Detection and Quantification of 8-Hydroxydeoxyguanosine Adducts in Peripheral Blood of People Exposed to Ionizing Radiation

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Ionizing radiation produces a variety of damaging insults to nucleic acids, including the promutagenic lesion 8-hydroxydeoxyguanosine. In the present study, the 8-hydroxydeoxyguanosine content of peripheral blood leukocyte DNA isolated from individuals exposed to therapeutic doses of ionizing radiation was determined by a HPLC-coupled 32P-postlabeling assay. Peripheral blood leukocyte DNA from individuals irradiated with 180–200 cGy were observed to contain 2–4.5 times as much 8-hydroxydeoxyguanosine as that from unexposed individuals. These results were confirmed by the use of a HPLC-coupled electrochemical detection system. Thus, human exposure to ionizing radiation significantly increased the circulating leukocyte DNA content of 8-hydroxydeoxyguanosine.

Introduction

Ionizing radiation, such as that used in radiation therapy, causes a wide variety of DNA damage, ranging from single- and double-strand breaks to DNA-protein cross-links, 8-hydroxydeoxyguanosine (8-OHdG), thymidine glycol, and other oxidative and free-radical damage (1). Aside from single-strand breaks, 8-OHdG appears to be the major adduction product of radiation damage to cellular DNA (1,2). This lesion, 8-OHdG, is known to be removed from DNA by repair mechanisms (3,4), which is not surprising considering that it is a promutagenic lesion (5,6). Thus, the ability to monitor individuals for 8-OHdG levels would be valuable to the assessment of DNA damage caused by ionizing radiation and other oxidative and free-radical inducing agents.

Techniques have recently been developed by us and others (3,7–9) for the sensitive detection and quantitation of 8-OHdG residues in biological samples. This paper describes the quantitation of this oxidative DNA damage in circulating, nucleated blood cells in humans exposed to ionizing radiation by the use of a 32P-postlabeling assay.

Methods and Results

Venous blood samples (7–10 mL) were collected in EDTA-coated vacutubes from untreated individuals and patients who just received a therapeutic dose of ionizing radiation. The blood was drawn within 45 min (between 15 and 45 min) of completion of the radiation exposure, the buffy coat separated (between 15 min and 3 hr of blood collection), and the DNA isolated by standard phenol extraction methods (9). The phenol was freshly neutralized and buffered with 0.1 M Tris-HCl, pH 8.0, and 0.1% β-hydroxyquinoline was added before use.

Postlabeling methods for 8-OHdG adduct analysis of DNA were developed by modifying our previously published methods (9–11). Briefly, DNA samples (100 μg) were enzymatically digested to deoxynucleotide-3′-monophosphates (3′dNMP) as previously described (9–11), and then individually resolved by HPLC (Fig. 1). The appropriate HPLC elution fractions for 3′dGMP and 3′8-OHdMP were separately pooled and one-thousandth of the total unmodified 3′dGMP was added to the 3′-OHdGMP pooled fractions and lyophilized to dryness. The 3′-OHdGMP plus the 3′dGMP were dissolved in sterile H2O and 32P-postlabeled as previously described (9–11), except that apyrase was replaced by ATPase (0.01–0.02 U) in the presence of 0.1 mM ZnSO4 and 0.1 mM of each K+, Mg2+, and Cl−, and incubated at 37°C for 2 hr. The 32P was precipitated by the addition of cold (−4°C), concentrated formic acid and a freshly prepared solution of 400 mM Na tungstate/500 mM tetraethyammonium HCl/50 mM procaine and centrifuged for 10 min at 4°C. The supernatant was neutralized with the addition of 50% triethylamine/H2O, lyophilized, reconstituted in sterile...
FIGURE 1. HPLC elution profile of standard deoxynucleotide-5'-monophosphates including 8-hydroxydeoxyguanosine-5'-monophosphate, detected by absorbance at 254 nm. These nucleotides were resolved with a Waters C18 μBondapak 30 cm × 3.9 mm column coupled with a 4.6 mm × 10 mm Econosil 5 μm C18 guard column, isocratically eluted at 1 mL/min in 98% 0.1 M triethylamine (pH 7.0) and 2% acetonitrile. The deoxynucleotide-3'-monophosphates elute in the same respective times as the deoxynucleotide-5' monophosphate (9,10).

water, applied to prewashed PEI cellulose plates, and the nucleotides resolved by two-dimensional TLC (Fig. 2). The appropriate spots were scraped and the radioactivity determined by liquid scintillation counting. The level of 8-OHdG was observed to be greater in irradiated than in unexposed individuals (Table 1).

