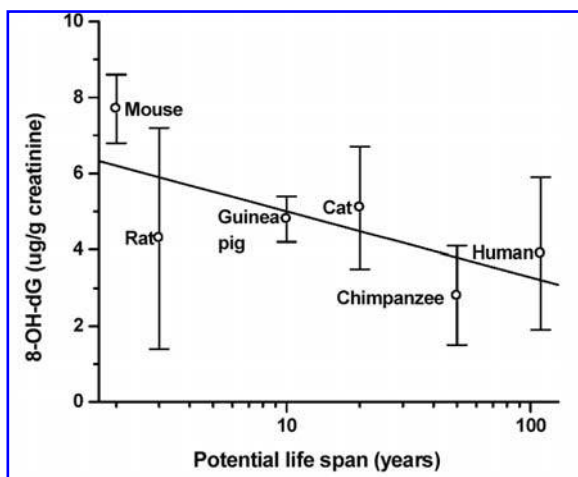


FIG. 2. No significant correlation ($r = -0.71$, $p = 0.11$) between species' potential life span and urinary 8-OH-dG content (normalized to urinary creatinine).

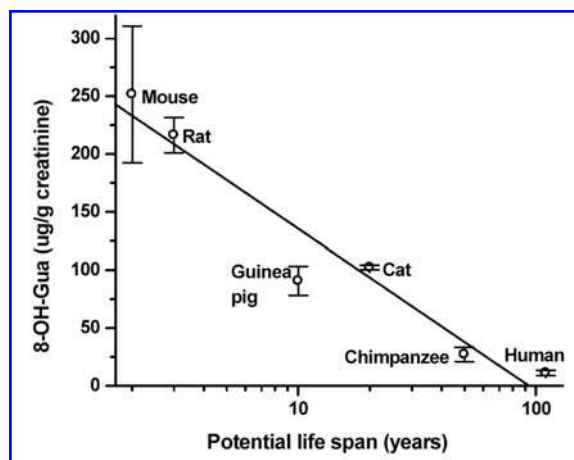


FIG. 3. Significant correlation ($r = -0.97$, $p = 0.002$) between species' potential life span and urinary 8-OH-Gua content (normalized to urinary creatinine).

TABLE 3. DATA AND RESULTS FOR THE MAMMALS USED FOR 8-OH-GUA ANALYSES

Species	Potential life span (years)	<i>n</i> ^a	Age during experiment (years)	Body weight (kg)	SMR (cal/g/day)	Urinary 8-OH-Gua (μg/g creatinine)
Mouse	2	13	0.15 ± 0.07	0.018 ± 0.003	191 ± 7.8	252 ± 59
Rat	3	3	0.20 ± 0.00	0.373 ± 0.014	89 ± 0.9	216 ± 15
Guinea pig	10	6	1.0 ± 0.0	1.2 ± 0.1	67 ± 1.7	91 ± 13
Cat	20	3	1.1 ± 0.6	2.8 ± 1.1	55 ± 5.7	102 ± 2.0
Chimpanzee	50	6	22 ± 3.3	51 ± 6.1	26 ± 0.8	27 ± 6.2
Human	110	7	41 ± 9.8	66 ± 16	25 ± 1.5	11 ± 2.4

Mean values ± standard deviations are presented.

^aNumber of individual animals/humans (*n*) used for urine collection.

found in human urine, 11 ± 2.4 (μg/g creatinine), and the highest levels existed in the urine of the mouse, 205 ± 16 (μg/g creatinine) and rat 216 ± 15 (μg/g creatinine) (Table 3).

A recalculation of the mean values for urinary 8-OH-dG and 8-OH-Gua in Tables 2 and 3 to molar amounts yielded the following molar ratios for 8-OH-Gua/8-OH-dG: mouse = 55, rat = 85, guinea pig = 32, cat = 34, chimpanzee = 16, and human = 5.

The SMR (cal/g/day) was calculated from the animals' and humans' weight data, as presented in Tables 2 and 3. The correlations between the different species' SMR and the urinary levels of 8-OH-dG ($r = 0.91$, $p = 0.01$) and 8-OH-Gua ($r = 0.90$, $p = 0.01$) were both significant (Figs. 4 and 5).

