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Detection and Quantitation of Acrolein-Derived 1,N^2-Propanodeoxyguanosine Adducts in Human Lung by Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry

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Abstract

Acrolein, a widely distributed environmental pollutant, reacts with dGuo in DNA to form two pairs of 1,N^2-propano-dGuo adducts: (6*R*/*S*)-3-(2′-deoxyribos-1′-yl)-5,6,7,8-tetrahydro-6-hydroxypyrimido[1,2-*a*]purine-10(3*H*)one (α-OH-Acr-dGuo) and (8*R*/*S*)-3-(2′-deoxyribos-1′-yl)-5,6,7,8-tetrahydro-8-hydroxypyrimido[1,2-*a*]purine-10(3*H*)one (γ-OH-Acr-dGuo). α-OH-Acr-dGuo is the more mutagenic and induces mainly G→T transversions. A recent study demonstrated that acrolein DNA adducts are preferentially formed in *p53* mutational hotspots in human lung cancer, but there are no reports on the presence of these adducts in human lung. To directly investigate this question, we have developed a sensitive and specific liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) method for quantitative analysis of Acr-dGuo adducts in DNA. Our method is based on the enzymatic hydrolysis of DNA isolated from human lung in the presence of [$^{13}C_{10}$,$^{15}N_5$]Acr-dGuo as internal standards. Acr-dGuo adducts are enriched from the hydrolysates by solid phase extraction and analyzed by LC-ESI-MS/MS using selected reaction monitoring. The method is accurate and precise, and the identity of the adducts was confirmed by monitoring different transitions from the same parent ion, and by carrying out reactions with NaOH and $NaBH_4$, which produced N^2-(3-hydroxypropyl)dGuo or 1,N^2-(1,3-propano)dGuo from γ-OH-Acr-dGuo and α-OH-Acr-dGuo, respectively. Thirty DNA samples from lung tissue were analyzed and Acr-dGuo adducts were detected in all samples. Both α-OH- and γ-OH-Acr-dGuo were observed in most of the samples; total adduct concentrations ranged from 16 – 209 adducts/10^9 nucleotides. These results demonstrate for the first time that both types of Acr-dGuo adducts are present in human lung DNA. There was no difference in adduct levels between current and ex-smokers. Collectively, the results support a plausible role for acrolein as one cause of *p53* mutations in human lung.

Introduction

Acrolein, a highly reactive α,β-unsaturated aldehyde, is a widely distributed environmental pollutant (1) and is also formed endogenously through lipid peroxidation (2). Its concentration

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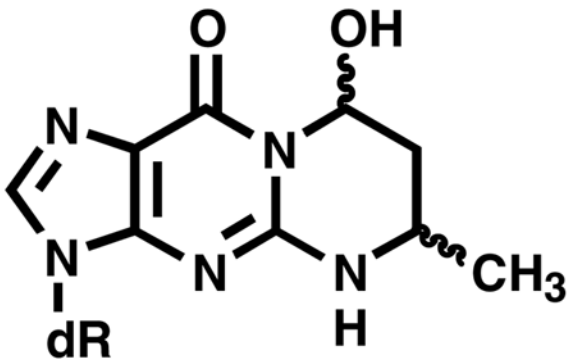
[1]Abbreviations: α-OH-Acr-dGuo, (6*R*/*S*)-3-(2′-deoxyribos-1′-yl)-5,6,7,8-tetrahydro-6-hydroxypyrimido[1,2-*a*]purine-10(3*H*)one; γ-OH-Acr-dGuo, (8*R*/*S*)-3-(2′-deoxyribos-1′-yl)-5,6,7,8-tetrahydro-8-hydroxypyrimido[1,2-*a*]purine-10(3*H*)one; Acr-dGuo, both α-OH-Acr-dGuo and γ-OH-Acr-dGuo; Cro-dGuo, (*6S, 8S*)- and (*6R, 8R*)-3-(2′-deoxyribos-1′-yl)-5,6,7,8-tetrahydro-8-hydroxy-6-methylpyrimido[1,2-*a*]purine-10(3*H*)one; LC-ESI-MS/MS, liquid chromatography-electrospray ionization-tandem mass spectrometry; LOQ, limit of quantitation; SPE, solid phase extraction; SRM, selected reaction monitoring.

in cigarette smoke is relatively high, about 18 - 98 μg per cigarette (3). It is mutagenic in bacteria (4–6) and in cultured human cells (7,8). However, it is generally considered non-carcinogenic, except that one study reported the induction of bladder tumors in rats treated with acrolein (9,10). The weak carcinogenicity of acrolein may be due to efficient detoxification by glutathione or other sulfhydryls. Nonetheless, acrolein is strongly suspected to be responsible for the induction of secondary bladder tumors in cyclophosphamide-treated patients (11).

Acrolein reacts readily with dGuo in DNA to form cyclic 1,N^2-propanodeoxyguanosine adducts (Acr-dGuo, Scheme 1). Depending on the direction of the initial Michael addition, two pairs of stereoisomers of (6*R*/*S*)-3-(2′-deoxyribos-1′-yl)-5,6,7,8-tetrahydro-6-hydroxypyrimido[1,2-*a*]purine-10(3*H*)one (α-OH-Acr-dGuo) and (8*R*/*S*)-3-(2′-deoxyribos-1′-yl)-5,6,7,8-tetrahydro-8-hydroxypyrimido[1,2-*a*]purine-10(3*H*)one (γ-OH-Acr-dGuo) are formed (12). α-OH-Acr-dGuo in particular is mutagenic in human cells and induces predominantly G→T transversions (8). Acr-dGuo adducts have been detected in various human tissues as well as tissues from untreated animals (13–16). Early studies used ^{32}P-postlabeling coupled with HPLC and detected γ-OH-Acr-dGuo as the major adduct *in vivo*. Its level was 3-fold higher in oral tissues of cigarette smokers compared with non-smokers (15). Although extremely sensitive, ^{32}P-postlabeling lacks internal standards for quantitation. A capillary liquid chromatography nanoelectrospray isotope dilution tandem MS method was recently developed for analysis of γ-OH-Acr-dGuo in DNA hydrolysates (16). Its levels were significantly higher in brain tissues from Alzheimer's disease subjects compared with age-matched controls.