Verification of increased measurable levels of 8-OHdG in circulating blood cells of patients receiving radiation therapy was obtained by using an electrochemical detector (Coulouchem Model 5100A, ESA, Inc., Bedford, MA) coupled to the HPLC. The DNA was isolated from the separated buffy coat in a Model 340A Applied Biosystems DNA extractor programmed for the same basic protocol.

FIGURE 2. Autoradiogram of two-dimensional TLC separation of a 32P-postlabeled patient sample, following HPLC fractionation and mixing of 8-hydroxydeoxyguanosine-3'-monophosphate (3,8'-OHdGMP) plus one-thousandth of the HPLC 3'dGMP fraction. PEI plates (20 × 20 cm) were prewashed in MeOH and dried before spotting with 32P-labeled sample and standards (quantities of 5'dGMP and 5'8-OHdGMP that were sufficient to be detected by UV nm at 254 mm were also spotted on these TLC plates). The spotted plates were developed in 1 M ammonium isobutyrate, pH 7/10% isopropanol (D1) and washed in MeOH and dried before turning the plate 90 degrees and developing in 0.5 M LiCl/0.2 × SSC/5% butanol (D2). The plates were washed again in MeOH and dried before redeveloping in ammonium sulfate/ammonium bisulfate, pH 3.5/1 M ammonium formate/2% isopropanol, in the same direction as D2. The final plates were washed in MeOH, dried, and the 5'dGMP and 5'8-OHdGMP spots detected by UV light. Film was exposed for 30 min.
Table 1. 8-Hydroxydeoxyguanosine (8-OHdG) content of DNA from human peripheral blood.

| Donor | Cancer | Exposed field | Radiation dose, cGy | 8-OHdG/10^6 deoxyguanosine |
|-------|--------|---------------|---------------------|----------------------------|
| HPLC/32P-postlabeling/TLC | | | | |
| 1 | None | — | 0 | 8 + 1 (3)* |
| 2 | Lung | Chest/spine | 180 | 35 (2) |
| 3 | Prostate | Pelvis | 200 | 16 + 1 (3) |
| 4 | Prostate | Pelvis | 200 | 28 + 3 (3) |
| HPLC/electrochemical detection | | | | |
| 1 | None | — | 0 | 8 (2) |
| 5 | Breast | Chest/spine | 240 | 112 (2) |
| 6 | Prostate | Pelvis | 200 | 19 (2) |

*Values represent the average or mean + SD of (n) samples.

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REFERENCES

1. Ward, J. F. DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and repairability. Prog. Nucl. Acid Res. Mol. Biol. 35: 95–125 (1988).

2. Floyd, R. A. Role of oxygen free radicals in carcinogenesis and brain ischemia. FASEB J. 4: 2587–2597 (1990).

3. Kasai, H., Crain, P. F., 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).

4. Tchou, J., Kasai, H., Shibutani, S., Chung, M.-H., LaVal, J., Grollman, A. P., and Nishimura, S. 8-Oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. Proc. Natl. Acad. Sci. U.S.A. 88: 4690–4694 (1991).

5. Wood, M. L., Dizdaroglu, M., Gajewski, E., and Essigmann, J. M. Mechanistic studies of ionizing radiation and oxidative mutagenesis: genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. Biochemistry 29: 7024–7032 (1990).

6. Shibutani, S., Takeda, M., and Grollman, A. P. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxoG. Nature 349: 431–434 (1991).

7. Floyd, R. A., and Wong, P. K. Electrochemical detection of hydroxyl free radical adducts to deoxyguanosine. In: DNA Repair. A Laboratory Manual of Research Procedures, Vol. 3 (E. C. Friedberg and P. C. Hanawalt, Eds.), Marcel Dekker, Inc., New York, 1988, pp. 419–426.

8. Richter, C., Park, J. W., and Ames, B. N. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc. Natl. Acad. Sci. U.S.A. 85: 6465–6467 (1988).

9. Powe, A. C., Wilson, V. L., Taffe, B. G., Wood, M. L., Essigmann, J. M., and Harris, C. C. Detection and quantitation of 8-hydroxydeoxyguanosine residues by 32P-postlabeling. Proc. Am. Assoc. Cancer Res. 30: 201 (1989).

10. Wilson, V. L., Smith, R. A., Atrup, H., Krokkan, H., Musci, D. E., Le, N.-N.-T., Longoria, J., Ziska, D., and Harris, C. C. Genomic 5-methylcytosine determination by 32P-postlabeling analysis. Anal. Biochem. 152: 275–284 (1986).

11. Wilson, V. L., Busi, A. K., Essigmann, J. M., Smith, R. A., and Harris, C. C. O^5-methyldeoxyguanosine detection by 32P-postlabeling and nucleotide chromatography. Cancer Res. 48: 2156–2161 (1988).