DISCUSSION

According to the "Rate of living theory" an organism's life span is dependent on its metabolic rate (17). A higher metabolic rate is thus correlated with higher oxygen consumption, which leads to the production of more reactive oxygen species (20). It has been shown that the levels of 8-OH-dG in mitochondrial DNA are inversely correlated with the maximum life span potentials of different mammalian species, in

agreement with the lower amount of free radical generation in long-lived species (2).

In this article, we report the levels of the oxidative damage markers 8-OH-dG and 8-OH-Gua in the urine of seven mammalian species (see Tables 2 and 3). As reported previously, 8-OH-Gua is mainly formed by the process of BER (base excision repair) (5). On the other hand, 8-OH-dG may be considered as a more general marker of oxidative damage, since it originates from NER (nucleotide excision repair) and is an end product of the dephosphorylation of 8-hydroxydeoxyguanosine-5'-triphosphate (8-OH-dGTP) from the cellular nucleotide pool (5). The contribution of 8-OH-dG from the cellular nucleotide pool should not be underestimated, since during cell replication, the dGTP needed for the synthesis of new DNA would also be a significant target for oxygen radicals. There may also be some contribution from RNA degradation/depurination and the oxidation of free guanine (nonoxidized) found in urine. In addition, we must consider the possibility that there may be some increase in urinary 8-OH-Gua levels, due to the purine content in the diet. Although it has been shown that in humans and mice, diet has no effect on urinary 8-OH-Gua levels (7, 18), rats will show increased urinary 8-OH-Gua levels due to the effect of diet (10, 16).

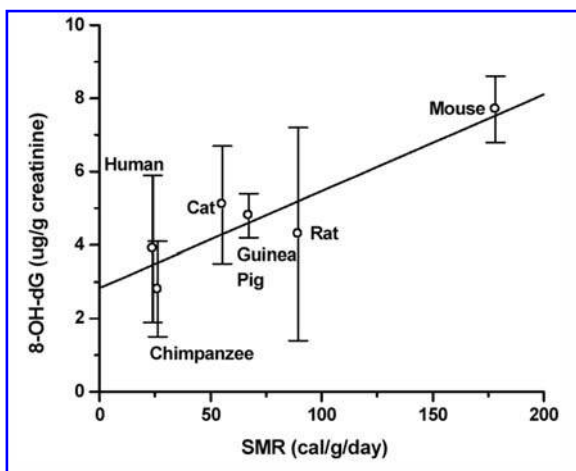


FIG. 4. Significant correlation ($r = 0.91$, $p = 0.01$) between species' specific metabolic rate (SMR) and urinary 8-OH-dG content (normalized to urinary creatinine).

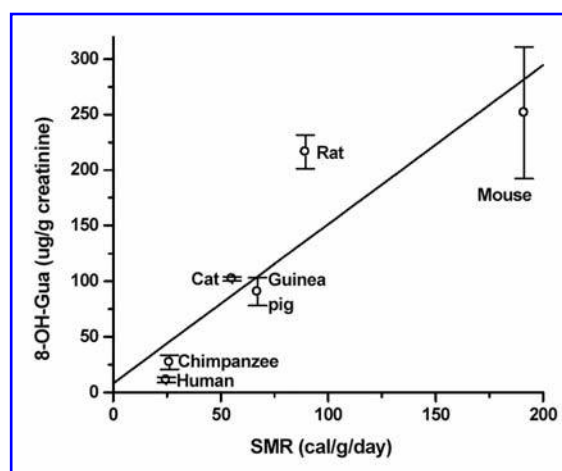


FIG. 5. Significant correlation ($r = 0.90$, $p = 0.01$) between species' specific metabolic rate (SMR) and urinary 8-OH-Gua content (normalized to urinary creatinine).

