previously shown that 8-OH-dG and 8-OH-Guo induce SCE in human lymphocytes at a low concentration (100 nM) (6). The ribonucleoside 8-OH-Guo induced a higher frequency of SCE than 8-OH-dG. Recently, the incorporation of ^{14}C-labeled 8-OH-dG into F-7 human breast cancer cell DNA, as measured by an accelerator mass spectrometry assay, was reported (4). This result suggested that 8-OH-dG is incorporated into cellular DNA *via* a salvage pathway. Chung and his collaborators reported that 8-OH-dG is incorporated into OGG1-deficient human leukemia cell DNA and induces apoptosis due to its accumulation in DNA (5).

The most important point of our findings is that considerably large amounts of genotoxic, oxidatively damaged nucleoside analogues are present in commonly consumed foods, although their genotoxicity are not as strong as those of N^4-aminocytidine and 2-amino-N^6-hydroxyadenine. These 8-OH-Gua compounds may be produced by oxidation of their precursors, dG, Guo and Gua, during the drying process by heating or under sunlight. The higher levels of these 8-OH-Gua compounds in processed fish food products, as compared to those in meat products, may be due to higher levels of their precursor molecules, dG, Guo, Gua, dGMP and GMP, in fish. By broiling or heating, the precursor nucleosides, dG and guanosine, may be further oxidized to 8-OH-dG and 8-OH-Guo. 8-OH-Gua may be heat labile and thus be degraded. It should be pointed out that in addition to its natural presence in these products, GMP is also added to various processed foods with 5′-inosine monophosphate (5′-IMP) as a flavour enhancer, and therefore Guo and Gua may be easily produced from GMP during the cooking or storage of foods.

Epidemiological studies in Korea revealed a positive correlation between the intake of broiled fish and stomach cancer (10,11). The repeated consumption of these 8-OH-Gua compounds from daily food, in addition to other mutagens such as heterocyclic amines, may stimulate cancer induction in the digestive tract. Further studies on the carcinogenicity of these 8-OH-Gua compounds are required to assess their risk to human health.

Acknowledgment: This work was supported in part by grant-in-aid (15–22) for cancer research from the Ministry of Health, Labor and Welfare of Japan.

References

1 Kasai H, Nishimura, S. Hydroxylation of guanine in nucleosides and DNA at the C-8 position by heated glucose and oxygen radical forming agents. Environ Health Perspect. 1986; 67: 111–6.
2 Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb LA. 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G→T and A→C substitutions. J Biol Chem. 1992; 267: 166–72.
3 Kasai H. Analysis of a form of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. Mutat Res. 1997; 387: 147–63.
4 Hah SS, Kim HM, Sumbad RA, Henderson PT. Hydantoin derivative formation from oxidation of 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxodG) and incorporation of ^{14}C-labeled 8-oxodG into the DNA of human breast cancer cells. Bioorg Med Chem Lett. 2005; 15: 3627–31.
5 Hyun JW, Jung YC, Kim HS, Choi EY, Kim JE, Yoon BH, Yoon SH, Lee YS, Choi J, You HJ, Chung MH. 8-hydroxydeoxyguanosine causes death of human leukemia cells deficient in 8-oxoguanine glycosylase 1 activity by inducing apoptosis. Mol Cancer Res. 2003; 1: 290–9.
6 Arashidani K, Iwamoto-Tanaka N, Muraoka M, Kasai H. Genotoxicity of ribo-and deoxyribonucleosides of 8-hydroxyguanine, 5-hydroxycytosine, and 2-hydroxyadenine: induction of SCE in human lymphocytes and mutagenicity in *Salmonella typhimurium* TA 100. Mutat Res. 1998; 403: 223–7.
7 Kasai H. A new automated method to analyze urinary 8-hydroxydeoxyguanosine by a high-performance liquid chromatography-electrochemical detector system. J Radiat Res. 2003; 44: 185–9.
8 Nagao M, Nakayasu M, Aonuma S, Wakabayashi K, Hirose M, Sugimura T. Mutagenic properties of 2-amino-N^6-hydroxyadenine in Salmonella and in Chinese hamster lung cells in culture. Mutat Res. 1991; 253: 97–102.
9 Negishi K, Harada C, Ohara Y, Oohara K, Nitta N, Hayatsu H. N^4-aminocytidine, a nucleoside analog that has an exceptionally high mutagenic activity. Nucleic Acids Res. 1983; 11: 5223–33.
10 Ahn YO. Diet and stomach cancer in Korea. Int J Cancer. 1997; Suppl 10: 7–9.
11 Lee JK, Park BJ, Yoo KY, Ahn YO. Dietary factors and stomach cancer: a case-control study in Korea. Int J Epidemiol. 1995; 24: 33–41.

Genes and Environment, Vol. 28, No. 2 pp. 62–67 (2006)

Regular article

Fragmentation of the DNA Repair Enzyme, OGG1, in Mouse Nonparenchymal Liver Cells by Arsenic Compounds

Takeshi Hirano[1,2], Kazuaki Kawai[1], Yuko Ootsuyama[1] and Hiroshi Kasai[1,3]

[1]Department of Environmental Oncology, University of Occupational and Environmental Health, Japan, Fukuoka, Japan

(Received March 7, 2006; Accepted April 14, 2006)

Our previous work demonstrated that arsenic compounds increased 8-hydroxyguanine (8-OH-Gua) in the DNA of cultured human cells (A549) and reduced the endonuclease nicking activity for 8-OH-Gua, suggesting that arsenic compound-induced carcinogenesis was the consequence of the inhibition of DNA repair. However, the exact mechanism by which the repair systems were disturbed was unknown. To elucidate the mechanism, we analyzed mouse 8-oxoguanine DNA glycosylase 1 (mOGG1) expression in mouse nonparenchymal liver cells, NCTC, treated with arsenic compounds (arsenic trioxide, sodium arsenite, and sodium hydrogen arsenate). We detected a cleaved form of mOGG1 (35 kDa) in addition to normal mOGG1 (type 1a, 38 kDa) in NCTC treated with arsenic compounds. These results are similar to our previous results which showed that fragmentation of mOGG1 by etoposide was related to caspase-dependent apoptosis, and was accompanied by increased 8-OH-Gua accumulation. Taken together, our results suggested that arsenic compounds might increase 8-OH-Gua accumulation by inhibiting 8-OH-Gua repair, due to mOGG1 cleavage.

