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Cigarette smoking induces an increase in oxidative DNA damage, 8-hydroxydeoxyguanosine, in a central site of the human lung

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To evaluate the effect of cigarette smoking on oxidative stress in lung tissues, we compared the levels of a type of oxidative DNA damage, 8-hydroxydeoxyguanosine (8-OH-dG), in tissue obtained from the central sites of lungs from 14 current smokers, seven ex-smokers and nine non-smokers. The mean level of 8-OH-dG in the lung tissues from smokers was 1.43-fold higher than that of the non-smokers (the difference was statistically significant, $P = 0.0262$). A positive correlation between the 8-OH-dG levels in normal lung tissues and the Brinkman index was obtained from smokers and ex-smokers ($r = 0.525$; $P = 0.0134$). A positive correlation was also obtained for the 8-OH-dG levels in normal lung tissues and the number of cigarettes smoked per day ($r = 0.436$; $P = 0.0132$). These results suggest that oxidative DNA damage is induced in lung DNA by cigarette smoking.

Introduction

Oxygen radicals are generated by environmental agents, such as ionizing radiation and chemical carcinogens, and also by endogenous processes. These oxygen radicals cause extensive damage to DNA, including single-strand breaks and the formation of modified bases and DNA–protein cross links (1–3). Among the various forms of oxidative DNA damage, 8-hydroxydeoxyguanosine (8-OH-dG*) (4) has been most extensively investigated, because it can be quantitated with high sensitivity by high performance liquid chromatography coupled with electrochemical detection (HPLC-ECD) (5,6). There is a growing volume of data that provides evidence that 8-OH-dG is a key biomarker relevant to carcinogenesis in both animal models and human studies (7–10).

Using the tobacco-attributed mortality rates in 1995, there will be ~3.4 million deaths from tobacco in 2025, as estimated from population growth (11). Epidemiological studies have established a causal relationship between cigarette smoking and various sites of human cancers (12), especially lung cancer (13). Cigarette smoke contains not only potent carcinogens, such as benzo[*a*]pyrene and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), but also a large amount of oxygen radical forming substances, such as catechol and hydroquinone. These substances may enhance lung carcinogenesis by free radical-mediated reactions, although the actual mechanism of tobacco-associated carcinogenesis in humans is not fully understood. It has been reported that a high intake of antioxidants contributes to a reduction of lung cancer risk in animals and humans (14,15). We have recently reported a positive correlation between the number of cigarettes smoked per day and the 8-OH-dG levels and its repair activities in human peripheral leukocytes (16). To understand the actual role of oxygen radicals in cigarette smoking-induced lung carcinogenesis, we investigated the 8-OH-dG levels in DNA from central sites of human lung tissues.

***Abbreviations:** 8-OH-dG, 8-hydroxydeoxyguanosine; ECD, electrochemical detection.

Materials and methods

Chemicals

A DNA Extractor WB Kit was purchased from Wako Biochemicals (Osaka). Nuclease P1 and acid phosphatase (type XA, P-1435) were from Sigma Chemicals Co. Other chemicals were of the highest purity commercially available. Milli-Q water was used for all experiments.

Tissue sampling

Samples of lung cancer tissue and normal lung tissue were obtained from 30 previously untreated lung cancer patients, by surgical lobectomy or pneumonectomy performed at Kyushu Kouseinenkin Hospital. Non-cancerous tissues were obtained from a central site of the lung that was 3–10 cm distant from the cancer tissue. These samples were ~8–10 mm in diameter and consisted of mixtures of both bronchial and alveolar tissues. Histopathological diagnosis was performed by hospital pathologists according to the WHO lung carcinoma classification (17). After surgical removal, all tissues were immediately frozen and stored at –70°C until the measurement of 8-OH-dG in the DNA. The average patient age was 67 years, and the population consisted of 19 males and 11 females. The types of cancer consisted of 10 squamous cell carcinomas and 20 adenocarcinomas. Based on the hospital case records, we grouped the individuals into three tobacco smoking categories: current smokers, ex-smokers (the time since smoking cessation ranged from 6 months to 20 years), and non-smokers (never smoked cigarettes regularly). A total of 90% (nine individuals) of the patients with squamous cell carcinoma were either current smokers or ex-smokers, and 40% (eight individuals) of the patients with adenocarcinoma were non-smokers.