The human (healthy nonsmokers) urinary levels of 3.9 μg 8-OH-dG/g creatinine presented in this study (Table 2), and also presented previously (9), correlate well with the data from other studies using EC or GC-MS methods (3.3–4.0 μg /g creatinine) (3, 11). For mice, the level of urinary 8-OH-dG, 7.7 μg /g creatinine (Table 2), is similar to the previously published value of 6.9 μg /g creatinine obtained by EC-detection (3). For rats, our value of 4.3 μg 8-OH-dG/g creatinine in urine (Table 2), is lower than that previously published (7.8 μg /g creatinine), which was determined by EC-detection (3). In a more recent study comparing urinary DNA repair products in different mammalian species, using the GC-MS method, the human 8-OH-dG level (recalculated from nmol/mmol creatinine to μg /g creatinine) was found to be 5.3 μg 8-OH-dG/g creatinine (6). Although the reported value (6) for 8-OH-dG in human urine is similar to ours, the reported levels of urinary 8-OH-dG in mice and rats (6) are up to four times higher than our levels (Table 2). This discrepancy might be attributed to different strains of animals being used and/or to the different methods employed for sample preparation and analysis.

We found 11 μg 8-OH-Gua/g creatinine in human urine (Table 3), which is three times higher than the level determined by other researchers using the method of EC-detection (3.8 μg 8-OH-Gua/g creatinine) (21), and equals the value found using the GC-MS method (recalculated to 12 μg 8-OH-Gua/g creatinine) (6). Our levels of 8-OH-Gua/g creatinine in mouse and rat urine are between 1.5 to 3 times higher than those previously published (6, 21). In animals, especially rodents, the urinary 8-OH-Gua level is also probably influenced by the type of strain used and the nucleic acid content of the diet, which could explain some of the differences in the published values. The content of urinary 8-OH-Gua in humans is reportedly not to be influenced by diet (7), and the values found in the literature would thus be expected to be more similar. Urinary 8-OH-Gua may be rather insoluble, especially in the concentrated rodent urine. Thus, one possibility for the lower levels presented by other authors could be that the 8-OH-Gua recovery from urine was not optimal during the various cleaning and concentration steps used before analysis.

Our results regarding the levels of urinary 8-OH-dG and 8-OH-Gua, in human and various animals, show a significant positive correlation with the species SMR (Figs. 4 and 5). A higher SMR would be expected to increase the level of oxidative stress and the formation of DNA damage. These results are also in agreement with the conclusions from previous authors, who found a significant correlation between 8-OH-dG or 8-OH-Gua and SMR, although they did not analyze the urine from any long-lived animals, such as the chimpanzee, in their study (6). In addition, the urinary excretion of 8-OH-dG from humans, rats, and mice positively correlates with the species-specific oxygen consumption (19). Thus, mice, with the highest oxygen consumption and metabolic rate, had a threefold higher level of urinary 8-OH-dG as compared to that of humans (19).

In terms of the possible correlation between urinary 8-OH-dG or 8-OH-Gua and species' potential life spans, only 8-OH-Gua significantly correlated (negatively) with the potential life spans (Fig. 4). Although 8-OH-dG showed a tendency toward a negative correlation, it was not significant (Fig. 3).

These results agree with the previously mentioned report (6), which analyzed the same relationships and found a significant correlation only for urinary 8-OH-Gua and species' maximum potential life spans. Since a higher SMR is generally associated with smaller, short-lived animals (Tables 2 and 3), it would be expected that the levels of oxidative stress markers, such as 8-OH-dG and 8-OH-Gua, should be lower in the urine of long-lived animals or human, as compared to short-lived animals. This is the case for the level of urinary 8-OH-Gua, which is 20 times lower in human urine as compared to rat urine (Table 3). On the other hand, for 8-OH-dG, the urinary levels are almost the same for rat and human (Table 2). The nonsignificant correlation between potential life span and 8-OH-dG may be due to larger variations in the origin and/or repair pathways of this particular type of damage, as compared to 8-OH-Gua, which would be expected to be derived mainly from BER. 8-OH-Gua may thus be a better marker than 8-OH-dG for comparisons of oxidative damage between different species.

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ABBREVIATIONS

8-OH-dG, 8-hydroxydeoxyguanosine; 8-OH-dGTP, 8-hydroxydeoxyguanosine triphosphate; 8-OH-G, 8-hydroxyguanosine; 8-OH-Gua, 8-hydroxyguanine; BER, base excision repair; dGTP, deoxyguanosine triphosphate; EC, electrochemical; HPLC, high-performance liquid chromatography; NER, nucleotide excision repair; SMR, specific metabolic rate; SOD, superoxide dismutase.

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