Key words: mOGG1, arsenic compounds, 8-hydroxyguanine

Introduction

Arsenic compounds are known to be toxic or carcinogenic to humans and are associated with various diseases, such as cancer, hepatic damage and arteriosclerosis. In particular, they are associated with the development of several kinds of tumors, including lung, skin, kidney, bladder and liver tumors (1–5). Recently, arsenic trioxide was shown to induce DNA damage, such as 8-hydroxyguanine (8-OH-Gua) and DNA strand breaks, in cultured human cells (6,7). It was also reported that the urinary 8-OH-dG level is increased with exposure to arsenic compounds (8,9). The DNA damage induced by arsenic compounds was extensively investigated, and the contributions of reactive oxygen species (ROS) and nitric oxide generated by arsenic compounds to the carcinogenic mechanisms have been suggested (10–12). However, the exact role of DNA damage in arsenic compound-related carcinogenesis is still unclear.

8-OH-Gua is a major premutagenic form of oxidative DNA damage that induces GC to TA transversion type point mutation in DNA (13). Therefore, GC to TA point mutations, which are often detected in various cancer cells, may be induced by ROS. In order to prevent such mutations, several repair systems are ubiquitously present, from bacteria to human (14,15). However, when excessive amounts of ROS are generated or the repair systems are inhibited, 8-OH-Gua might accumulate in nuclear or mitochondrial DNA, leading to the subsequent carcinogenesis, because the accumulation level of 8-OH-Gua depends on the balance between 8-OH-Gua generation and repair.

Recent studies revealed that heavy metals, such as cadmium chloride, reduced DNA repair activity (16,17). Arsenic compounds also interact with DNA repair systems, leading to the accumulation of DNA damage (18). Furthermore, it was reported that metabolites of inorganic arsenic, monomethylarsonous- and dimethylarsinous acids, inhibit the activity of the Fpg protein (9), which functions as a glycosylase and an AP-lyase and also has a 5′-terminal deoxyribosephosphate excising activity (14). Hence, it is likely that 8-OH-Gua repair might be inhibited by arsenic compounds, leading to an accumulation of 8-OH-Gua that could be responsible for carcinogenesis. However, the detailed mechanisms of the inhibition of DNA repair systems are still unknown.

The present study was performed to examine the expression of a DNA repair protein, mouse 8-oxoguanine DNA glycosylase 1 (mOGG1). Insights relevant to the inhibition mechanisms of DNA repair in cells treated with arsenic compounds are discussed.

[2]Present address: Graduate School of Environmental Engineering, University of Kitakyushu, Kitakyushu, Fukuoka, 808-0135, Japan
[3]Correspondence to: Hiroshi Kasai, Department of Environmental Oncology, University of Occupational and Environmental Health, Japan, Kitakyushu, Fukuoka, 807-8555, Japan. Tel: +81-93-603-1611, Fax: +81-93-601-2199, E-mail: h-kasai@med.uoeh-u.ac.jp

Materials and Methods

Cell culture and treatment with arsenic compounds: NCTC, nonparenchymal liver cells, were obtained from the Health Science Research Resources Bank (HSRRB, Osaka, Japan) and were cultured in NCTC135 medium (ICN Biomedicals Inc., Ohio) with 10% horse serum. When the cells were ~70% confluent, arsenic compounds (arsenic trioxide, sodium arsenite, and sodium arsenate), cadmium chloride as a reference, and dimethyl sulfoxide (DMSO) as a control were added into the culture medium to a final concentration of 10 μM each. After 24 and 48 h of cultivation, the cells were harvested, washed with sterile PBS, and subjected to analyses.

Sample preparation and Western blotting: NCTC were homogenized with a Potter-type homogenizer in cold buffer (50 mM Tris-HCl, 50 mM KCl, 3 mM EDTA, 5 mM magnesium acetate, and 3 mM β-mercaptoethanol) containing 5 μg/mL each of protease inhibitors (leupeptin, antipain, pepstatin, and chymostatin). The homogenates were centrifuged (12000 g, 30 min), and the supernatants were used for Western blot analysis. The Western blotting method was described elsewhere. Protein concentrations were determined using a Bio-Rad Protein Assay kit (Bio-Rad, Richmond, CA). Proteins (40 μg) were fractionated on 4–12% SDS-polyacrylamide gels (NuPAGE, NOVEX, CA), and blotted onto PVDF membranes (Millipore, MA). The filters were blocked with a buffer (150 mM Tris-HCl, pH 7.6, containing Triton X-100 and bovine serum albumin) overnight at 37°C, and then were incubated with anti-mOGG1 antiserum overnight at 37°C. After two washes, the blots were incubated with an alkaline phosphatase-coupled secondary antibody (EY Laboratories, Inc., CA) at 37°C for 3 h. The antigen-antibody complexes were visualized with a BCIP-NBT solution kit (Nakalai Tesque, Inc., Kyoto). The anti-mOGG1 antiserum was prepared as described in our previous work (19). The amino acid sequence of the peptide used as the antigen was QSFRWKEOSP (aa 43–52).

Endonuclease nicking assay: The base excision repair activity was assayed by using a previously described method. Briefly, the cells were homogenized in cold buffer (50 mM Tris-HCl, 50 mM KCl, 3 mM EDTA, 5 mM magnesium acetate, and 3 mM β-mercaptoethanol) containing 5 μg/mL each of protease inhibitors (leupeptin, antipain, pepstatin, and chymostatin), and the lysates were centrifuged (12000 g, 30 min) to obtain crude extracts. The extracts were incubated with a 22-mer, fluorescently labeled, synthetic oligonucleotide containing an 8-OH-Gua residue in its sequence. These mixtures were electrophoresed on a 20% denaturing polyacrylamide gel. The excised fragments, generated as a consequence of base excision repair activity, were analyzed with a Pharmacia ALF DNA sequencer (Fragment Manager, Ver. 1.1; Amersham Pharmacia Biotech, Uppsala, Sweden).