Analysis of 8-OH-dG formation

The DNA was extracted from the nuclear fraction with the DNA Extractor WB Kit (Wako, Japan) (18). To the extracted nuclear DNA (~ 100 μg/100 ml of 0.1 mM EDTA), 1 μl of 2 M sodium acetate, 4 μl of nuclease P1 (5 mg/ml, Yamasa Co., Japan, YA7801) and 2 μl of acid phosphatase (47 mg/ml, suspension in 1.8M $(NH_4)_2SO_4$, Sigma, P-1435) were added and incubated at 37°C for 30 min. The 8-OH-dG content in the digested DNA was measured by an HPLC-ECD systems, as described previously (16).

Statistical analysis

The data are presented as means ± SD. Differences between groups were tested by the ANOVA factorial with Fisher's PLSD at a 5% significance level. The linear correlation of the paired data was calculated by means of Pearson's correlation coefficient *r*. All analyses were carried out using the Stat View 4.5 program (Berkeley, CA).

Results

Table I shows the amount of 8-OH-dG in lung cancer tissue and non-cancerous lung tissue, age, sex, smoking status, tumor type and Brinkman index. The mean 8-OH-dG levels in non-cancerous lung tissues in smokers, ex-smokers and non-smokers were $0.74 \pm 0.21/10^5$ dG, $0.60 \pm 0.21/10^5$ dG and $0.52 \pm 0.25/10^5$ dG respectively (Figure 1). The mean level of 8-OH-dG in smokers was 1.43-fold higher than

Table I. Characteristics of patients

Case no.	Age (yr)	Sex	Tumor type	Level of 8-OH-dG[a]		Smoking status	Brinkman index[b]
				CA	Non-CA		
1	76	M	SQ	0.51	0.69	C	1200
2	72	M	SQ	0.60	0.86	EX	1200
3	82	M	AD	0.44	0.73	C	900
4	71	M	SQ	0.65	0.81	C	2000
5	75	F	AD	0.87	0.81	N	–
6	75	F	AD	0.74	0.93	N	–
7	67	M	SQ	0.30	0.56	C	940
8	67	M	AD	0.50	0.59	C	940
9	80	F	SQ	0.54	0.50	EX	400
10	68	F	SQ	0.43	0.99	C	1400
11	44	M	AD	0.54	0.50	C	520
12	84	M	AD	0.25	0.89	EX	1000
13	67	F	AD	0.41	0.39	EX	60
14	59	F	AD	0.92	0.62	N	–
15	66	F	AD	0.70	0.31	N	–
16	49	M	AD	0.45	0.72	C	900
17	81	M	SQ	0.51	0.62	EX	1040
18	71	F	AD	0.20	0.15	N	–
19	30	F	AD	0.45	0.46	N	–
20	72	M	AD	0.90	0.56	EX	1060
21	45	M	AD	0.38	0.52	N	–
22	74	M	SQ	0.99	0.71	C	1250
23	76	F	AD	0.21	0.27	N	–
24	65	M	AD	0.47	1.10	C	900
25	51	M	AD	0.60	0.40	C	825
26	73	M	AD	0.30	0.35	EX	450
27	75	M	AD	0.60	1.09	C	800
28	76	M	AD	1.57	0.71	C	1000
29	52	F	SQ	0.30	0.58	N	–
30	79	M	SQ	0.90	0.78	C	1400

Abbreviations: M, male; F, female; SQ, squamous cell carcinoma; AD, adenocarcinoma; CA, lung cancer tissue; non-CA, non-cancerous lung tissue; C, current smoker; EX, ex-smoker; N, non-smoker.
[a]Number of 8-OH-dG residues/10^5 dG. [b]Cigarettes/day×years.

