Increase in the 8-Hydroxyguanine Repair Activity in the Rat Kidney after the Administration of a Renal Carcinogen, Ferric Nitrilotriacetate

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One type of oxidative DNA damage, 8-hydroxyguanine (8-OH-Gua), is known to increase in rat kidney DNA after the administration of a renal carcinogen, ferric nitrilotriacetate (Fe-NTA). To determine the involvement of oxygen radicals in Fe-NTA carcinogenesis, we examined whether the 8-OH-Gua repair enzymes are induced in the rat kidney after Fe-NTA administration, in addition to our analysis of the 8-OH-Gua levels in the DNA, because the 8-OH-Gua repair activity is known to be induced in mammalian cells by oxidative stress due to ionizing radiation. The 8-OH-Gua repair enzyme activity was determined with an endonuclease assay using a 22-mer double strand DNA, which contains 8-OH-Gua at a specific position. A significant increase in the 8-OH-Gua repair activity was observed in the rat kidney after a single intraperitoneal injection of Fe-NTA ($p < 0.01$). This is the first report on the induction of the repair activity for 8-OH-Gua after treatment with a chemical carcinogen. This assay will be useful for evaluating the carcinogenicity of oxygen radical-forming chemicals. — Environ Health Perspect 104(Suppl 3):651–653 (1996)

Key words: 8-hydroxyguanine, DNA repair, endonuclease nicking assay, ferric nitrilotriacetate, oxidative DNA damage

Introduction

Many environmental chemical carcinogens damage DNA by generating oxygen radicals in cells. One of the major types of oxidative DNA damage is 8-hydroxyguanine (8-OH-Gua) (1). The formation of 8-OH-Gua in nuclear DNA may play an important role in carcinogenesis because it causes GC → TA transversions (2). Many

researchers have reported that 8-OH-Gua is a useful marker of oxidative DNA damage and can be used to estimate the carcinogenicity of oxygen radical-forming chemicals. In addition to specific types of chemical carcinogens such as peroxisome proliferators (3), well-known carcinogens such as benzo[a]pyrene (plus light) (4) and aflatoxin (5), also have been reported to increase the 8-OH-Gua levels in animal cellular DNA.

The 8-OH-Gua repair activity in mammalian systems is induced after cells are exposed to oxidative stress by ionizing radiation (6). We hypothesized that the induction of the 8-OH-Gua repair activity could be another biological marker of cellular oxidative stress, in addition to the increase in 8-OH-Gua in the DNA.

In this study, we treated rats with a renal carcinogen, ferric nitrilotriacetate (Fe-NTA), which causes renal proximal tubular necrosis as a consequence of iron-mediated peroxidation of the membrane lipids (7), and finally leads to a high incidence of

renal adenocarcinoma in rats and mice (8). The level of 8-OH-Gua increases in the rat kidney DNA after the administration of Fe-NTA (9). To confirm the involvement of oxygen radicals in Fe-NTA carcinogenesis, we measured the levels of 8-OH-Gua repair activities in the rat kidney after Fe-NTA administration.

Materials and Methods

Animal Treatment

Six-week-old male Wistar rats were sacrificed at 1, 6, 24, and 72 hr after the injection of Fe-NTA (15 mg Fe/kg body weight [bw], intraperitoneal [ip] injection) and control rats were sacrificed at 24 hr after the injection of saline (7.5 ml/kg bw, ip).

Determination of 8-Hydroxyguanine in DNA

The 8-OH-Gua level in the DNA was determined according to the method of Nakae et al. (10) with some modifications. Briefly, the organs were immediately homogenized by a potter-type homogenizer and were then centrifuged at $1,500 \times g$ for 15 min at 4°C to separate the nuclear fraction. The DNA was extracted from the nuclear fraction with a DNA Extractor WB Kit (Wako, Japan). Using this kit, more purified DNA was isolated from small samples (about 50 mg), as compared to previous methods. The extracted DNA was digested with nuclease P1 and acid phosphatase in a solution of 1 mM EDTA and 10 mM sodium acetate (pH 4.5). After incubation at 37°C for 30 min, the mixture was treated with ion exchange resin, Muromac (Muromachi Kagaku, Kogyo, Japan), and was centrifuged at $15,000 \times g$ for 5 min. The supernatant was transferred to a tube with a filter (Millipore Samprep C, 0.2 µm), centrifuged at $5,000 \times g$ for 5 min, and injected into a high-performance liquid chromatography (HPLC) column (Beckman Ultrasphere-ODS, 5 µm, 4.6×250 mm) equipped with an electrochemical detector (ESA Coulochem II). Deoxyguanosine (dG; 0.5 mg/ml) and 8-hydroxydeoxyguanosine (8-OH-dG; 5 ng/ml) solutions were used as standard samples. The value of 8-OH-dG was calculated as the number of residues per 10^5 dG.