Analysis of 8-OH-Gua in cellular DNA: The assay for measuring the 8-OH-Gua levels was described elsewhere. Briefly, NCTC treated with 10 μM arsenic trioxide for 24 h were divided into two cell groups: adherent cells and floating cells. The cellular genomic DNA of each cell group was isolated by the sodium iodide method, using a DNA Extraction WB Kit (Wako Pure Chemical Industries, Ltd., Japan). For cell homogenization, a lysis solution containing 1 mM desferal (deferoxamine mesylate, Sigma Chemical Co., MO, USA) was used. The isolated DNA was digested with nuclease P1 (Yamasa Corp., Choshi, Japan) and alkaline phosphatase (Roche Diagnostics GmbH, Mannheim, Germany) to obtain a deoxynucleoside mixture. The solution was filtered with an Ultrafree-Probind filter (Millipore, Bedford, MA) and was injected into a high-performance liquid chromatography (HPLC) column (Shiseido Capcell Pak C18 MG) equipped with an electrochemical detector (ECD) (Coulochem II, ESA, USA). The 8-OH-Gua value in the DNA was calculated as the number of 8-OH-Gua per 10^6 guanine (Gua).

Statistical analysis: Analyses were performed using the Stat View-J 4.5 program (Berkeley, CA). Statistical significance was determined by the one-way analysis of variance (ANOVA), followed by the Scheffe test.

Results

Cell viability: As shown in Fig. 1, the viability of the NCTC treated with arsenic trioxide and sodium arsenite significantly decreased during the 48 h treatment, as compared to the DMSO-treated control cells. The floating cells revealed the significant reduction in viability with these compounds.

Endonuclease nicking assay: We analyzed endonuclease nicking activity using 8-OH-Gua containing DNA to examine whether arsenic compounds inhibit 8-OH-Gua repair activity. We observed that endonuclease nicking activities decreased in a crude extract from arsenic trioxide-treated cells during 48 h (Fig. 2).

Analyses of mOGG1 expression: We analyzed the mOGG1 protein expression in the cells treated with arsenic trioxide, sodium arsenite, sodium hydrogen arsenate, cadmium chloride, and DMSO as a control. Fragmentation of the 38 kDa mOGG1 to the 35 kDa form was detected in the cells treated with arsenic trioxide for 24 and 48 h, and with sodium arsenite for 48 h, but not with sodium hydrogen arsenate, cadmium chloride, and DMSO (Fig. 3). It is worth mentioning that the fragmentation of mOGG1 was detected in the cells treated with arsenic trioxide even at 24 h, suggesting that mOGG1 fragmentation might occur at the early

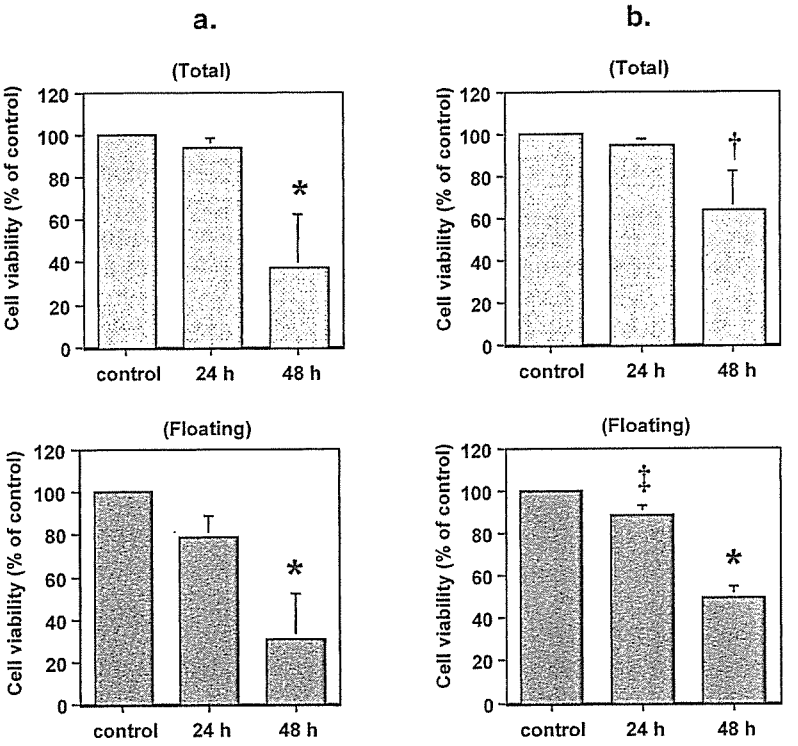

Fig. 1. Viability of NCTC. (a) The viabilities of total cells and floating cells of arsenic trioxide (10 μM)-treated NCTC (24 and 48 h). The ratio (%) of the viability of treated cells to that of the DMSO-treated control cells is shown. (b) The viability of total cells and floating cells of sodium arsenite (10 μM)-treated NCTC (24 and 48 h). Cell viability was assayed by means of the trypan blue exclusion test. The volume of DMSO was equal to that of the arsenic trioxide or sodium arsenite solutions. Means ± SD, n = 4–5. *$p < 0.0001$; †$p = 0.0007$; ‡$p = 0.0017$; significantly lower than the control group.