that of the non-smokers (the difference was statistically significant, $P = 0.0262$). A positive correlation between the 8-OH-dG levels in non-cancerous lung tissues and the Brinkman index was obtained in smokers and ex-smokers ($r = 0.525$; $P = 0.0134$; Figure 2). A positive correlation was also observed between the 8-OH-dG levels and the number of cigarettes smoked per day ($r = 0.444$; $P = 0.0132$; Figure 3). Non-smokers showed a 6.2-fold interindividual variation in the lung tissue 8-OH-dG levels. The amount of 8-OH-dG in the cancer tissue was compared between squamous cell carcinoma, which is the prevailing type of smoking-related lung cancer, and adenocarcinoma, but no difference in the 8-OH-dG level was observed between these tumor types. The mean level of 8-OH-dG in the non-cancerous lung tissue from the patients with squamous cell carcinoma was also not significantly different from that of the patients with adenocarcinoma. The mean level of 8-OH-dG in the lung cancer tissues was slightly lower than that of the non-cancerous lung tissues, and there was no correlation between the 8-OH-dG levels in the lung cancer tissues and the smoking habits. There was also no difference between males and females in the 8-OH-dG levels in the lung cancer tissues.

Discussion

The mean level of 8-OH-dG in the lung tissues from smokers was significantly higher than that of the non-smokers. We found a positive correlation between the 8-OH-dG levels in the lung tissues and the Brinkman index or the number of cigarettes smoked per day.

The higher levels of oxidative DNA damage in the lung tissues from smokers and ex-smokers support the hypothesis that oxygen radicals are persistently produced in lung tissues because of cigarette smoking. Our data, which show a positive correlation between daily cigarette consumption or long-term use of cigarettes and levels of 8-OH-dG, support the conclusions of epidemiological studies that cigarette smoking may be one of the causes of lung cancer.

Various studies have already established that carcinogen–DNA adduct levels in human lung tissues are increased in smokers (19,20). Some investigators have described differences in lung cancer susceptibility, even in individuals with similar smoking status histories. Several studies have also indicated that individual variations in gene polymorphisms, for example, in cytochrome P4501A1 (CYP1A1) and glutathione *S*-transferase class mu (GSTM1), were partially responsible for susceptibility to smoking-related lung cancer (21,22). In the present study, there were interindividual variations in the 8-OH-dG levels in all smoking habit groups. We have previously reported a positive association between cigarette smoking and oxidative DNA damage in human leukocytes and also interindividual variations in the 8-OH-dG levels and repair activities (16). Further studies of the effects of patients' occupations, life-styles, living

