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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 ^{32}P-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 ^{32}P-postlabeling assay.

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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 deoxynucteotide-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′8-OHdGMP pooled fractions and lyophilized to dryness. The 3′8-OHdGMP plus the 3′dGMP were dissolved in sterile H_2O and ^{32}P-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 $ZnSO_4$ and 0.1 mM of each K^+, Mg^+, and Cl^-, and incubated at 37°C for 2 hr. The $^{32}\hat{P}i$ was precipitated by the addition of cold (−4°C), concentrated formic acid and a freshly prepared solution of 400 mM Na tungstate/500 mM tetraethylammonium HCl/50 mM procaine and centrifuged for 10 min at 4°C. The supernatant was neutralized with the addition of 50% triethylamine/H_2O, lyophilized, reconstituted in sterile

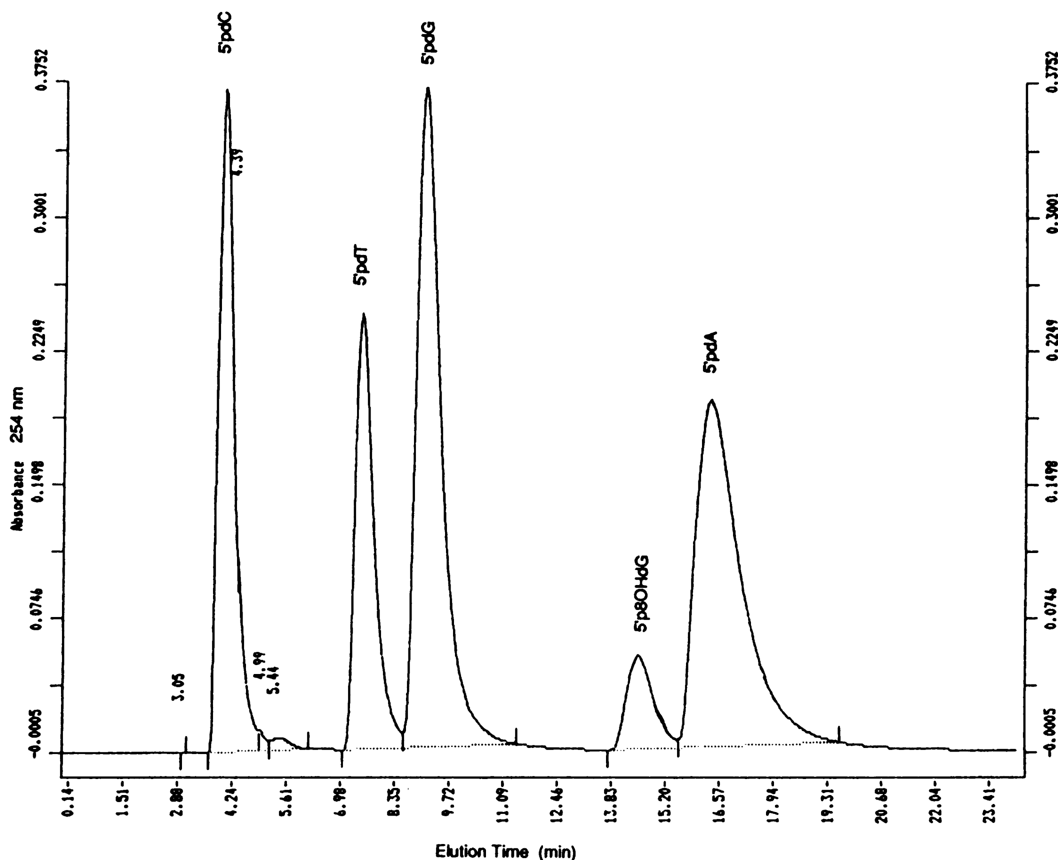
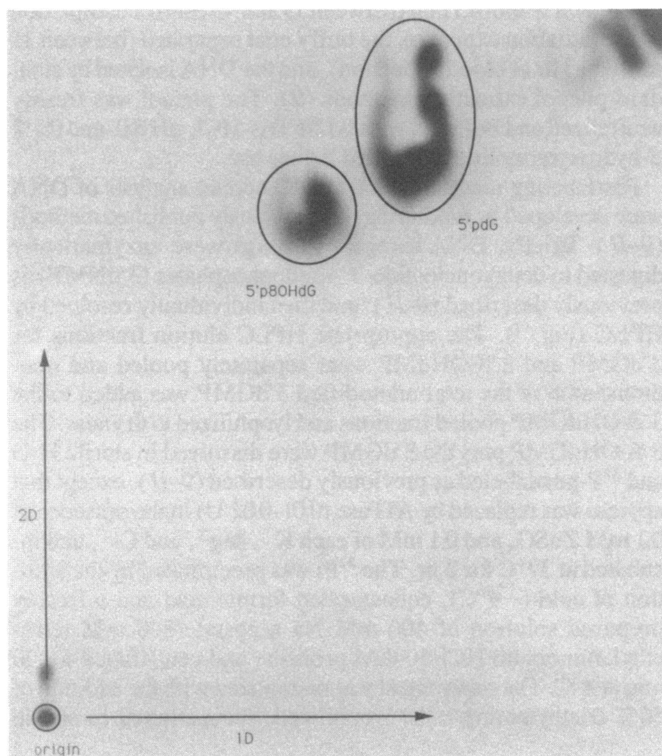