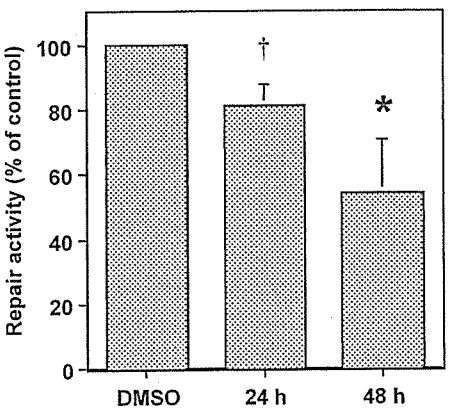

Fig. 2. 8-OH-Gua repair activity in NCTC treated with arsenic trioxide. Repair activity was analyzed in the cells treated with arsenic trioxide by an endonuclease nicking assay. The value of the repair activity of treated cells is expressed as the ratio (%) to that of the DMSO-treated control. Means ± SD, n = 6. *$p < 0.0001$; †$p = 0.022$; significantly lower than the control group.

stage of the inhibition of mOGG1 activity.

Accumulation of 8-OH-Gua in NCTC DNA: As arsenic compounds reportedly generate reactive oxygen species (ROS), the 8-OH-Gua level might increase in the DNA of arsenic compound-treated cells. Cultured NCTC cells were treated with 10 μM arsenic trioxide for 24 h, and the cells were divided into adherent and floating cell populations. We detected a significant increase in 8-OH-Gua in the DNA of the floating NCTC-cells in comparison with the control cells at 24 h (Fig. 4).

Discussion

Since 8-OH-Gua is a form of oxidative DNA damage that induces GC to TA point mutations in DNA, it is believed to be related to carcinogenesis. In this context, in order to reduce cancer risk it is important to prevent 8-OH-Gua accumulation in nuclear DNA. For this purpose, understanding the mechanisms of 8-OH-Gua generation is an important issue. As for arsenic compounds, many reports have indicated that they increased oxidative DNA damage in cultured cells (6,7). For example, hydroxyl radical-induced oxidative DNA damage was generated in arsenic trioxide-treated keratinocyes (10). In addition, elevated levels of 8-OH-dG were observed in the urine of the patients with arsenic poisoning (8,9). In the present study, we also detected an increased level of 8-OH-Gua in the DNA of NCTC treated with a physiologically relevant concen-

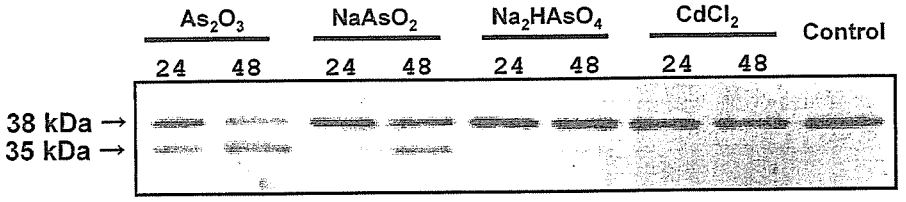

Fig. 3. Immunoblotting of mOGG1 in NCTC treated with arsenic trioxide (10 μM), sodium arsenite (10 μM), sodium arsenate (10 μM), and cadmium chloride (10 μM) for 24 and 48 h. Upper bands (38 kDa) represent normal mOGG1 and lower bands (35 kDa) are cleaved mOGG1. Control refers to data of DMSO-treated NCTC (24 h). As_2O_3, arsenic trioxide; $NaAsO_2$, sodium arsenite; Na_2HAsO_4; sodium hydrogen arsenate; $CdCl_2$, cadmium chloride.

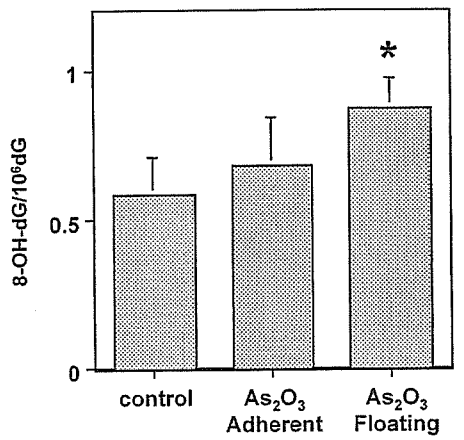

Fig. 4. Effect of arsenic trioxide on 8-OH-dG formation in NCTC cells. The results represent means ± SD, n = 3. *$p < 0.05$, significantly higher than the control group.

tration (10 μM) of arsenic trioxide.

It is noteworthy that the accumulation level of 8-OH-Gua depends on the balance between the generation and repair of 8-OH-Gua. Hence, the DNA repair activity and the 8-OH-Gua generation must both be considered. In fact, several recent reports have attributed the mechanism of DNA damage accumulation to the inhibition of DNA repair system as described below.

We and other groups have reported that some carcinogenic agents, such as cadmium compounds and diesel exhaust particles, disturbed 8-OH-Gua repair, leading to the subsequent accumulation of 8-OH-Gua (16,17,20). As for arsenic compounds, we also previously reported that sodium arsenite decreased the 8-OH-Gua base excision repair activity and increased the 8-OH-Gua level in the DNA of culture cells (6). Another group also indicated that arsenic compounds increased the accumulation of oxidative DNA damage along with the interference of repair systems (18). Besides the DNA repair system, defense systems against ROS, such as superoxide dismutase (SOD) and GSH-Px, were also disrupted by arsenic compounds (21). These findings suggest that arsenic compounds disrupt the defense mechanisms, including DNA repair systems, against oxidative stresses. However, the detailed mechanisms are still unknown.