A recent study demonstrated that acrolein DNA adducts are preferentially formed at *p*53 mutational hotspots in human lung cancer and inhibit DNA repair (17). This study challenged the hypothesis that the *p53* mutations are due to reactions with polycyclic aromatic hydrocarbon diol epoxides. These results raise the possibility that acrolein, which occurs in quantities up to 10,000 times as great as benzo[*a*]pyrene in cigarette smoke, may be a major etiological agent for cigarette smoking-related lung cancer. However, there are no reports in the literature on the presence of acrolein-DNA adducts in human lung tissue. In a previous study, we developed a liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) method to analyze the crotonaldehyde- and acetaldehyde-derived 1,N^2-propanodeoxyguanosine (Cro-dGuo), a structurally related adduct (18).



Cro-dGuo

dR = 2'-deoxyribosyl

In the present study, we investigated Acr-dGuo adducts in human lung DNA using a similar method. The results demonstrate their presence in relatively high quantities.

Experimental Section

HPLC-UV analysis

This was carried out using Waters Associates (Milford, MA) instruments equipped with a UV detector (Shimadzu Scientific Instruments, Columbia, MD) operated at 254 nm or a model 996 photodiode array detector. System 1 used a 4.6 mm × 25 cm 5 μm Luna C18 column (Phenomenex, Torrance, CA) with isocratic elution by 5% acetonitrile in H_2O at a flow rate of 0.7 mL/min. This system was used for the purification of Acr-dGuo and [$^{13}C_{10}$,$^{15}N_5$]Acr-dGuo. System 2 used the same column as system 1 with a gradient from 5 to 40% CH_3OH in H_2O over the course of 35 min at a flow rate of 0.7 mL/min. This system was used for the analysis of dGuo.

Chemicals and Enzymes

[$^{13}C_{10}$,$^{15}N_5$]dGuo was obtained from Spectra Stable Isotopes (Columbia, MD). Ethanol was obtained from AAPER Alcohol and Chemical Co. (Shelbyville, KY). 2-Propanol was purchased from Acros Organics (Morris Plains, NJ). Puregene DNA purification solutions were procured from Gentra Systems (Minneapolis, MN). Calf thymus DNA, micrococcal nuclease, and phosphodiesterase II were obtained from Worthington Biochemical Co. (Lakewood, NJ). Alkaline phosphatase was obtained from Roche Diagnostics Corporation (Indianapolis, IN). All other chemicals were purchased from Sigma-Aldrich.

Acr-dGuo and [$^{13}C_{10}$,15N_5]Acr-dGuo

Acr-dGuo standards were prepared as described (12) from the reaction of dGuo and acrolein. In brief, acrolein (56 mg, 1 mmol) was allowed to react with dGuo (25 mg, 0.09 mmol) in 10 mL of 0.1 M phosphate buffer (pH 7) at 37 ºC overnight. The products were purified by HPLC system 1 (15). α-OH-Acr-dGuo eluted earlier as two interchanging peaks of equal height, corresponding to the two diastereomers, while γ-OH-Acr-dGuo eluted as a single peak. The two peaks of α-OH-Acr-dGuo were collected together, and both α-OH- and γ-OH-Acr-dGuo were characterized and quantified by ^{1}H NMR, using toluene as an internal standard. The NMR spectra were consistent with published results (*11*, see Supporting Information). α-OH-Acr-dGuo: UV λ_{max} (ε) 259 nm (16800); positive ESI-MS *m/z* 324 $[M + H]^+$; MS/MS of *m/z* 324 (collision energy 30 eV): *m/z* (relative intensity) 208 $[BH]^+$ (100), 190 $[BH - H_2O]^+$ (53), 152 $[Gua + H]^+$. γ-OH-Acr-dGuo: UV λ_{max} (ε) 259 nm (18000); positive ESI-MS *m/z* 324$[M + H]^+$; MS/MS of *m/z* 324 (collision energy 30 eV): *m/z* (relative intensity) 208 $[BH]^+$ (48), 190 $[BH - H_2O]^+$ (29), 164 $[BH - CH_3CHO]^+$ (100), 152$[Gua + H]^+$(18), 135 $[Gua- NH_3 + H]^+$ (13). The approximate yields were 5% for each isomer. [$^{13}C_{10}$,$^{15}N_5$]Acr-dGuo was prepared the same way from [$^{13}C_{10}$,$^{15}N_5$]dGuo and quantified by UV at 254 nm. The amount of Acr-dGuo in [$^{13}C_{10}$,$^{15}N_5$]Acr-dGuo, as determined by LC-MS/MS, was less than 0.5%.

Human tissue samples

This study was approved by the University of Minnesota Research Subjects' Protection Programs Institutional Review Board Human Subjects Committee. Thirty lung samples were obtained from The Cancer Center Tissue Procurement Facility. The samples were histologically confirmed as normal tissue. They were obtained at surgery, immediately frozen in liquid N_2, and stored at -80 ºC until DNA isolation. Urine samples were also obtained from some subjects just prior to surgery. They were analyzed for nicotine and cotinine as described previously (19).

DNA Isolation

This was performed as previously described (20), following the "DNA Purification from 1 g Animal Tissue" protocol (Gentra Systems) with several modifications. Isolated DNA was stored at −20 ºC until sample preparation. For the artifact study, lung tissue samples were split into 2 portions. One was isolated as usual, and the other was homogenized in cell lysis solution containing 100 mM $NaBH_3CN$. For this portion of the sample, isopropanol, ethanol, and 70% ethanol all contained 100 mM $NaBH_3CN$.