Measurement of 8-OH-Gua Repair Activity

The endonuclease activities of the rat organs were assayed by a previously

described method (*11,12*) with some modifications. Briefly, 100 mg of each organ was homogenized in 300 µl of 50 mM Tris–HCl buffer (pH 7.5) containing protease inhibitors (5 µg/ml each of pepstatin, leupeptin, antipain, and chymostatin) with a potter-type homogenizer. Homogenates were centrifuged at $10,000 \times g$ to obtain the crude extracts; then the total protein concentrations were determined with a protein assay kit (Bio-Rad), using bovine serum albumin as the standard. The total protein concentration of the crude extracts was adjusted to 5 mg/ml. A 22 mer double stranded DNA containing 8-OH-Gua (5′-GGTGGCCTGACG*CATTCC-CCAA-3′; G*=8-OH-Gua), prepared by the method of Bodepudi et al. (*13*), was used as the substrate for this assay. The crude extract (50 µg protein) was incubated with 0.05 pmol of the [^{32}P]-end-labeled double-stranded DNA substrate at 25°C for 1.5 hr. After two ethanol precipitations, the pellet was dried, dissolved in 10 µl of loading buffer (80% formamide, 10 mM NaOH, 1 mM EDTA, 0.1% xylene cyanol, 0.1% bromophenol blue), and denatured by heating at 90°C for 3 min. Ten microliters of the sample was applied to a 20% denaturing polyacrylamide gel for electrophoresis. For comparison, a hot piperidine-treated oligonucleotide was also analyzed as a fragment marker. After electrophoresis, the autoradiograms were processed and the radioactivity was analyzed using a Bioimage analyzer system (Fujix BAS 2000). The repair activity was expressed as the ratio of the excised fragment activity to the total activity (substrate activity plus fragment activity).

Results and Discussion

The 8-OH-Gua levels in the DNA increased significantly at 6 hr ($p < 0.01$ vs control) after the administration of Fe-NTA
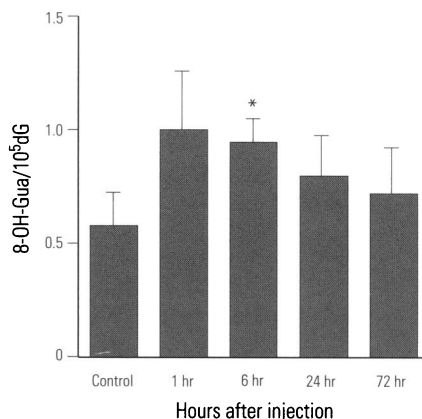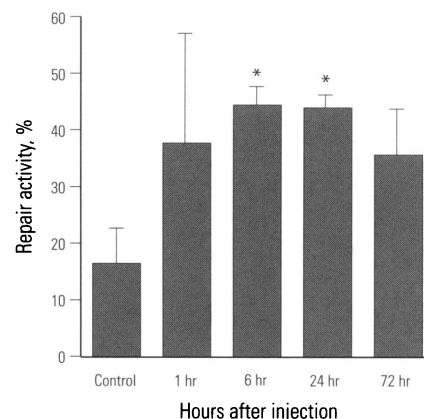

Figure 1. Change in the 8-hydroxyguanine levels in rat kidney DNA (15 mg Fe/kg bw, ip). Values are mean ±SD; $n=5$. *$p < 0.01$ vs control as evaluated by applying the Wilcoxon Rank Sum Test (using the exact table).

(15 mg Fe/kg bw, ip) (Figure 1), and their repair activities also increased significantly at 6 and 24 hrs ($p < 0.01$ vs control) (Figure 2). The 8-OH-Gua levels in the DNA increased to a maximum level more rapidly than the repair activities. Thereafter, both of the 8-OH-Gua levels in the DNA and their repair activities decreased.

It has been reported that oxidative stress plays an important role in Fe-NTA carcinogenesis. Umemura et. al. (*9*) reported that oxidative DNA damage, as measured by the 8-OH-Gua levels, increased in the rat kidney DNA after the administration of Fe-NTA. In their study, the 8-OH-Gua level in the DNA was 2 to 4/10⁵ Gua. Using the new DNA isolation method, we also observed an increase in 8-OH-Gua, but its level was 0.5 to 1/10⁵ Gua. Therefore, the background 8-OH-Gua level was reduced in our analysis.

