Advances in Brief

1,N^2-Propanodeoxyguanosine Adducts: Potential New Biomarkers of Smoking-induced DNA Damage in Human Oral Tissue[1]

Raghu G. Nath, Joseph E. Ocando, Joseph B. Guttenplan, and Fung-Lung Chung[2]

American Health Foundation, Division of Carcinogenesis and Molecular Epidemiology, Valhalla, New York 10595 [R. G. N., J. E. O., F-L. C.], and Division of Basic Science, New York University, College of Dentistry and Department of Environmental Medicine, New York University Medical School, New York, New York 10010 [J. B. G.]

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,N^2-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 ^{32}P-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 ($P = 0.0003$). This study shows the detection of the potentially promutagenic 1,N^2-propanoguanine 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 qualitative and 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 polyaromatic 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 bioactivation by forming cyclic propano adducts (16, 17). Both acrolein and crotonaldehyde are found in cigarette smoke and automobile exhaust and are produced by burning fatty food (18, 19). AdG[3] 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,N^2-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 ^{32}P-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/isoamyl alcohol extractions and treatment

---

[2] To whom correspondence should be addressed, at American Health Foundation, Division of Carcinogenesis and Molecular Epidemiology, 1 Dana Road, Valhalla, NY 10595. Phone: (914) 789-7161; Fax: (914) 592-6317; E-mail: ChungAHF.aol.com.

[3] The abbreviations used are: AdG, acrolein-derived 1,N^2-propanodeoxyguanosine; CdG, crotonaldehyde-derived 1,N^2-propanodeoxyguanosine; GSH, reduced glutathione; HPLC, high-performance liquid chromatography.
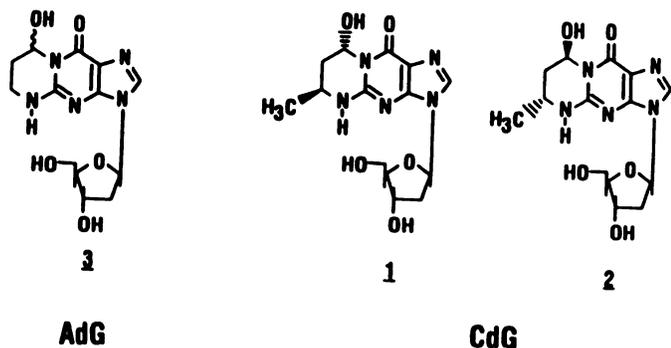
Fig. 1. Structures of isomers of AdG and CdG detailed *in vivo*. AdG 1 and AdG 2 are not shown. The diastereomers of CdG 1 and CdG 2 are arbitrarily assigned.

Table 1 *AdG and CdG levels in the gingival DNA of nonsmokers and smokers*

| | Age (yr) | Sex | Cigarettes/day | μmol/mol guanine | | |
				AdG 3	CdG 1	CdG 2
Nonsmokers						
1	45	M		0.424	0.026	0.038
2	43	F		0.409	0.039	0.048
3	41	M		0.404	0.040	0.101
4	33	M		0.335	0.033	0.119
5	27	F		0.409	0.104	0.216
6	21	F		0.747	0.108	0.351
7	38	M		0.321	0.030	0.071
8	47	F		0.410	0.020	0.177
9	65	M		0.328	0.031	0.210
10	45	M		0.237	0.041	0.151
11	66	M		0.287	ND[c]	1.337
12	31	M		1.167	0.262	0.891
Smokers						
1[a]	58	M	NA[b]	0.644	0.242	0.591
2	39	F	15	0.977	0.331	1.024
3	43	F	10–15	1.243	0.633	1.444
4	43	F	10	1.022	0.319	0.749
5	30	M	5–7	1.506	1.692	4.334
6	42	F	5–10	2.488	0.924	3.935
7	55	M	NA	1.030	0.372	1.270
8	51	M	20	1.202	0.559	2.118
9	51	F	10–12	0.808	0.333	1.149
10	45	F	10–15	0.517	0.329	0.913
11	36	F	12	3.558	0.119	1.359

[a] Buccal mucosal DNA was used.
[b] NA, not available.
[c] ND, not detected.

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 ^{32}P-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).

