1,N2-Propanodeoxyguanosine Adducts: Potential New Biomarkers of Smoking-induced DNA Damage in Human Oral Tissue

Raghu G. Nath, Joseph E. Ocando, Joseph B. Guttenplan, and Fung-Lung Chung

American Health Foundation, Division of Carcinogenesis and Molecular Epidemiology, Valhalla, New York 10595. Phone: (914) 789-7161; Fax: (914) 592-6317; E-mail: ChungAHF.aol.com.

Abstract

Highly DNA-reactive α,β-unsaturated aldehydes such as acrolein and crotonaldehyde are common environmental pollutants present in cigarette smoke and automobile exhaust and are also released endogenously by lipid peroxidation. Acrolein- and crotonaldehyde-derived 1,N2-propanodeoxyguanosine (AdG and CdG, respectively) have been detected in the tissues of carcinogen-treated rodents and as background lesions in DNA from humans and untreated rodents. To determine whether cigarette smoking increases the levels of AdG and CdG, gingival tissue DNA from 11 smokers (4 males and 7 females; 30–58 years old) and 12 nonsmokers (8 males and 4 females; 21–66 years old) was analyzed using a previously described 32P-postlabeling high-performance liquid chromatography method. The results showed that the mean AdG levels in smokers were significantly higher than those in nonsmokers (1.36 ± 0.90 μmol/mol guanine in smokers versus 0.46 ± 0.26 μmol/mol guanine in nonsmokers; P = 0.003). The mean CdG 1 levels in smokers and nonsmokers were 0.53 ± 0.44 and 0.06 ± 0.07 μmol/mol guanine, respectively, corresponding to an 8.8-fold increase for smokers (P = 0.0015). Similar to CdG 1, levels of CdG 2 were increased 5.5-fold in smokers as compared to nonsmokers, from 0.31 ± 0.40 to 1.72 ± 1.26 μmol/mol guanine (P = 0.0014). Furthermore, the total levels of cyclic adduct (AdG and CdG) in smokers were 4.4-fold greater than those in nonsmokers (P = 0.0083). This study shows the detection of the potentially promutagenic 1,N2-propano guanine adducts in human oral tissues and demonstrates for the first time an increase of structurally identified adducts in oral tissue DNA by cigarette smoking.

Introduction

Cigarette smoking has been causally associated with cancers at several sites in humans, including the oral cavity (1, 2). The development of convenient biomarkers for DNA damage caused by cigarette smoking is important in identifying the segment of smokers who are at high risk of developing neoplastic malignancies. Although information about exposure to various tobacco carcinogens may be obtained from analysis of urinary metabolites and/or hemoglobin or protein adducts (3–6), specific genetic biomarkers are perhaps the most direct and relevant to cancer risks. These biomarkers could provide valuable quantitative information about structures and potential mutagenic characteristics of the persistent DNA damage resulting from exposure to carcinogens in cigarette smoke. Cigarette smoke contains a number of known carcinogens, including polycyclic hydrocarbons, nitrosamines, and heterocyclic amines (7). Whereas some studies showed an increase of the nonpolar polycyclic aromatic hydrocarbons-type adducts in DNA obtained from oral biopsy samples and the exfoliated mucosal cells of smokers (8, 9), others failed to show such an increase (10–12). There is, however, a general consensus that DNA isolated from various tissues of smokers, such as the lungs, contains higher levels of polycyclic aromatic hydrocarbons-like adducts as compared to nonsmokers (13–15), but the structures of these DNA modifications have not been identified.

In addition to these carcinogens, cigarette smoke also contains relatively high concentrations of reactive aldehydes. Among them are acrolein and crotonaldehyde, the two simplest α,β-unsaturated aldehydes or enals. Enals are known to modify DNA bases without covalent linking. Acrolein and crotonaldehyde-derived 1,N2-propanodeoxyguanosine adducts have been detected by immunoassay in Salmonella tester strains TA 100 and TA 104 on incubation with acrolein under conditions known to induce revertants (20). Moreover, acrolein may initiate bladder cancer in rats under certain conditions (21). AdG was also found in the lymphocytic DNA of a cyclophosphamide-treated dog (22). CdG adducts have been detected in the liver of rats treated with crotonaldehyde or N-nitrosopyrrolidine, a hepatocarcinogen that yields crotonaldehyde on metabolism (23). Crotonaldehyde has been shown to induce liver tumors in rats (24). Consistent with their potential in mutagenesis and carcinogenesis, site-specific mutagenesis studies have shown that cyclic 1,N2-propanoguanine adducts, if present in DNA, are likely to induce mutations (25, 26). Our studies have shown that diastereomers of AdG and CdG, shown in Fig. 1, are present in the tissues of humans and untreated rodents as background DNA lesions (27–29). Endogenous enals produced by lipid peroxidation are likely to contribute to their formation, which may perhaps constitute a more important source than environmental enals (28). Because these DNA lesions may play a role in cancer development, it is important to assess them as biomarkers for monitoring DNA damage in humans. To this end, in this study, we used a 32P-postlabeling-HPLC method developed previously for exocyclic adducts (27, 29) to analyze levels of AdG and CdG in the gingival DNA of smokers and nonsmokers.