Analysis of DNA for Acr-dGuo

For enzymatic hydrolysis, DNA (0.1 – 1.0 mg) was dissolved in 900 μL of 10 mM sodium succinate/5 mM $CaCl_2$ buffer (pH 7.0) to which 25 fmol of each isomer of [$^{13}C_{10}$,$^{15}N_5$]Acr-dGuo was added as internal standard. The mixture was heated at 100 ºC for 30 min and cooled to room temperature. Enzymatic hydrolysis was performed by incubation with 75 units of micrococcal nuclease (from *Staphylococcus aureus*) and 0.45 unit of phosphodiesterase II (from bovine spleen) at 37 ºC for 6 h. Then, 150 units of alkaline phosphatase (from calf intestine) were added, and the mixture was incubated at 37 ºC overnight. A 10 μL aliquot was removed for dGuo quantitation, and the remaining hydrolysate was purified using a solid phase extraction (SPE) cartridge [Strata-X, 33 μm, 30 mg/1 mL (Phenomenex)]. After the sample was applied, the cartridge was washed with 1 mL H_2O and 1 mL 5% CH_3OH/H_2O, and the analyte was eluted with 1 mL 70% CH_3OH/H_2O. The eluants were evaporated to dryness, and dissolved in 20 μL of H_2O for LC-ESI-MS/MS analysis. A buffer control which lacked DNA was prepared each time and processed in the same way to exclude any contamination; while a calf thymus DNA (0.5 mg) sample was prepared and served as a positive control.

LC-ESI-MS/MS analysis was carried out with an Agilent 1100 capillary flow HPLC (Agilent Technologies, Palo Alto, CA) equipped with a 100 mm × 0.5 mm 1.8 μm particle size C18 column (Agilent Zorbax SB-C18) and coupled to either a Finnigan Quantum Ultra AM or Discovery Max (ThermoElectron, San Jose, CA) triple quadrupole mass spectrometer. The solvent elution program was a gradient from 5 to 25% CH_3OH in 15 mM ammonium acetate buffer in 20 min at a flow rate of 10 μL/min at 50 ºC. The ESI source was set in the positive ion mode as follows: voltage, 3.7 kV; current, 3 μA; and heated ion transfer tube, 275 ºC. The adducts were analyzed by MS/MS using selected reaction monitoring (SRM). Ion transitions of *m/z* 324 → *m/z* 208 (Acr-dGuo) and *m/z* 339 → *m/z* 218 ([$^{13}C_{10}$,$^{15}N_5$]Acr-dGuo) with collision energy of 12 eV were used for quantitation and those of *m/z* 324 → *m/z* 164 and *m/z* 324 → *m/z* 190 (Acr-dGuo) and *m/z* 339 → *m/z* 174 and *m/z* 339 → *m/z* 200 ([$^{13}C_{10}$,$^{15}N_5$]Acr-dGuo) with collision energy of 32 eV were used for structural confirmation. Other MS parameters were optimized to achieve maximum signal intensity.

Calibration curves were constructed before each analysis using standard solutions of Acr-dGuo and [$^{13}C_{10}$,$^{15}N_5$]Acr-dGuo. A constant amount of [$^{13}C_{10}$,$^{15}N_5$]Acr-dGuo (10 fmol) was mixed with differing amounts of Acr-dGuo (0.5 – 100 fmol) and analyzed by LC-ESI-MS/MS-SRM. dGuo content was determined by HPLC system 2, and total nucleotides calculated from the amount of dGuo, considering that dGuo accounts for 19.9% of the nucleotides in human DNA (21). The adduct levels were expressed per 10^9 nucleotides.

Reaction of Acr-dGuo with NaOH and $NaBH_4$

The eluant from SPE containing the adducts was dissolved in 0.5 mL of 0.5 N NaOH containing an excess of $NaBH_4$. The resulting mixture was heated at 100 °C for 1 h, cooled to room temperature, and neutralized to pH 7 with 1 N HCl. The mixture was loaded on another Strata-X SPE cartridge and washed with 2 mL of H_2O to remove salts. The corresponding products were eluted by 1 mL 70% CH_3OH/H_2O and analyzed by LC-ESI-MS/MS, with the following ion transitions: *m/z* 326 → *m/z* 210 [N^2-(3-hydroxypropyl)-dGuo], *m/z* 341 → *m/z* 220

{[$^{13}C_{10}$,$^{15}N_5$]N^2-(3-hydroxypropyl)-dGuo}, *m/z* 308 → *m/z* 192 [1,N^2-(1,3-propano)-dGuo], and *m/z* 323 → *m/z* 202 {[$^{13}C_{10}$,$^{15}N_5$]1,N^2-(1,3-propano)-dGuo}.

Results

Standard characterization and calibration curves

Acr-dGuo standards were prepared as described (12), and characterized by NMR (see Supporting Information). They exist as two regioisomers, α-OH- and γ-OH-Acr-dGuo. The two diastereomers of α-OH-Acr-dGuo eluted earlier on HPLC as two interchanging peaks, while γ-OH-Acr-dGuo eluted as a single peak. The internal standard for our analysis was [$^{13}C_{10}$,$^{15}N_5$]Acr-dGuo, prepared by reacting acrolein with [$^{13}C_{10}$,$^{15}N_5$]dGuo, and characterized by UV and LC-ESI-MS, and comparison to Acr-dGuo. For LC-ESI-MS/MS-SRM analysis, the transitions monitored were *m/z* 324 → *m/z* 208 for Acr-dGuo and *m/z* 339 → *m/z* 218 for [$^{13}C_{10}$,$^{15}N_5$]Acr-dGuo. Calibration curves were plotted for the concentration ratios versus the integrated peak area ratios of analyte and internal standards. The two peaks corresponding to α-OH-Acr-dGuo were both integrated to reflect the total amount of this isomer, and linear responses were observed for both α-OH- and γ-OH-Acr-dGuo.