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
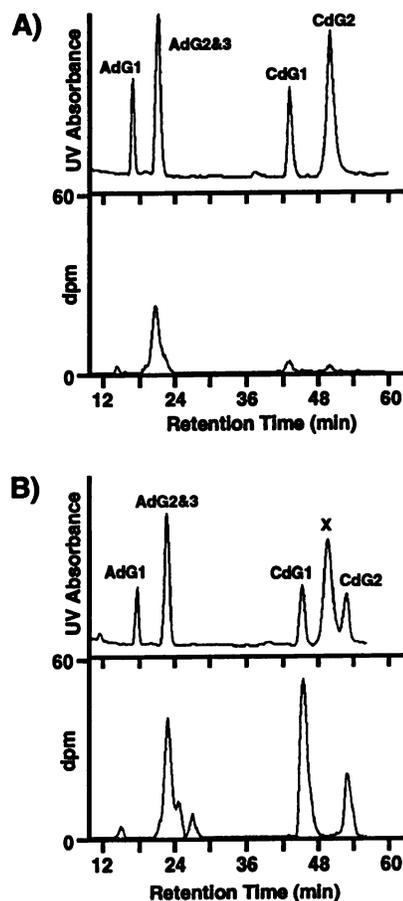

Fig. 2. Typical HPLC chromatograms showing the comigration of purified radioactive peaks obtained from (*A*) a nonsmoker's DNA (63 μg) and (*B*) a smoker's DNA (30.8 μg) with the synthetic UV standards. The UV peak marked *X* in *B* is an unidentified compound present in some samples.
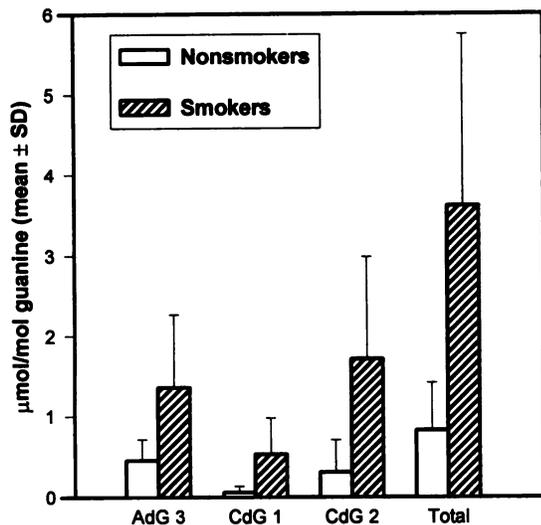
582

Fig. 3. Comparison of the mean levels of cyclic DNA adducts [AdG 3 ($P = 0.003$), CdG 1 ($P = 0.0015$), CdG 2 ($P = 0.0014$) and total adduct levels ($P = 0.0003$)] in oral tissue between smokers and nonsmokers.

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 direct contact with oral tissue during smoking, and they do not need bioactivation for the formation of AdG and CdG (16). However, this does not explain the greater increases in CdG than in AdG, because the concentration of acrolein (3–220 μg/cigarette) in cigarette smoke is comparable, if not higher, than that of crotonaldehyde (10–228 μg/cigarette; Refs. 18 and 19), and acrolein is more reactive toward DNA than crotonadehyde (16). It is tempting to speculate that the greater increase of CdG could be due in part to the presence of 1,3-butadiene (16–70 μg/cigarette) and N-nitrosopyrrolidine (30–390 μg/cigarette) in cigarette smoke (31, 32). Both are known to be metabolized to crotonaldehyde (33, 34). Although the metabolism of these compounds by oral tissue has not been reported, it is conceivable that crotonaldehyde as a metabolite could conjugate with GSH, which is then transported to the gingival tissue via circulation.

An alternate route of exposure to enals is through their generation by stimulated lipid peroxidation in smokers. The pro-oxidant state in smokers has been previously characterized by the higher plasma

levels of malondialdehyde, the lower GSH levels, and increased 8-hydroxydeoxyguanine levels in leukocyte, oral mucosa, and lung DNA (35–38). The plasma GSH levels return to normal on smoking cessation (39). The depletion of hepatic GSH by treatment with L-buthionine-(S,R)-sulfoximine caused a dramatic increase in the $1,N^2$-propanodeoxyguanosine adduct levels in rat liver DNA (40). Interestingly, in that study, the increases of CdG in the L-buthionine-(S,R)-sulfoximine-treated rats were much more pronounced than those in AdG, a result similar to that obtained from the present study in smokers. These observations seem to lend support to the possibility that endogenous pathways may play a major role in smoking-induced AdG and CdG levels. Regardless of the mechanism(s), this study is the first report of structurally identified DNA adducts in human oral tissue whose levels were significantly increased by smoking. In view of the promutagenic potential of these adducts, the detection of elevated levels of AdG and CdG in oral tissue DNA of smokers may provide a tool for monitoring the risk of smoking-induced oral cancer in humans.