OGG1, one of the main repair enzymes for 8-OH-Gua in mammalian cells, was cloned in 1996 (22–24). Although other repair enzymes besides OGG1 could also participate, OGG1 might play an important and central role in 8-OH-Gua repair. Therefore, analysis of OGG1 expression might provide useful information to understand the 8-OH-Gua repair systems. In the present study, the fragmentation of mOGG1 was detected in NCTC treated with arsenic compounds. Similar results were obtained in our previous studies, in which we detected the fragmentation of mOGG1 in carcinogen-treated mouse liver or during caspase-dependent apoptosis in cultured mouse nonparenchymal hepatocytes (19,25). In those cases and the present study, the 8-OH-Gua accumulation was increased, suggesting that the fragmentation of OGG1 resulted in the increased level of 8-OH-Gua accumulation. The smaller mOGG1 was suggested to be a product of posttranscriptional modification, rather than alternative splicing (19). We propose two mechanisms of mOGG1 fragmentation. First, the fragmentation might be due to caspase activity, because arsenic trioxide reportedly induces apoptosis through caspase-3 activation (26). Secondly, as arsenic trioxide produces more ROS in comparison to other arsenic compounds (27), we can speculate that mOGG1 is excised by ROS. The most notable point of our present results is that mOGG1 fragmentation occurred at the early stage of DNA repair inhibition, suggesting that mOGG1 fragmentation might be responsible for DNA repair inhibition. On the other hand, another possible mechanism for the inhibition of OGG1 activity was suggested by other groups. Recently, Youn *et al.* reported that the cadmium-mediated decrease in *hOGG1* transcription was the result of decreased binding of the transcription factor Sp1 to the *hOGG1* promoter (28). Whether the same mechanism also occurs in arsenic compound-treated cells or animals remains unknown. Further studies are required to clarify the detailed mechanism of DNA repair inhibition.

In conclusion, our results suggest that arsenic

compounds increased the accumulation of 8-OH-Gua by the cleavage of its repair enzyme, mOGG1.

Acknowledgements: This work was supported by a grant from the University of Occupational and Environmental Health, Japan, and a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1 Grimsrud TK, Berge SR, Haldorsen T, Andersen A. Can lung cancer risk among nickel refinery workers be explained by occupational exposures other than nickel? Epidemiology. 2005; 16: 146–54.
2 Rossman TG, Uddin AN, Burns FJ. Evidence that arsenite acts as a cocarcinogen in skin cancer. Toxicol Appl Pharmacol. 2004; 198: 394–404.
3 Hopenhayn-Rich C, Biggs ML, Smith AH. Lung and kidney cancer mortality associated with arsenic in drinking water in Cordoba, Argentina. Int J Epidemiol. 1998; 27: 561–9.
4 Hernandez-Zavala A, Cordova E, Del Razo LM, Cebrian M. E, Garrido E. Effects of arsenite on cell cycle progression in a human bladder cancer cell line. Toxicology. 2005; 207: 49–57.
5 Lu SN, Chow NH, Wu WC, Chang TT, Huang WS, Chen SC, Lin CH, Carr BI. Characteristics of hepatocellular carcinoma in a high arsenicism area in Taiwan: a case-control study. J Occup Environ Med. 2004; 46: 437–41.
6 Mei N, Kunugita N, Hirano T, Kasai H. Acute arsenite-induced 8-hydroxyguanine is associated with inhibition of repair activity in cultured human cells. Biochem Biophys Res Commun. 2002; 297: 924–30.
7 Fujino Y, Guo X, Liu J, Matthews IP, Shirane K, Wu K, Kasai H, Miyatake M, Tanabe K, Kusuda T, Yoshimura T; Japan Inner Mongolia Arsenic Pollution Study Group. Chronic arsenic exposure and urinary 8-hydroxy-2′-deoxyguanosine in an arsenic-affected area in Inner Mongolia, China. J Expo Anal Environ Epidemiol. 2005; 15: 147–52.
8 Yamauchi H, Aminaka Y, Yoshida K, Sun G, Pi J, Waalkes MP. Evaluation of DNA damage in patients with arsenic poisoning urinary 8-hydroxydeoxyguanine. Toxicol Appl Pharmacol. 2004; 198: 291–6.
9 Schwerdtle T, Walter I, Mackiw I, Hartwig A. Induction of oxidative DNA damage by arsenite and its trivalent and pentavalent methylated metabolites in cultured human cells and isolated DNA. Carcinogenesis. 2003; 24: 967–74.
10 Shi H, Hudson LG, Ding W, Wang S, Cooper KL, Liu S, Chen Y, Shi X, Liu KJ. Arsenite causes DNA damage in keratinocytes *via* generation of hydroxyl radicals. Chem Res Toxicol. 2004; 17: 871–8.
11 Gurr JR, Yih LH, Samikkannu T, Bau DT, Lin SY, Jan KY. Nitric oxide production by arsenite. Mutat Res. 2003; 533: 173–82.
12 Chien YH, Bau DT, Jan KY. Nitric oxide inhibits DNA-adduct excision in nucleotide excision repair. Free Radic Biol Med. 2004; 36: 1011–7.
13 Kasai H, Nishimura S. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. Nucleic Acids Res. 1984; 12: 2137–45.
14 Tchou J, Kasai H, Shibutani S, Chung MH, Laval J, Grollman AP, Nishimura S. 8-Oxoguanine (8-hydoxyguanine) DNA glycosylase and its substrate specificity. Proc Natl Acad Sci USA. 1991; 88: 4690–4.
15 Boiteux S, Gajewski E, Laval J, Dizdaroglu M. Substrate specificity of the *Escherichia coli* Fpg protein (formamidepyrimidine-DNA glycosylase): excision of purine lesions in DNA produced by ionizing radiation or photosensitization. Biochemistry. 1992; 31: 106–10.
16 Hirano T, Yamaguchi Y, Kasai H. Inhibition of 8-hydroxyguanine repair in testes after administration of cadmium chloride to GSH-depleted rats. Toxicol Appl Pharmacol. 1997; 147: 9–14.
17 Porter DW, Yakushiji H, Nakabeppu Y, Sekiguchi M, Fiyash MJ, Kasprzak KS. Sensitivity of *Escherichia coli* (MutT) and human (MTH1) 8-oxo-dGTPases to *in vitro* inhibition by the carcinogenic metals, nickel(II), copper (II), cobalt(II) and cadmium(II). Carcinogenesis. 1997; 18: 1785–91.
18 Hartwig A, Blessing H, Schwerdtle T, Walter I. Modulation of DNA repair processes by arsenic and selenium compounds. Toxicology. 2003; 193: 161–9.
19 Hirano T, Kudo H, Doi Y, Nishino T, Fujimoto S, Tsurudome Y, Ootsuyama Y, Kasai H. Detection of a smaller, 32-kDa 8-oxoguanine DNA glycosylase 1 in 3′-methyl-4-dimethylamino-azobenzene-treated mouse liver. Cancer Sci. 2004; 95: 118–22.
20 Tsurudome Y, Hirano T, Yamato H, Tanaka I, Sagai M, Hirano H, Nagata N, Itoh H, Kasai H. Changes in levels of 8-hydroxyguanine in DNA, its repair and OGG1 mRNA in rat lungs after intratracheal administration of diesel exhaust particles. Carcinogenesis. 1999; 20: 1573–6.
21 Shen ZY, Shen WY, Chen MH, Shen J, Zeng Y. Reactive oxygen species and antioxidants in apoptosis of esophageal cancer cells induced by As_2O_3. Int J Mol Med. 2003; 11: 479–84.
22 Rosenquist TA, Zharkov DO, Grollman AP. Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. Proc Natl Acad Sci USA. 1997; 94: 7429–34.
23 Radicella JP, Dherin C, Desmaze C, Fox MS, Boiteux S. Cloning and characterization of *hOGG1*, a human homolog of *OGG1* gene of *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA. 1997; 94: 8010–5.
24 Tani M, Shinmura K, Kohno T, Shiroishi T, Wakana S, Kim SR, Nohmi T, Kasai H, Takenoshita S, Nagamachi Y, Yokota J. Genomic structure and chromosomal localization of the mouse *Ogg1* gene that is involved in the repair of 8-hydroxyguanine in DNA damage. Mamm Genome. 1998; 9: 32–7.
25 Hirano T, Kawai K, Ootsuyama Y, Orimo H, Kasai H. Detection of a mouse OGG1 fragment during caspase-dependent apoptosis: oxidative DNA damage and apoptosis. Cancer Sci. 2004; 95: 634–8.
26 Park JW, Cjoi YJ, Jang MA, Baek SH, Lim JH, Passaniti T, Kwon TK. Arsenic trioxide induces G2/M growth