FIGURE 1. HPLC elution profile of standard deoxynucleotide-5'-monophosphates including 8-hydroxydeoxyguanosine-5'-monophosphate, detected by absorbance at 254 nm. These nucleotides were resolved with a Waters C18 μBondaPak 30 cm × 3.9 mm column coupled with a 4.6 mm × 10 mm Econosil 5 μm C18 guard column, 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 (Coulochem 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 ^{32}P-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 ^{32}P-labeled sample and standards (quantities of 5'dGMP and 5'8-OHdGMP that were sufficient to be detected by UV mm 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/10^5 deoxyguanosine
HPLC/^{32}P-postlabeling/TLC				
1	None	—	0	8 + 1 (3)[a]
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)

[a]Values represent the average or mean + SD of (n) samples.

described above, except that the procedure was performed under helium. Samples of DNA (100 μg) were enzymatically digested as described above. The digests were filtered through Millipore Ultrafree MC (10,000 molecular weight cutoff) filters and lyophilized to dryness. Each sample was dissolved in 30 μL of HPLC eluant and aliquots analyzed by HPLC with electrochemical detection. Samples were isocratically eluted with 20 mM sodium phosphate, pH 5.5 / 5% MeOH, at a flow rate of 1.0 mL/min. Again, increased levels of 8-OHdG were observed in irradiated patients (Table 1).

Discussion

The results indicate that people exposed to a therapeutic dose of ionizing radiation have higher levels of 8-OHdC in their circulating blood leukocyte DNA than nonirradiated individuals. HPLC/^{32}P-postlabeling and TLC analysis of human peripheral blood DNA samples from exposed individuals showed between 2 and 4.4 times the content of this oxidative damage found in control samples. Similar increases in 8-OHdG levels in irradiated patients were also observed using electrochemical detection techniques. However, the basal level of 8-OHdG in circulating nucleated blood cells was approximately 5 times higher than previously suggested (*3,7,8*). The protracted time frame of the DNA isolation, HPLC fractionation, postlabeling, and TLC procedures may have allowed excessive oxidation of the deoxyguanosine to have occurred, forming an abnormally high background.

Future studies of patients will require optimization of conditions, both in the choice of patient (field of exposure) and in the time frame of sample procurement and processing. First, the vascularity and the volume of blood irradiated varies with tissue and tumor, so that the actual dose of radiation that peripheral blood cells receive may vary from person to person. Second, the pharmacokinetics of 8-OHdG formation and removal in circulating nucleated cells in humans following exposure to ionizing radiation is not known. Thus, the optimal time of sampling from patients needs to be discerned. And last, the optimal conditions and time frame for processing blood samples needs to be determined to avoid spurious increases in *in vitro* oxidative production of 8-OHdG.

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