Materials and Methods

Samples of oral tissue obtained from surgery at the periodontal clinic of New York University Dental Center were frozen at −80°C. In all cases, gingival tissues were used for this study, except for one case (see Table 1) in which buccal mucosa was used. Table 1 shows the age and sex of the patients and the self-reported number of cigarettes smoked. The tissues (50–300 mg each) from 12 nonsmokers and 11 smokers were thawed, washed with saline, minced, suspended in 2 ml of PBS, and incubated with 0.5 mg/ml collagenase (Sigma; Type IV) and 5 mM CaCl2 for 10 min at 37°C. Tissues were then washed twice with saline, and DNA was extracted using a procedure involving sequential phenol and chloroform/isooamyl alcohol extractions and treatment...
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Fig. 1. Structures of isomers of AdG and CdG detailed in vivo. AdG 1 and AdG 2 are not shown. The diastereomers of CdG 1 and CdG 2 are arbitrarily assigned.

Table 1 AdG and CdG levels in the gingival DNA of nonsmokers and smokers (nmol/mol guanine)

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Sex</th>
<th>Cigarettes/day</th>
<th>AdG 3</th>
<th>CdG 1</th>
<th>CdG 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsmokers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>45</td>
<td>M</td>
<td>0.424</td>
<td>0.036</td>
<td>0.038</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
<td>F</td>
<td>0.409</td>
<td>0.039</td>
<td>0.048</td>
</tr>
<tr>
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<td>M</td>
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<td>0.040</td>
<td>0.101</td>
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<tr>
<td>4</td>
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<td>M</td>
<td>0.335</td>
<td>0.033</td>
<td>0.119</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>F</td>
<td>0.409</td>
<td>0.104</td>
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<tr>
<td>6</td>
<td>21</td>
<td>F</td>
<td>0.747</td>
<td>0.108</td>
<td>0.351</td>
</tr>
<tr>
<td>7</td>
<td>38</td>
<td>M</td>
<td>0.321</td>
<td>0.030</td>
<td>0.071</td>
</tr>
<tr>
<td>8</td>
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<td>F</td>
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<td>0.020</td>
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<tr>
<td>9</td>
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<td>M</td>
<td>0.328</td>
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<td>10</td>
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<td>M</td>
<td>0.237</td>
<td>0.041</td>
<td>0.151</td>
</tr>
<tr>
<td>11</td>
<td>66</td>
<td>M</td>
<td>0.287</td>
<td>ND</td>
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<tr>
<td>12</td>
<td>31</td>
<td>M</td>
<td>1.167</td>
<td>0.262</td>
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</table>

Smokers

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Sex</th>
<th>Cigarettes/day</th>
<th>AdG 3</th>
<th>CdG 1</th>
<th>CdG 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1†</td>
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<td>M</td>
<td>0.644</td>
<td>0.242</td>
<td>0.591</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>F</td>
<td>0.977</td>
<td>0.331</td>
<td>1.024</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>F</td>
<td>1.243</td>
<td>0.633</td>
<td>1.444</td>
</tr>
<tr>
<td>4</td>
<td>43</td>
<td>F</td>
<td>1.022</td>
<td>0.319</td>
<td>0.749</td>
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<tr>
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<td>30</td>
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<td>1.906</td>
<td>1.692</td>
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<tr>
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<td>0.924</td>
<td>3.935</td>
</tr>
<tr>
<td>7</td>
<td>55</td>
<td>M</td>
<td>1.030</td>
<td>0.372</td>
<td>1.270</td>
</tr>
<tr>
<td>8</td>
<td>51</td>
<td>M</td>
<td>1.202</td>
<td>0.559</td>
<td>2.118</td>
</tr>
<tr>
<td>9</td>
<td>51</td>
<td>F</td>
<td>0.808</td>
<td>0.333</td>
<td>1.149</td>
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<tr>
<td>10</td>
<td>45</td>
<td>F</td>
<td>0.517</td>
<td>0.329</td>
<td>0.913</td>
</tr>
<tr>
<td>11</td>
<td>36</td>
<td>F</td>
<td>3.558</td>
<td>0.119</td>
<td>1.359</td>
</tr>
</tbody>
</table>

* Buccal mucosal DNA was used.
† NA, not available.
ND, not detected.