arrest and apoptosis after caspase-3 activation and bcl-2 phosphorylation in promonocytic U937 cells. Biochem Biophys Res Commun. 2002; 286: 726–34

27 Tsai SH, Hsieh MS, Chen L, Liang YC, Lin JK, Lin SY. Suppression of Fas ligand expression on endothelial cells by arsenite through reactive oxygen species. Toxicol Lett. 2001; 123: 11–9

28 Youn CK, Kim SH, Lee do Y, Song SH, Chang IY, Hyun. JW, Chung MH, You HJ. Cadmium down-regulates human OGG1 through suppression of Sp1 activity. J Biol Chem 2005; 280: 25185–95.

ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 5 & 6, 2006

Forum Original Research Communication

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

PETER SVOBODA,[1] MUNEYUKI MAEKAWA,[1] KAZUAKI KAWAI,[1]
TOSHIKAZU TOMINAGA,[2] KIRSTI SAVELA,[3] and HIROSHI KASAI[1]

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-

[1]Department of Environmental Oncology, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Kitakyushu, Japan.
[2]Kumamoto Primates Research Park, Sanwa Kagaku Kenkyusho Co. Ltd., Kumamoto, Japan.
[3]Finnish Institute of Occupational Health, Helsinki, Finland.

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 prepurification 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-dihydroxypurine) 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 μm, 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 × 50 mm) and a main column (1.5 × 150 mm). For the analysis of 8-OH-dG in the second HPLC separation, a reversed phase column (CAPCELL PAK C18, 5 μm, 4.6 × 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 nonsmokers, 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 $[m^7Gua] = 0.0284 \times [\text{creatinine}] + 0.0065$ (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 μl) were manually mixed with an equal volume of a 4% acetonitrile, sodium acetate buffer containing the ribonucleoside marker (120 μg/ml), and were stored at +5°C overnight. After centrifugation at 13,000 rpm for 5 min, a 20 μl 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 (calories/gram/day) = 393 × (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