Acknowledgments

References

1. Wynder, E. L., and Stellman, S. D. Comparative epidemiology of tobacco-related cancer. Cancer Res., 37: 4608–4622, 1977.
2. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 38: Tobacco Smoking, p. 421. Lyon, France: IARC, 1986.
3. Brunnemann, K. D., Prokopczyk, B., Djordjevic, M. V., and Hoffmann, D. Formation and analysis of tobacco-specific N-nitrosamines. Crit. Rev. Toxicol., 26: 121–137, 1996.
4. Hecht, S. S., Carmella, S. G., Murphy, S. E., Foiles, P. G., and Chung, F-L. Carcinogen biomarkers related to smoking and upper aerodigestive tract cancer. J. Cell. Biochem., 17 (Suppl.): 27–35, 1993.
5. Bryant, M. S., Skipper, P. I., Tannenbaum, S. R., and Maclure, M. Hemoglobin adducts of 4-aminobiphenyl in smokers and nonsmokers. Cancer Res., 47: 602–608, 1987.
6. Melikian, A. A., Prahalad, A. K., and Secker-Walker, R. H. Comparison of the levels of the urinary benzene metabolite trans, trans-muconic acid in smokers and non-smokers and the effect of pregnancy. Cancer Epidemiol. Biomark. Prev., 3: 239–244, 1994.
7. Hoffmann, D., and Wynder, E. L. Chemical constituents and bioactivity of tobacco smoke. IARC Sci. Publ. No. 74, pp. 145–165, 1986.
8. Zhang, Y-J., Hsu, T-M., and Santella, R. M. Immunoperoxidase detection of poly-cyclic aromatic hydrocarbon-DNA adducts in oral mucosa cells of smokers and nonsmokers. Cancer Epidemiol. Biomark. Prev., 4: 133–138, 1995.
9. Stone, J. G., Jones, N. J., McGregor, A. D., and Waters, R. Development of a human biomonitoring assay using buccal mucosa: comparison of smoking-related DNA adducts in mucosa versus biopsies. Cancer Res., 55: 1267–1270, 1995.
10. Chacko, M., and Gupta, R. C. Evaluation of DNA damage in the oral mucosa of tobacco users and non-users by ^{32}P-adduct assay. Carcinogenesis (Lond.), 9: 2309–2313, 1988.
11. Dunn, B. P., and Stich, H. F. ^{32}P-postlabelling analysis of aromatic DNA adducts in human oral mucosal cells. Carcinogenesis (Lond.), 7: 1115–1120, 1986.
12. Foiles, P. G., and Miglietta, L. M. Evaluation of ^{32}P-postlabeling analysis of DNA from exfoliated oral mucosa cells as a means of monitoring exposure of the oral cavity to genotoxic agents. Carcinogenesis (Lond.), 10: 1429–1434, 1989.
13. Randerath, E., and Randerath, K. Monitoring tobacco smoke-induced DNA damage by ^{32}P-postlabeling. IARC Scientific Publ. No. 124, pp. 305–314, 1993.
14. Phillips, D. H. DNA adducts in human tissues: biomarkers of exposure to carcinogens in tobacco smoke. Environ. Health Perspect., 104 (Suppl. 3): 453–458, 1996.
15. Santella, R. M., Grinsberg-Funes, R. A., Young, T. C., Drickey, C., Singh, V. H., Wang, L. W., and Perara, F. P. Cigarette smoking related to polycyclic aromatic hydrocarbons-DNA adducts in peripheral mononuclear cells. Carcinogenesis (Lond.), 13: 2041–2045, 1992.
16. Chung, F-L., Young, R., and Hecht, S. S. Formation of cyclic $1,N^2$-propanodeoxy-guanosine adducts in DNA upon reaction with acrolein or crotonaldehyde. Cancer Res., 44: 990–995, 1984.
17. Marnett, L. J. DNA adducts of α, β-unsaturated aldehydes and dicarbonyl compounds. IARC Sci. Publ. No. 125, pp. 151–156, 1994.
18. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Acrolein, pp. 337–372. Lyon, France: IARC, 1995.
19. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Crotonaldehyde, pp. 373–391. Lyon, France: IARC, 1995.
20. Foiles, P. G., Akerkar, S. A., and Chung, F-L. Application of an immunoassay for

583

cyclic acrolein deoxyguanosine adducts to assess their formation in DNA of *Salmonella typhimurium* under conditions of mutation induction by acrolein. Carcinogenesis (Lond.), *10:* 87–90, 1989.