Method Development and Validation

The method was developed based on our previous study on the analysis of Cro-dGuo adducts (18), with some modifications. DNA was enzymatically hydrolyzed in the presence of [$^{13}C_{10}$,$^{15}N_5$]Acr-dGuo as internal standard, and the analytes were enriched from the hydrolysate by SPE. The enzymatic hydrolysis procedures were optimized to reduce background noise and signal suppression in the MS analysis. Our previous method used DNase I, phophodiesterase I, and alkaline phosphatase with incubation at 37 °C for 1 h. When calf thymus DNA was hydrolyzed in this way, significant signal suppression was observed for Acr-dGuo, which affected quantitation. This was probably due to the polarity of Acr-dGuo, which eluted close to unmodified dAdo. Our current method employed heating DNA to 100 °C for 30 min, followed by the addition of micrococcal nuclease, phosphodiesterase II, and alkaline phosphatase, and overnight incubation (22). Heat-denatured DNA was hydrolyzed much more rapidly by micrococcal nuclease (23,24). In addition, alkaline phosphatase contained a small amount of adenosine deaminase, which converted dAdo to dIno during the longer incubation. When heating was omitted, the measured level of Acr-dGuo was much lower than when heating was included (data not shown). Overall, this method gave more complete hydrolysis of DNA and removed dAdo which interfered with the Acr-dGuo analysis. This resulted in lower background and less signal suppression. Also, various HPLC conditions were investigated for better separation of the three isomeric peaks of Acr-dGuo. LC-ESI-MS/MS-SRM chromatograms obtained upon analysis of untreated calf thymus DNA are shown in Figure 1 (Panel A). All three product peaks were observed in the transition *m/z* 324 → *m/z* 208 and they coeluted with the internal standards peaks in the transition *m/z* 339 → *m/z* 218. No peaks were observed at this retention time in a buffer control which lacked DNA (data not shown). When the collision energy was increased to 32 eV, a peak at the retention time of γ-OH-Acr-dGuo was observed in the transition *m/z* 324 → *m/z* 164 [BH - CH_3CHO]$^+$, while all three peaks were observed in the transition of *m/z* 324 → *m/z* 190 [BH - H_2O]$^+$. Identical peaks were observed for the internal standards at the corresponding transitions. These results were fully consistent with the MS/MS analysis of standards. To further investigate peak identity, eluants from SPE were treated with NaOH and $NaBH_4$. Under these conditions, α-OH- and γ-OH-Acr-dGuo react differently (Scheme 2). γ-OH-Acr-dGuo undergoes base-catalyzed ring-opening followed by reduction of the intermediate aldehyde, producing N^2-(3-hydroxypropyl)-dGuo, while treatment of α-OH-Acr-dGuo with NaOH and $NaBH_4$ results in the elimination of H_2O followed by reduction, giving unsubstituted 1,N^2-(1,3-propano)-dGuo (12). The results of analysis of calf thymus DNA after the reaction with NaOH and $NaBH_4$ are shown in Figure 1

(Panel B). The peaks in the transition *m/z* 324 → *m/z* 208 disappeared. By comparing with standards that underwent the same procedures, the peak eluting at 14.1 min in the transition *m/z* 308 → *m/z* 192 was assigned as 1,N^2-(1,3-propano)-dGuo, which came from reduction of α-OH-Acr-dGuo, while the peak eluting at 12.3 min in the transition *m/z* 326 → *m/z* 210 was N^2-(3-hydroxypropyl)-dGuo, which came from reduction of γ-OH-Acr-dGuo. Both peaks coeluted with peaks in the transitions *m/z* 323 → *m/z* 202 and *m/z* 341 → *m/z* 220, from the internal standards. These results unambiguously demonstrate the identity of the adducts as those shown in Scheme 1.

Accuracy was determined by analyzing calf thymus DNA spiked with differing levels of Acr-dGuo standards. Each sample was analyzed in triplicate. The results are summarized in Figure 2, which shows good agreement between expected and measured values. Precision of the method was investigated by analyzing calf thymus DNA in six replicates on three separate days. The interday CVs were 5% for α-OH-Acr-dGuo and 7% for γ-OH-Acr-dGuo, as summarized in Table 1. The limit of quantitation (LOQ) for pure standard was 0.5 fmol injected on column (S/N = 10) as well as a linear MS response. LOQ in DNA samples was estimated as 4 adducts/10^9 nucleotides, starting from 0.5 mg of DNA. The recovery of 25 fmol of internal standard during sample processing was 88% and 84% for α-OH-Acr-dGuo and →-OH-Acr-dGuo, respectively. The stability of the analytes was investigated by analyzing 25 fmol of standards that were processed through the same enzymatic hydrolysis procedure, and no decomposition was observed.

It was possible that Acr-dGuo adducts could have been formed as artifacts from the reaction of intracellular acrolein with DNA during DNA isolation. In this case, acrolein could be pre-existing in the cell or produced from lipid peroxidation during DNA isolation. We tested this possibility by adding $NaBH_3CN$ to the DNA isolation solutions. $NaBH_3CN$ would reduce any acrolein released during DNA isolation. Three lung tissue samples were each divided into two portions and DNA was isolated using solutions containing or not containing $NaBH_3CN$. In other respects, the enzymatic hydrolysis, SPE, and LC-MS/MS analysis procedures were identical. The results indicated that levels of both α-OH- and γ-OH-Acr-dGuo were similar under these two conditions, which eliminated the possibility of artifactual formation of Acr-dGuo adducts.

Analysis of Human Lung DNA for Acr-dGuo

Lung tissue samples were obtained at surgery from 30 subjects (Table 2). Ten were male, 19 were female, and gender information was not available for one subject. Ages ranged from 37 – 81 years (mean ± S.D. 62.2 ± 12), and the age of one subject was unknown. All subjects were current or ex-smokers, based on self report. Urinary nicotine and cotinine levels were available for 14 subjects. These analyses established that 5 of the subjects – numbers 1, 2, 8, 9, 10 – were current smokers, and disagreed with self-report for 2 subjects – numbers 8 and 13 (although we cannot exclude the possibility that the subjects with positive urinary cotinine and nicotine may have been using nicotine replacement therapy). Acr-dGuo adducts were found in all samples. Figure 3 shows a representative chromatogram. Five DNA samples were also analyzed after reaction with NaOH and $NaBH_4$, which gave similar chromatograms to those shown in Figure 1, Panel B. Both α-OH- and γ-OH-Acr-dGuo were detected in all samples except one in which only γ-OH-Acr-dGuo was observed. Adduct levels ranged from ND – 154 adducts/10^9 nucleotides for α-OH-Acr-dGuo and 6.4 – 159 adducts/10^9 nucleotides for γ-OH-cr-dGuo, which were much higher than the Cro-dGuo levels we analyzed before (18). There was no difference in adduct levels between confirmed current smokers (N = 5) and non-smokers (N = 9), nor was there any relationship of adduct levels to self-reported time since cessation of smoking, gender, or age.