Results and Discussion

Fig. 2 shows typical HPLC chromatograms of comigration of the radioactive peaks obtained from a nonsmoker's and a smoker's DNA with the authentic UV standards of AdG 3, CdG 1, and CdG 2. These results showed, for the first time, the presence of these exocyclic adducts in human oral tissue DNA. Of the three isomers of AdG, AdG 3 was the predominant isomer detected in oral tissue DNA. This observation was consistent with previous results obtained from analysis of other human tissues (27, 29). Table 1 shows the levels of AdG 3, CdG 1, and CdG 2 of each subject from both smokers and nonsmokers. The significant variations for the background adduct levels in nonsmokers are likely caused by individual variability, although the intrinsic variability of the method may also contribute to the differences observed. Other reasons for the background variability could include factors known to influence lipid peroxidation, such as alcohol consumption, dietary fat, intake of antioxidant supplements, and nutritional factors. Unfortunately, lifestyle information other than smoking status was not available. In smokers, we did not see a positive correlation between the number of cigarettes smoked and the adduct levels. A number of reasons, such as the small sample size, inaccurate self-reported number of cigarettes smoked, and assay variability, could account for the lack of correlation. Of course, individual differences in detoxification and repair may be also important.

In Fig. 3, the mean levels of AdG 3, CdG 1, CdG 2, and total adducts (AdG and CdG) were compared between smokers and nonsmokers. The AdG level in smokers and nonsmokers was 1.36 ± 0.90 with ribonuclease A, T1, and protease (30). Typical DNA yields were ~1 μg/mg tissue, and the purity was checked by the ratios of absorbance at 260/280 (1.8–2.0). The procedures used for detection and quantification of AdG and CdG in DNA were described previously (27, 28). For purification and analysis by reverse-phase HPLC, C18 ODS-3 columns (4.5 × 250 mm; Phenomenex, Torrance, CA) were used. Briefly, 23–122 μg of DNA were enzymatically hydrolyzed and analyzed by HPLC, and the fractions with retention times corresponding to those of AdG- and CdG 3’-monophosphates were collected. Portions of DNA digest (equivalent to ~10 μg of DNA) from each sample were used to quantify the amount of DNA and to determine the efficiency of enzyme hydrolysis. AdG and CdG fractions were treated with nuclease P1 and 32P-postlabeled in the presence of T4 polynucleotide kinase. The labeled digests were purified first by one-dimensional TLC followed by sequential reverse-phase and ion-pair HPLC analysis. The purified adduct bisphosphates were finally analyzed by reverse-phase HPLC with detection by a radioflow detector, and adduct levels were quantified from radioactivity in the peaks by adjusting for the recovery of simultaneously labeled AdG and CdG as external standards. For confirmation of the identity of the adducts, portions of each purified AdG and CdG adduct bisphosphate from individual samples in each group were pooled into one sample and enzymatically converted to the corresponding 5’-monophosphates. The comigration of labeled adduct 5’-monophosphates obtained with the authentic UV standards was considered confirmation of identity of the in vivo adducts (27, 29).
and 0.46 ± 0.26 µmol/mol guanine, respectively, corresponding to a 3.0-fold increase in smokers \((P = 0.003)\). The CdG 1 level in smokers was 0.53 ± 0.44 µmol/mol guanine, equivalent to an 8.8-fold increase from 0.06 ± 0.07 µmol/mol guanine found in nonsmokers \((P = 0.0015)\). The increase in CdG 2 level from 0.31 ± 0.40 µmol/mol guanine in nonsmokers to 1.72 ± 1.26 µmol/mol guanine in smokers indicates a 5.5-fold increase \((P = 0.0014)\). When the total adduct levels were examined, smokers showed a 4.4-fold increase as compared with nonsmokers. These results showed that AdG and CdG levels in oral tissues were significantly elevated by smoking, and the increases in CdG levels seemed to be greater than those of AdG 3. Comparable increases in smokers’ adduct levels were also seen after the enzymatic conversion to the corresponding 5’-monophosphates in the confirmation experiments.