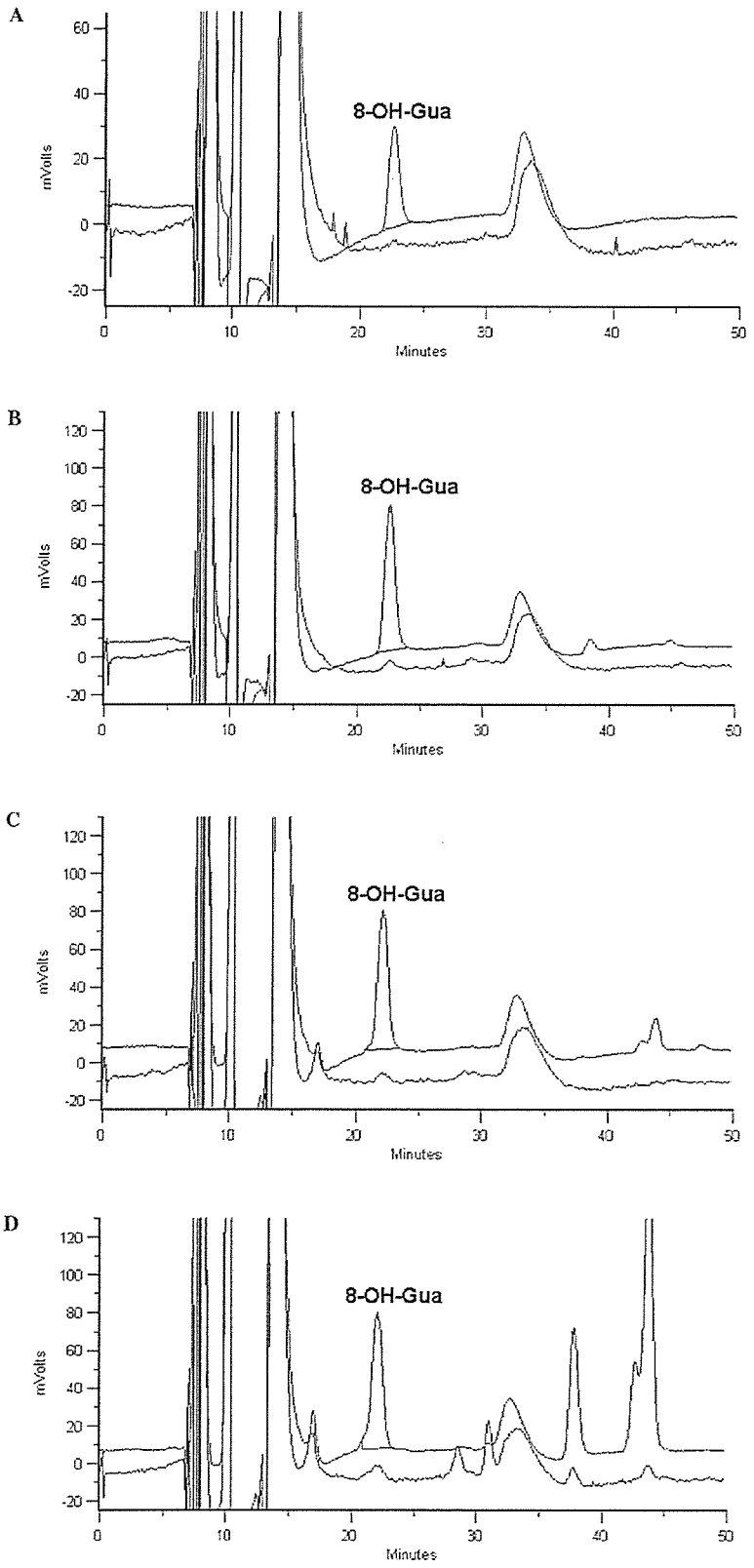

FIG. 1. Chromatograms of HPLC-2 electrochemical detection of 8-OH-Gua at applied potentials of E1 = 190 mV (*lower line*) and E2 = 300 mV (*upper line*): (A) 8-OH-Gua standard (5 ng/ml); (B) mouse (diluted ×50); (C) chimpanzee (diluted ×5); and (D) human (diluted ×5).

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) 8-OH-dG[a] (ng/ml)*	*Recovery 8-OH-dG[a] (%)*	*Control 8-OH-Gua[b] (ng/ml)*	*Spiked + 8-OH-Gua (4 ng/ml) 8-OH-Gua[b] (ng/ml)*	*Recovery 8-OH-Gua[b] (%)*
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

[a]For 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 ($n = 3$).

[b]For 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)*	*n[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.

[a]Number of individual animals/humans (n) used for urine collection.

[b]Data 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 urinary 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

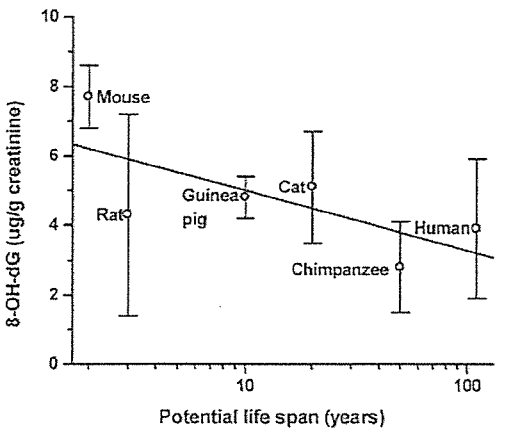

FIG. 2. No significant correlation ($r = -0.71$, $p = 0.11$) between species' potential life span and urinary 8-OH-dG content (normalized to urinary creatinine).

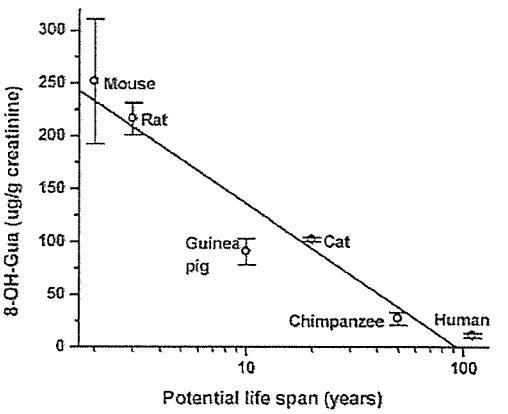

FIG. 3. Significant correlation ($r = -0.97$, $p = 0.002$) between species' potential life span and urinary 8-OH-Gua content (normalized to urinary creatinine).

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

Species	*Potential life span (years)*	*n*[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.
[a]Number 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).

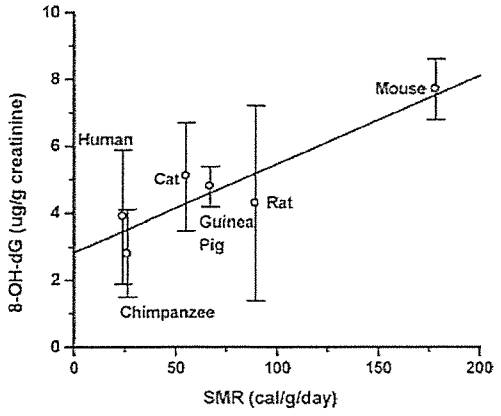

FIG. 4. Significant correlation ($r = 0.91$, $p = 0.01$) between species' specific metabolic rate (SMR) and urinary 8-OH-dG content (normalized to urinary creatinine).

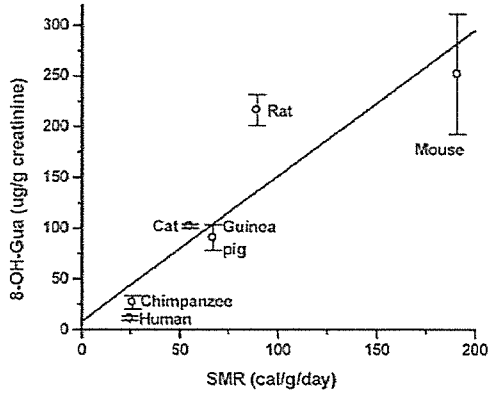

FIG. 5. Significant correlation ($r = 0.90$, $p = 0.01$) between species' specific metabolic rate (SMR) and urinary 8-OH-Gua content (normalized to urinary creatinine).