Discussion

The results of this study demonstrate for the first time that acrolein-DNA adducts, including the mutagenic adduct α-OH-Acr-dGuo, are present in human lung. This is significant in view of a recent study which showed that acrolein produces a spectrum of DNA damage in the *p53* gene that is remarkably similar to the spectrum of mutations found in this gene in lung tumors from smokers (17). The total levels of acrolein-DNA adducts quantified here, about 1 per 10^7 nucleotides, are higher than "PAH-DNA adducts" reported in lung tissue by ELISA, typically about 0.3 adducts per 10^7 nucleotides, and benzo[*a*]pyrene diol epoxide – DNA adducts, which are frequently undetectable and typically amount to 0.1 – 0.5 per 10^7 nucleotides when they are detected (25–27). Collectively, these results indicate that acrolein could contribute significantly to the mutations seen in the *p53* gene in lung cancer and challenge the widely held view that these mutations are due to polycyclic aromatic hydrocarbon diol epoxides (28). The weak or non-existent carcinogenicity of acrolein and the similarity in adduct levels between a limited number of confirmed current and ex-smokers on the other hand argue against a significant direct role for this compound in tobacco smoke - induced lung cancer, and further work is clearly needed to resolve these issues (29).

The structure of acrolein DNA adducts was first characterized in 1984 (12). Using ^{32}P-postlabeling/HPLC, Chung and co-workers have detected Acr-dGuo adducts in various human and untreated animal tissues (13–15), suggesting the existence of an endogenous source. γ-OH-Acr-dGuo was the major adduct detected, and the levels of α-OH-Acr-dGuo were too low to be quantified or detected in most tissues. ^{32}P-Postlabeling has certain limitations, such as the inability to provide unambiguous structural identification and the lack of internal standards for reliable quantitation. In the present study, we have developed a sensitive and specific LC-ESI-MS/MS method for the quantitative analysis of Acr-dGuo adducts. Compared with the method described by Liu et al (16), ours does not involve the setup of a nanospray source for the mass spectrometer, and uses commercially available capillary columns. Although the sensitivity is slightly lower than the previously published method, it is still sufficient to detect these adducts in human tissues.

The levels of Acr-dGuo in human lung were higher than the structurally related Cro-dGuo adducts which we have analyzed before (18). This is consistent with previous studies by Chung and co-workers, who also detected higher levels of Acr-dGuo than Cro-dGuo (13,14). Cigarette smoking is not the only source of these adducts. Acrolein from the environment and from endogenous formation through lipid peroxidation should also contribute. As reported by Pan and Chung (30), the rate of Acr-dGuo formation from polyunsaturated fatty acids under oxidative conditions was much higher than that of Cro-dGuo, consistent with our results.

We failed to observe a relationship between levels of Acr-dGuo adducts and urinary nicotine and cotinine, or time since cessation of smoking based on self-report. However, nicotine and cotinine data were available only for a small number of subjects, and self-report can be unreliable. Nothing is known about the kinetics of Acr-dGuo adduct removal after smoking cessation. Exposures to acrolein other than cigarette smoking as well as endogenous formation of acrolein from lipid peroxidation could contribute to adduct levels and will vary among people. Furthermore, individuals will have different abilities to detoxify acrolein and to repair Acr-dGuo adducts. Further research is needed to establish the relationship, if any, of Acr-dGuo adducts to cigarette smoking.

In summary, we have developed a sensitive and specific MS method for the quantitative analysis of Acr-dGuo adducts in human tissue DNA. Our results clearly demonstrate the presence of Acr-dGuo adducts in human lung DNA. Additional research is required to assess the contribution of acrolein to lung cancer caused by cigarette smoking.

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References

1. Izard C, Libermann C. Acrolein. Mutat Res 1978;47:115–138. [PubMed: 415230]
2. Chung FL, Chen HJC, Nath RG. Lipid peroxidation as a potential source for the formation of exocyclic DNA adducts. Carcinogenesis 1996;17:2105–2111. [PubMed: 8895475]
3. Roemer E, Stabbert R, Rustemeier K, Veltel DJ, Meisgen TJ, Reininghaus W, Carchman RA, Gaworski CL, Podraza KF. Chemical composition, cytotoxicity and mutagenicity of smoke from US commercial and reference cigarettes smoked under two sets of machine smoking conditions. Toxicology 2004;195:31–52. [PubMed: 14698566]
4. Lutz D, Eder E, Neudecker T, Henschler D. Structure-mutagenicity relationship in α,β-unsaturated carbonylic compounds and their corresponding allylic alcohols. Mutat Res 1982;93:305–315.
5. Eder E, Henschler D, Neudecker T. Mutagenic properties of allylic and α,β-unsaturated compounds: consideration of alkylating mechanisms. Xenobiotica 1982;12:831–848. [PubMed: 6763406]
6. Marnett LJ, Hurd HK, Hollstein MC, Levin DE, Esterbauer H, Ames BN. Naturally occurring carbonyl compounds are mutagens in salmonella tester strain TA104. Mutat Res 1985;148:25–34. [PubMed: 3881660]
7. Curren RD, Yang LL, Conklin PM, Grafstrom RC, Harris CC. Mutagenesis of xeroderma pigmentosum fibroblasts by acrolein. Mutat Res 1988;209:17–22. [PubMed: 3173398]
8. Yang IY, Chan G, Miller H, Huang Y, Torres MC, Johnson F, Moriya M. Mutagenesis by acrolein-derived propanodeoxyguanosine adducts in human cells. Biochemistry 2002;41:13826–13832. [PubMed: 12427046]
9. The Carcinogenic Potency Database (CPDB). 2007. http://potency.berkeley.edu
10. Cohen SM, Garland EM, John M, Okamura T, Smith RA. Acrolein intiates rat urinary bladder carcinogenesis. Cancer Res 1992;52:3577–3581. [PubMed: 1617627]
11. Gomes, R.; Meek, ME.; Eggleton, M. Concise International Chemical Assessment Document No. 43; World Health Organization, Geneva. 2002.
12. Chung FL, Young R, Hecht SS. Formation of cyclic 1, N^2-propanodeoxyguanosine adducts in DNA upon reaction with acrolein or crotonaldehyde. Cancer Res 1984;44:990–995. [PubMed: 6318992]
13. Nath RG, Chung FL. Detection of exocyclic 1, N^2-propanodeoxyguanosine adducts as common DNA lesions in rodents and humans. Proc Natl Acad Sci, USA 1994;91:7491–7495. [PubMed: 8052609]
14. Nath RG, Ocando JE, Chung FL. Detection of 1, N^2-propanodeoxyguanosine adducts as potential endogenous DNA lesions in rodent and human tissues. Cancer Res 1996;56:452–456. [PubMed: 8564951]
15. Nath RG, Ocando JE, Guttenplan JB, Chung FL. 1, N^2-Propanodeoxyguanosine adducts: Potential new biomarkers of smoking-induced DNA damage in human oral tissue. Cancer Res 1998;58:581–584. [PubMed: 9485001]
16. Liu X, Lovell MA, Lynn BC. Development of a method for quantification of acrolein-deoxyguanosine adducts in DNA using isotope dilution-capillary LC/MS/MS and its application to human brain tissue. Anal Chem 2005;77:5982–5989. [PubMed: 16159131]
17. Feng Z, Hu W, Hu Y, Tang MS. Acrolein is a major cigarette-related lung cancer agent: Preferential binding at p53 mutational hotspots and inhibition of DNA repair. Proc Natl Acad Sci U S A 2006;103:15404–15409. [PubMed: 17030796]
18. Zhang S, Villalta PW, Wang M, Hecht SS. Analysis of crotonaldehyde-and acetaldehyde-derived 1, N^2-propanodeoxyguanosine adducts in DNA from human tissues using liquid chromatography-electrsopray ionization-tandem mass spectrometry. Chem Res Toxicol 2006;19:1386–1392. [PubMed: 17040109]