21. Cohen, S. M., Garland, E. M., St. John, M., Okamura, T., and Smith, R. A. Acrolein initiates rat urinary bladder carcinogenesis. Cancer Res., *52:* 3577–3581, 1992.

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 ^{32}P-postlabeling and nucleotide chromatography. Carcinogenesis (Lond.), *12:* 1483–1490, 1991.

23. Chung, F-L., Young, R., and Hecht, S. S. Detection of cyclic $1,N^2$-propanodeoxyguanosine adducts in DNA of rats treated with N-nitrosopyrrolidine and mice treated with crotonaldehyde. Carcinogenesis (Lond.), *10:* 1291–1297, 1989.

24. Chung, F-L., Tanaka, T., and Hecht, S. S. Induction of liver tumors in F344 rats by crotonaldehyde. Cancer Res., *46:* 1285–1289, 1986.

25. Hashim, M. F., and Marnett, L. J. Sequence-dependent induction of base-pair substitutions and frameshifts by propanodeoxyguanosine during *in vitro* DNA replication. J. Biol. Chem., *271:* 9160–9165, 1996.

26. Moriya, M., Zhang, W., Johnson, F., and Grollman, A. P. Mutagenic potency of exocyclic DNA adducts: marked differences between *Escherichia coli* and simian kidney cells. Proc. Natl. Acad. Sci. USA, *91:* 11899–11903, 1994.

27. Nath, R. G., and Chung, F-L. Detection of exocyclic $1,N^2$-propanodeoxyguanosine adducts as common DNA lesions in rodents and humans. Proc. Natl. Acad. Sci. USA, *91:* 7491–7495, 1994.

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 $1,N^2$-propanodeoxyguanosine adducts as potential endogenous DNA lesions in rodent and human tissues. Cancer Res., *56:* 452–456, 1996.

30. Gupta, R. C. Nonrandom binding of the carcinogen N-hydroxy-2-acetylaminofluorene to repetitive sequences of rat liver DNA *in vivo*. Proc. Natl. Acad. Sci. USA, *81:* 6943–6947, 1984.

31. Brunnemann, K. D., Kagan, M. R., Cox, J. E., and Hoffmann, D. Analysis of 1,3-butadiene and other selected gas-phase components in cigarette mainstream and sidestream smoke by gas chromatography-mass selective detection. Carcinogenesis (Lond.), *11:* 1863–1868, 1990.

32. National Research Council. The Health Effects of Nitrate, Nitrite, and N-Nitroso Compounds, p. 529. Washington DC: National Academy Press, 1981.

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 Metab. Dispos., *20:* 658–664, 1992.

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:* 205–208, 1993.

36. Hulea, S. A., Olinescu, R., Nita, S., Crocnan, D., and Kummerow, F. A. Cigarette smoking causes biochemical changes in blood that are suggestive of oxidative stress: a case-control study. J. Environ. Pathol. Toxicol. Oncol., *14:* 173–180, 1995.

37. Asami, S., Hirano, T., Yamaguchi, R., Tomioka, Y., Itoh, H., and Kasai, H. Increase of a type of oxidative DNA damage, 8-hydroxyguanine, and its repair activity in human leukocytes by cigarette smoking. Cancer Res., *56:* 2546–2549, 1996.

38. Yarborough, A., Zhang, Y-J., Hsu, T-M., and Santella, R. M. Immunoperoxidase detection of 8-hydroxydeoxyguanosine in aflatoxin B_1-treated rat liver and human oral mucosal cells. Cancer Res. *56:* 683–688, 1996.

39. Lane, J. D., Opara, E. C., Rose, J. E., and Behm, F. Quitting smoking raises whole blood glutathione. Phys. Beh., *60:* 1379–1381, 1996.

40. Nath, R. G., Ocando, J. E., Richie, J. P., Jr., and Chung, F-L. Effect of glutathione depletion on exocyclic adduct levels in the liver DNA of F344 rats. Chem. Res. Toxicol., *10:* 1250–1253, 1997.

AACR American Association for Cancer Research

1,*N*²-Propanodeoxyguanosine Adducts: Potential New Biomarkers of Smoking-induced DNA Damage in Human Oral Tissue

Raghu G. Nath, Joseph E. Ocando, Joseph B. Guttenplan, et al.

Updated version	Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/58/4/581

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/58/4/581. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.