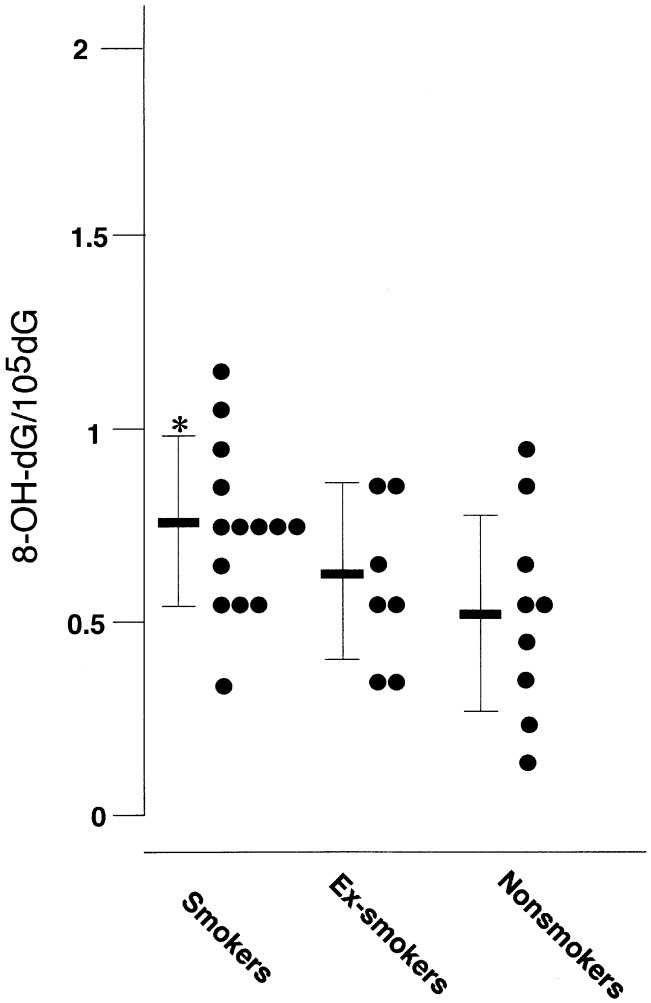

Fig. 1. Distribution of 8-OH-dG level in non-cancerous lung tissues of smokers, ex-smokers and non-smokers. DNA was obtained from a central site of the lung, which was distant from the cancer tissue. Each data point represents one individual. Bars represent means ± SD. Differences between groups were tested by the ANOVA factorial with Fisher's PLSD at the 5% significance level. $*P = 0.0262$ for difference from the non-smokers.

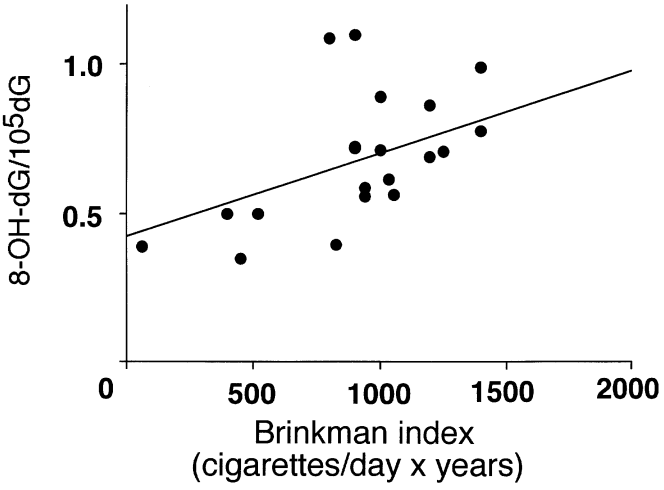

Fig. 2. Linear regression of the Brinkman index and the 8-OH-dG level in non-cancerous tissue obtained from a central site of the lung. Non-smokers were excluded from this analysis. A positive correlation between the Brinkman index and the 8-OH-dG level was obtained. Linear correlation coefficient, $r = 0.525$; $P < 0.05$.

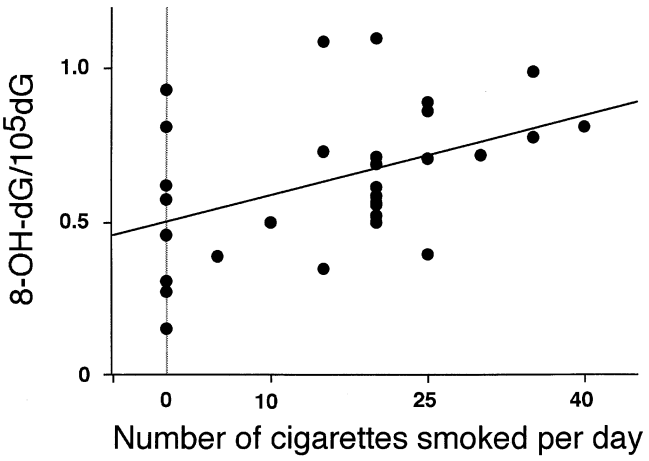

Fig. 3. Linear regression of the number of cigarettes smoked per day and the 8-OH-dG level in non-cancerous tissue obtained from a central site of the lung. A positive correlation between the number of cigarettes smoked per day and the 8-OH-dG level was obtained. Linear correlation coefficient, $r = 0.444$; $P < 0.05$.

environments and genetic differences on the 8-OH-dG levels are required to understand the actual role of oxygen radicals in human lung carcinogenesis.

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