19. Hecht SS, Carmella SG, Chen M, Dor Koch JF, Miller AT, Murphy SE, Jensen JA, Zimmerman CL, Hatsukami DK. Quantitation of urinary metabolites of a tobacco-specific lung carcinogen after smoking cessation. Cancer Res 1999;59:590–596. [PubMed: 9973205]
20. Wang M, Yu N, Chen L, Villalta PW, Hochalter JB, Hecht SS. Identification of an acetaldehyde adduct in human liver DNA and quantitation as N^2-ethyldeoxyguanosine. Chem Res Toxicol 2006;19:319–324. [PubMed: 16485909]
21. Snustad, DP.; Simmons, MJ.; Jenkins, JB. Principles of Genetics. John Wiley and Sons; New York: 1997. p. 189
22. Thomson NM, Mijal RS, Ziegel R, Fleischer NL, Pegg AE, Tretyakova NY, Peterson LA. Development of a quantitative liquid chromatography/electrospray mass spectrometric assay for a mutagenic tobacco specific nitrosamine-derived DNA adduct, O^6-[4-Oxo-4-(3-pyridyl)butyl]-2′-deoxyguanosine. Chem Res Toxicol 2004;17:1600–1606. [PubMed: 15606135]
23. von Hippel PH, Felsenfeld G. Micrococcal nuclease as a probe of DNA conformation. Biochemistry 1964;3:27–39. [PubMed: 14114500]
24. Cuatrecasas P, Fuchs S, Anfinsen CB. Catalytic properties and specificity of the extracellular nuclease of *Staphylococcus aureus*. J Biol Chem 1967;242:1541–1547. [PubMed: 4290246]
25. Boysen G, Hecht SS. Analysis of DNA and protein adducts of benzo[*a*]pyrene in human tissues using structure-specific methods. Mutation Res 2003;543:17–30. [PubMed: 12510015]
26. Kriek E, Rojas M, Alexandrov K, Bartsch H. Polycyclic aromatic hydrocarbon-DNA adducts in humans: relevance as biomarkers for exposure and cancer risk. Mutat Res 1998;400:215–231. [PubMed: 9685648]
27. Beland FA, Churchwell MI, Von Tungeln LS, Chen S, Fu PP, Culp SJ, Schoket B, Gyorffy E, Minarovits J, Poirier MC, Bowman ED, Weston A, Doerge DR. High-performance liquid chromatography electrospray ionization tandem mass spectrometry for the detection and quantitation of benzo[a]pyrene-DNA adducts. Chem Res Toxicol 2005;18:1306–1315. [PubMed: 16097804]
28. Pfeifer GP, Denissenko MF, Olivier M, Tretyakova N, Hecht SS, Hainaut P. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. Oncogene 2002;21:7435–7451. [PubMed: 12379884]
29. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 63. IARC; Lyon, FR: 1995. Acrolein; p. 337-372.
30. Pan J, Chung FL. Formation of cyclic deoxyguanosine adducts from omega-3 and omega-6 polyunsaturated fatty acids under oxidative conditions. Chem Res Toxicol 2002;15:367–372. [PubMed: 11896684]

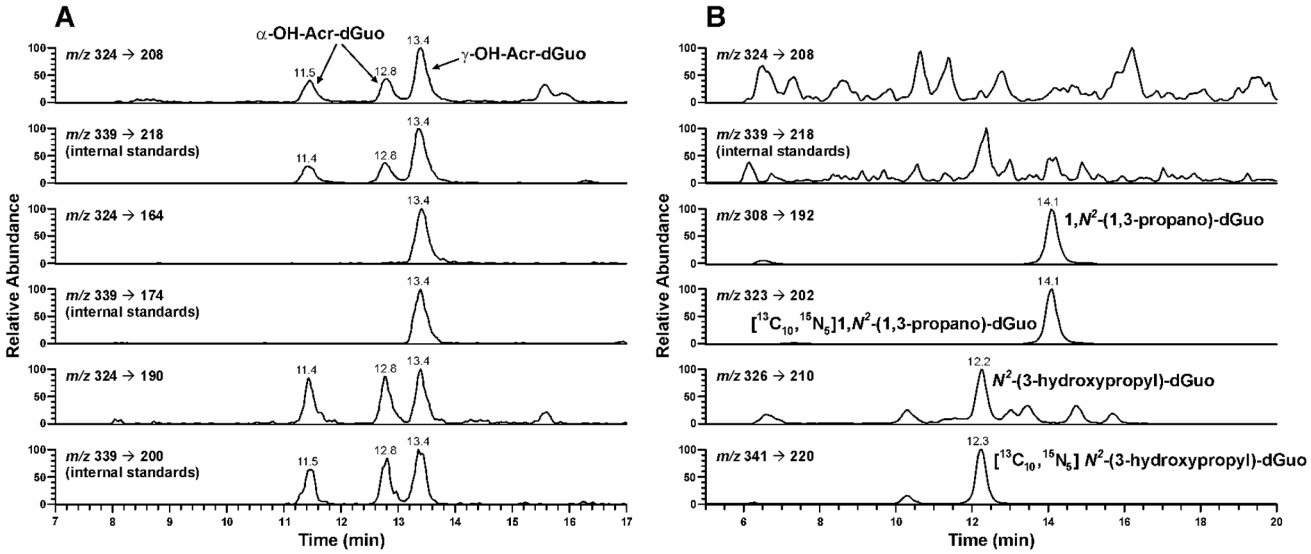


Figure 1.