In this study we found an increase in the 8-OH-Gua repair activity in the rat

Figure 2. Change in the 8-hydroxyguanine repair activities in rat kidneys (15 mg Fe/kg bw, ip). Values are mean ±SD; $n=5$. *$p < 0.01$ vs control as evaluated by applying the Wilcoxon Rank Sum Test (using the exact table).

kidney after Fe-NTA treatment. This is the first report on the induction of a repair activity for oxidative DNA damage after treatment with a chemical carcinogen. The 8-OH-Gua levels in the DNA increased rapidly to reach a maximum level at 1 hr after administration and then decreased. The 8-OH-Gua repair activities increased at 1 hr, and the higher levels were maintained for 72 hr. These results indicate that the oxidative DNA damage, the formation of 8-OH-Gua, rapidly induces its repair enzymes. In the liver (nontarget organ), neither the 8-OH-Gua levels in the DNA nor their repair activities were significantly changed (data not shown). Therefore, both the increase in 8-OH-Gua levels in the DNA and the increase in their repair activities reflected the organ-specific carcinogenic potency of Fe-NTA in rats. These assays will be useful for evaluating the carcinogenicity of oxygen radical-forming chemicals.

REFERENCES

1. Kasai H, Nishimura S. Formation of 8-hydroxyguanine by oxidative DNA damage, its repair and its mutagenic effects. In: Advances in Mutagenesis Research, Vol 4 (Obe G, ed). Berlin, Heidelberg: Springer-Verlag, 1993:31–45.
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 267:166–172 (1992).
3. Kasai H, Okada Y, Nishimura S, Rao MS, Reddy JK. Formation of 8-hydroxydeoxyguanosine in liver DNA of rats following long-term exposure to a peroxisome proliferator. Cancer Res 49:2603–2605 (1989).
4. Mauthe RJ, Cook VM, Coffing SL, Baird WM. Exposure of

mammalian cell cultures to benzo[*a*]pyrene and light results in oxidative DNA damage as measured by 8-hydroxydeoxyguanosine formation. Carcinogenesis 16:133–137 (1995).
5. Shen HM, Ong CN, Lee BL, Shi CY. Aflatoxin B₁-induced 8-hydroxydeoxyguanosine formation in rat hepatic DNA. Carcinogenesis 16:419–422 (1995).
6. Bases R, Franklin WA, Moy T, Mendez F. Enhanced excision repair activity in mammalian cells after ionizing radiation. Int J Radiat Biol 62:427–441 (1992).
7. Okada S, Hamazaki S, Ebina Y, Li JL, Midorikawa O. Nephrotoxicity and its prevention by vitamin E in ferric nitrilotriacetate-promoted lipid peroxidation. Biochim Biophys Acta 922:28–33 (1987).

8. Ebina Y, Okada S, Hamazaki S, Ogino F, Li JL, Midorikawa O. Nephrotoxicity and renal cell carcinoma after use of iron- and aluminum-nitrilotriacetate complexes in rats. J Natl Cancer Inst 76:107–113 (1986).

9. Umemura T, Sai K, Takagi A, Hasegawa R, Kurokawa Y. Formation of 8-hydroxydeoxyguanosine (8-OH-dG) in rat kidney DNA after intraperitoneal administration of ferric nitrilotriacetate (Fe-NTA). Carcinogenesis 11:345–347 (1990).

10. Nakae D, Mizumoto Y, Kobayashi E, Noguchi O, Konishi Y. Improved genomic/nuclear DNA extraction for 8-hydroxy-deoxyguanosine analysis of small amounts of rat liver tissue. Cancer Lett 97:233–239 (1995).

11. Chung MH, Kasai H, Jones DS, Inoue H, Ishikawa H, Ohtsuka E, Nishimura S. An endonuclease activity of *Escherichia coli* that specifically removes 8-hydroxyguanine residues from DNA. Mutat Res 254:1–12 (1991).

12. Yamamoto F, Kasai H, Bessho T, Chung MH, Inoue H, Ohtsuka E, Hori T, Nishimura S. Ubiquitous presence in mammalian cells of enzymatic activity specifically cleaving 8-hydroxyguanine-containing DNA. Jpn J Cancer Res 83:351–357 (1992).

13. Bodepudi V, Shibutani S, Johnson F. Synthesis of 2'-deoxy-7,8-dihydro-8-oxoguanosine and 2'-deoxy-7,8-dihydro-8-adenosine and their incorporation into oligomeric DNA. Chem Res Toxicol 5:608–617 (1992).