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.

ACKNOWLEDGMENTS

This work was supported by the Japan Society for the Promotion of Science. We are grateful to Mrs. Yuko Ootsuyama for kindly providing the cat urine samples.

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.

REFERENCES

1. Aitio A, Luotamo M, and Kiilunen M. *Biomonitoring of chemical exposure (in Finnish)*. Finnish Institute of Occupational Health, Finland, p.17, 1995.
2. Barja G and Herrero A. Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. *FASEB J* 14: 312–318, 2000.
3. Bogdanov MB, Beal MF, McCabe DR, Griffin RM, and Matson WR. A carbon column-based liquid chromatography electrochemical approach to routine 8-hydroxy-2'-deoxyguanosine measurements in urine and other biologic matrices: a one-year evaluation of methods. *Free Radic Biol Med* 27: 647–666, 1999.
4. Cooke MS, Evans MD, Herbert KE, and Lunec J. Urinary 8-oxo-2'-deoxyguanosine—source, significance, and supplements. *Free Radic Res* 32: 381–397, 2000.
5. Cooke MS, Evans MD, Dove R, Rozalski R, Gackowski D, Siomek A, Lunec J, and Olinski R. DNA repair is responsi-

ble for the presence of oxidatively damaged DNA lesions in urine. *Mutat Res* 574: 58–66, 2005.
6. Foksinski M, Rozalski R, Guz J, Ruszkowska B, Sztukowska P, Piwowarski M, Klungland A, and Olinski R. Urinary excretion of DNA repair products correlates with metabolic rates as well as with maximum life spans of different mammalian species. *Free Radic Biol Med* 37: 1449–1454, 2004.
7. Gackowski D, Rozalski R, Roszkowski K, Jawien A, Foksinski M, and Olinski R. 8-Oxo-7,8-dihydroguanine and 8-oxo-7,8-dihydro-2'-deoxyguanosine levels in human urine do not depend on diet. *Free Radic Res* 35: 825–832, 2001.
8. Harman D. Aging: overview. *Ann NY Acad Sci* 928: 1–21, 2001.
9. Harri M, Svoboda P, Mori T, Mutanen P, Kasai H, and Savela K. Analysis of 8-hydroxydeoxyguanosine among workers exposed to diesel particulate exhaust: comparison with urinary metabolites and PAH air monitoring. *Free Radic Res* 39: 963–972, 2005.
10. Helbock HJ, Beckman KB, Shigenaga MK, Walter PB, Woodall AA, Yeo HC, and Ames BN. DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. *Proc Natl Acad Sci USA* 95: 288–293, 1998.
11. Holmberg I, Stål P, and Hamberg M. Quantitative determination of 8-hydroxy-2′-deoxyguanosine in human urine by isotope dilution mass spectrometry: normal levels in hemochromatosis. *Free Radic Biol Med* 26: 129–135, 1999.
12. Ikehara M, Tada H, and Muneyama K. Synthesis of 8-hydroxypurine. *Chem Pharmcol Bull* 13: 1140–1142, 1965.
13. Kasai H, Crain PF, Kuchino Y, Nishimura S, Ootsuyama A, and Tanooka H. Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis* 7: 1849–1851, 1986.
14. Kasai H. A new automated method to analyze urinary 8-hydroxydeoxyguanosine by a high performance liquid chromatography–electrochemical detector system. *J Radiat Res* 44: 185–189, 2003.
15. Kleiber M. *The Fire of Life*. New York: John Wiley, 1961.
16. Park EM, Shigenaga MK, Degan P, Korn TS, Kitzler JW, Wehr CM, Kolachana P, and Ames BN. Assay of excised oxidative DNA lesions: isolation of 8-oxoguanine and its nucleoside derivatives from biological fluids with a monoclonal antibody column. *Proc Natl Acad Sci USA* 89: 3375–3379, 1992.
17. Pearl R. *The rate of living*. New York: Alfred Knopf, 1928.
18. Rozalski R, Siomek A, Gackowski D, Foksinski M, Gran C, Klungland A, and Olinski R. Diet is not responsible for the presence of several oxidatively damaged DNA lesions in mouse urine. *Free Radic Res* 38: 1201–1205, 2004.
19. Shigenaga MK, Gimeno CJ, and Ames BN. Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of *in vivo* oxidative DNA damage. *Proc Natl Acad Sci USA* 86: 9697–9701, 1989.
20. Sohal RS, Mockett RJ, and Orr WC. Mechanisms of aging: an appraisal of the oxidative stress hypothesis. *Free Radic Biol Med* 33: 575–586, 2002.
21. Suzuki J, Inoue Y, and Suzuki S. Changes in the urinary excretion level of 8-hydroxyguanine by exposure to reactive oxygen-generating substances. *Free Radic Biol Med* 18: 431–436, 1995.
22. Svoboda P and Kasai H. Simultaneous HPLC analysis of 8-hydroxydeoxyguanosine and 7-methylguanine in urine from humans and rodents. *Anal Biochem* 334: 239–250, 2004.
23. Tolmasoff JM and Ono T. Superoxide dismutase: correlation with life-span and specific metabolic rate in primate species. *Proc Natl Acad Sci USA* 77: 2777–2781, 1980.

Address reprint requests to:
Hiroshi Kasai
Department of Environmental Oncology
Institute of Industrial Ecological Sciences
University of Occupational and Environmental Health
1–1 Iseigaoka, Yahatanishi-ku
Kitakyushu 807–8555, Japan

E-mail: h-kasai@med.uoeh-u.ac.jp

Date of first submission to ARS Central, November 28, 2005; date of acceptance, December 10, 2005.