Chromatograms obtained upon LC-ESI-MS/MS analysis of calf thymus DNA. Calf thymus DNA was enzymatically hydrolyzed, purified by SPE, and analyzed (panel A); or the eluants from SPE were treated with NaOH and $NaBH_4$ and analyzed (panel B).

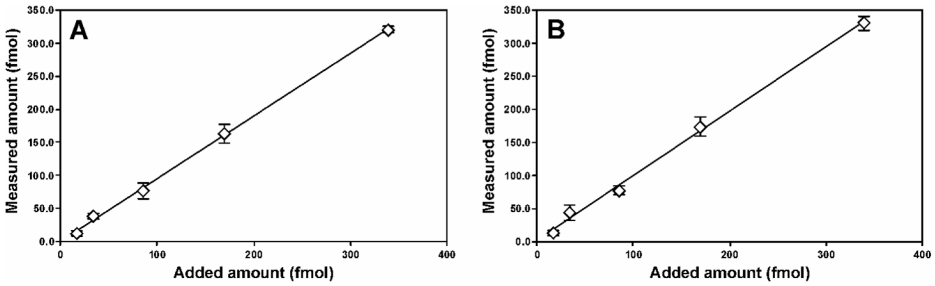


Figure 2.

Relationship of added to detected Acr-dGuo. Various amounts of α-OH- and γ-OH-Acr-dGuo standards (17, 34, 85, 170, and 340 fmol) were added to calf thymus DNA (0.5 mg) and analyzed by the method described in the text. Background levels in calf thymus DNA (40 adducts/10^9 nucleotides for α-OH-Acr-dGuo, and 30 adducts/10^9 nucleotides for γ-OH-Acr-dGuo) were subtracted from each amount detected. Each point represents a triplicate measurement. A, α-OH-Acr-dGuo, $R^2 = 1.0$; B, γ-OH-Acr-dGuo, $R^2 = 1.0$.

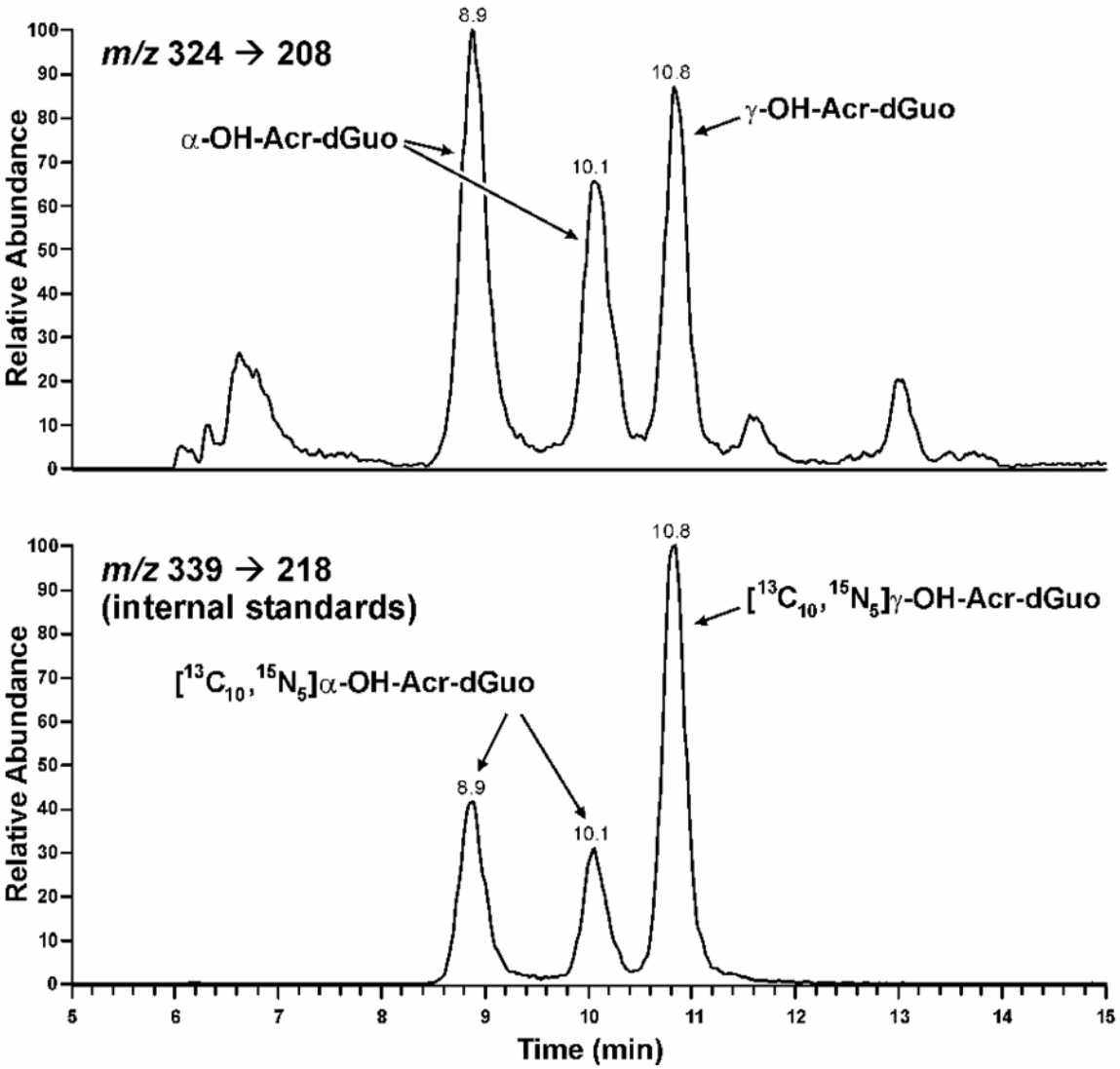
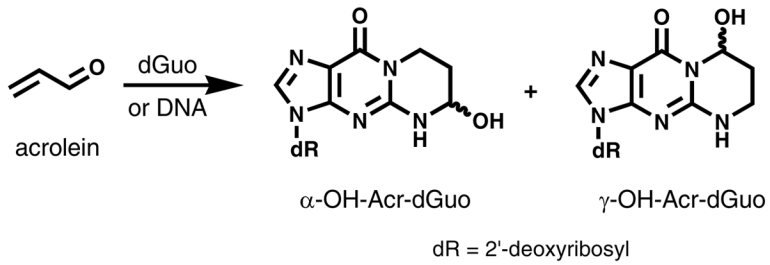
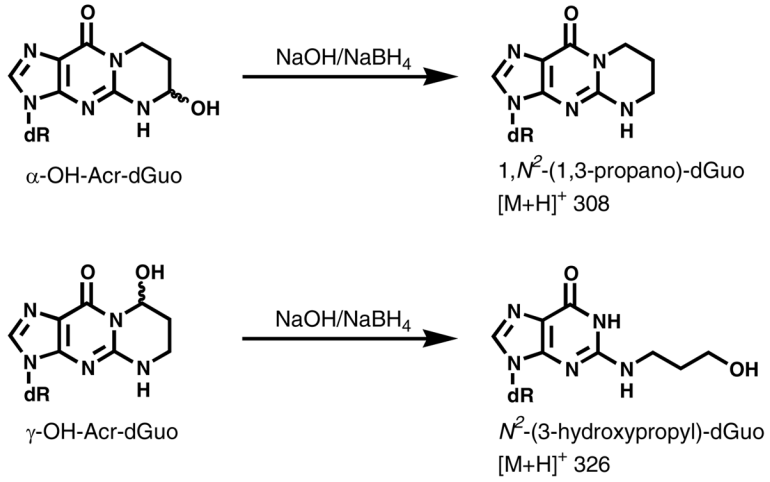