The background levels of AdG and CdG found in the gingival tissues of nonsmokers are believed to be mostly derived from the acrolein and crotonaldehyde released by endogenous oxidation of membrane fatty acids and, to a lesser extent, from environmental sources \((28)\). The increased AdG and CdG levels in smokers’ gingival DNA could result from direct exposure to acrolein and crotonaldehyde in the cigarette smoke and/or an increased production of endogenous enals via stimulation of lipid peroxidation by the oxidants in the cigarette smoke. The possibility that the increase is due to exposure to the aldehydes in smoke is supported by the fact that enals come in greater increase of CdG could be due in part to the presence of 1,3-butadiene \((3-220 \, \mu g/cigarette)\) in cigarette smoke. The possibility that the increase is due to exposure to the aldehydes in smoke is supported by the fact that enals come in greater increase of CdG could be due in part to the presence of 1,3-butadiene \((3-220 \, \mu g/cigarette)\) in cigarette smoke. The possibility that the increase is due to exposure to the aldehydes in smoke is supported by the fact that enals come in greater increase of CdG could be due in part to the presence of 1,3-butadiene \((3-220 \, \mu g/cigarette)\) in cigarette smoke. The possibility that the increase is due to exposure to the aldehydes in smoke is supported by the fact that enals come in greater increase of CdG could be due in part to the presence of 1,3-butadiene \((3-220 \, \mu g/cigarette)\) in cigarette smoke. The possibility that the increase is due to exposure to the aldehydes in smoke is supported by the fact that enals come in greater increase of CdG could be due in part to the presence of 1,3-butadiene \((3-220 \, \mu g/cigarette)\) in cigarette smoke. The possibility that the increase is due to exposure to the aldehydes in smoke is supported by the fact that enals come in greater increase of CdG could be due in part to the presence of 1,3-butadiene \((3-220 \, \mu g/cigarette)\) in cigarette smoke.

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References


cyclic acrolein deoxyguanosine adducts to assess their formation in DNA of *Salmo-
ella typhimurium* under conditions of mutation induction by acrolein. Carcinogen-
esis (Lond.), 10: 87-90, 1989.
22. Wilson, V. L., Foiles, P. G., Chung, F. L., Povey, A. C., Frank, A. A., and Harris,
C. C. Detection of acrolein and crotonaldehyde DNA adducts in cultured human cells
and canine peripheral blood lymphocytes by 32P-postlabeling and nucleotide chro-
23. Chung, F-L., Young, R., and Hecht, S. S. Detection of cyclic 1,N2-propanodeoxy-
guanosine adducts in DNA of rats treated with N-nitrosopyrrolidine and mice treated
24. Chung, F-L., Tanaka, T., and Hecht, S. S. Induction of liver tumors in F344 rats by
25. Hashim, M. F., and Mameli, L. J. Sequence-dependent induction of base-pair sub-
stitutions and frameshifts by propanodeoxyguanosine during *in vitro* DNA replica-
26. Mortya, M., Zhang, W., Johnson, F., and Grollman, A. P. Mutagenic potency of
exocyclic DNA adducts: marked differences between *Escherichia coli* and simian
27. Nath, R. G., and Chung, F-L. Detection of exocyclic 1,N2-propanodeoxyguanosine
adducts as common DNA lesions in rodents and humans. Proc. Natl. Acad. Sci. USA,
28. Chung, F-L., Chen, H-J. C., and Nath, R. G. Lipid peroxidation as a potential
endogenous source for the formation of exocyclic DNA adducts. Carcinogenesis
(Lond.), 17: 2105-2111, 1996.
29. Nath, R. G., Ocando, J. E., and Chung, F-L. Detection of 1,N2-propanodeoxy-
guanosine adducts as potential endogenous DNA lesions in rodent and human tissues.
30. Gupta, R. C. Nonrandom binding of the carcinogen N-hydroxy-2-acetylamino-
fluorene to repetitive sequences of rat liver DNA *in vivo*. Proc. Natl. Acad. Sci. USA,
1,3-butadiene and other selected gas-phase components in cigarette mainstream and
sidestream smoke by gas chromatography-mass selective detection. Carcinogenesis
33. Chung, F-L., Wang, M., and Hecht, S. S. Detection of exocyclic guanine adducts in
hydrolysates of hepatic DNA of rats treated with N-nitrosopyrrolidine and in calf
thymus DNA reacted with α-acetoxy-nitrosopyrrolidine. Cancer Res. 49: 2034-2041,
1989.
34. Sharer, J. E., Duescher, R. J., and Elfarra, A. A. Species and tissue differences in
the microsomal oxidation of 1,3-butadiene and the glutathione conjugation of butadiene
monoxide in mice and rats. Possible role in 1,3-butadiene-induced toxicity. Drug
35. Bridges, A. B., Scott, N. A., Parry, G. J., and Belch, J. J. Age, sex, cigarette
smoking and indices of free radical activity in healthy humans. Eur. J. Med., 2:
smoke causes biochemical changes in blood that are suggestive of oxidative stress:
of a type of oxidative DNA damage, 8-hydroxyguanine, and its repair activity in
detection of 8-hydroxydeoxyguanosine in aflatoxin B1-treated rat liver and human
39. Lane, J. D., Opara, E. C., Rose, J. E., and Behm, F. Quitting smoking raises whole
depletion on exocyclic adduct levels in the liver DNA of F344 rats. Chem. Res.
1,\textit{N}^2-\textit{Propanodeoxyguanosine Adducts: Potential New Biomarkers of Smoking-induced DNA Damage in Human Oral Tissue


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