Figure 3.
Chromatograms obtained upon LC-ESI-MS/MS-SRM analysis of DNA from human lung.



Scheme 1.
Structures of Acr-dGuo adducts.



Scheme 2.
Reaction of α-OH- and γ-OH-Acr-dGuo with NaOH and $NaBH_4$.

Table 1

Precision of the LC-ESI-MS/MS method for analysis of Acr-dGuo[a].

	Acr-dGuo level (adducts/10^9 nucleotides)				
	Day 1 Mean ± SD	**Day 2 Mean ± SD**	**Day 3 Mean ± SD**	**Average**	**%RSD**
α-OH	40 ± 7	37 ± 6	43 ± 5	40 ± 3	7
γ-OH	30 ± 7	27 ± 3	29 ± 6	29 ± 1	5

[a]Six aliquots of calf thymus DNA were analyzed on 3 separate days.

Table 2

Levels of Acr-dGuo detected in human lung DNA.

subject	gender	race	age at surgery	self-reported smoking history[a]	urinary biomarkers		amount of DNA analyzed (mg)[b]	Acr-dGuo level[c] (adducts/10^9 nucleotides)		
					cotinine (ng/ml)	nicotine (ng/ml)		α-OH	γ-OH	total
1	F	W	60	current	652	115	0.14	91	47	138
2	F	W	47	current	5084	1462	0.51	45	21	66
3	F	W	52	2 yr	6.8	1.3	1.25	18	9	27
4	M	W	69	2 yr	25.2	4.5	1.09	18	10	27
5	F	W	72	10 yr	3.7	0.7	0.20	25	49	74
6	F	W	79	10 yr	9.2	1.3	0.10	78	49	127
7	M	W	66	26 yr	4.3	1.5	0.03	154	55	209
8	F	W	51	3 months	2397	534	0.70	10	6	16
9	M	W	53	current	2346	410	0.81	15	10	25
10	M	W	53	current	692	117	0.54	ND	159	159
11	F	W	61	9 yr	26.7	4.1	0.29	75	47	121
12	F	B	52	6 yr	34.1	16.4	0.55	25	12	36
13	F	W	37	current	3.3	1.7	0.61	16	9	25
14	M	W	78	24 yr	7.2	0.6	0.09	85	53	138
15	F	W	68	1 month	NA	NA	0.65	33	23	56
16	F	W	72	2 months	NA	NA	0.49	20	13	33
17	M	W	78	NA	NA	NA	0.77	12	18	29
18	F	W	60	2 yr	NA	NA	0.10	44	24	68
19	M	W	72	7 yr	NA	NA	0.86	10	6	16
20	F	W	46	1 week	NA	NA	0.72	10	9	19
21	F	W	81	23 yr	NA	NA	0.48	17	17	34
22	F	W	58	18 yr	NA	NA	1.08	13	13	26
23	F	W	76	10 yr	NA	NA	0.04	110	82	192
24	M	W	66	17 yr	NA	NA	0.64	16	14	29
25	F	W	51	1 yr	NA	NA	0.53	10	13	24
26	F	W	56	1 yr	NA	NA	0.33	35	21	56
27	M	W	74	16 yr	NA	NA	0.79	15	11	26
28	M	W	69	9 yr	NA	NA	0.84	20	22	42
29	F	W	49	2 months	NA	NA	0.98	17	12	29
30	NA[e]	NA	NA	NA	NA	NA	0.04	111	47	158
Mean ± S.D.			62.2 ± 12				0.54 ± 0.35	40 ± 38	29 ± 31	68 ± 58

[a] Reported time since quitting

[b] Calculated based on dGuo

[c] Each value represents a single measurement. dGuo was determined by HPLC-UV.

[d] ND, not detected

[e] NA not available