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Advances in Brief

Different Susceptibility to Smoking-induced DNA Damage among Male and Female Lung Cancer Patients[1]

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Abstract

The levels of aromatic/hydrophobic DNA adducts were analyzed in normal lung tissue from 63 lung cancer patients and examined in relation to exposure and genetic factors. Adduct levels were significantly higher in smokers than in nonsmokers, but among smokers the number of cigarettes smoked per day had only low significance for the variation in adduct levels. An inverse correlation was found between years of smoking and DNA adduct levels ($r = 0.52$, $P = 0.001$). Thus, patients with high adduct levels generally had shorter duration of smoking and/or lower smoking dose before the clinical onset of the disease, which fits expected behavior of cancer susceptible individuals. The data indicated an excess of individuals with glutathione *S*-transferase M1 deficiency among male patients with high adduct levels. Among females the DNA adduct levels were higher than in males when adjusted for smoking dose. There was a highly significant difference in the distribution of males and females when smokers were divided into quartile groups according to adducts per pack year (trend test: 2-sided $P = 0.005$). This may indicate that women are at greater risk of tobacco-induced lung cancer.

Introduction

Several studies have indicated that individuals may differ in their susceptibility to carcinogenic compounds in the tobacco smoke (1, 2), and roughly only 1 of 10 lifetime smokers contracts lung cancer. A further characterization of biochemical/genetic features of these patients is important in order to understand disease development, particularly at low dose exposure. Large interindividual variations have been found in the activity of a number of enzymes involved in the biotransformation of carcinogens, including AHH[3] (3) and GSTs (4). It is therefore suggested that the ability to activate/detoxify carcinogens may modulate an individual's risk for lung cancer (1, 2). Similar importance has been attributed to the enzymes involved in the repair of damaged DNA (5).

Tobacco smoke contains at least 40 known carcinogens, many of which exert their biological effects after biotransformation and through the formation of DNA adducts in target tissues (6). Some studies have shown a correlation between tobacco smoke exposure and DNA adduct levels in the respiratory tract of lung cancer patients (7–9). However, the individual variation in adduct levels may be large even at similar exposure doses, since this level also is affected by the metabolism of mutagens as well as the repair of damaged DNA. Animal studies have indicated that there is no simple relation between adduct level and cancer risk; however, for a particular carcinogen, high levels of stable adducts in the target tissue appear to be related to the probability that tumors will occur (10). In the light of the high complexity of carcinogenic compounds in tobacco smoke and their metabolism in the body, the DNA adduct level may be a simple but potentially informative parameter in the study of predisposition to lung cancer. In the present study we have examined the relationship between lung DNA adduct level, exposure, and genetic factors in a group of lung cancer patients.

Materials and Methods

Samples of normal lung tissue were obtained from 63 previously untreated lung cancer patients undergoing surgery. The tissues were snap-frozen and stored at −80°C until DNA extraction. A questionnaire was filled out for each patient by the treating physician where information on smoking habits, other relevant exposures, occupation, and family history of cancer were recorded. All the tumors were non-small cell lung carcinomas. DNA were extracted from powdered nontumorous tissues by proteinase K/RNase digestion and phenol/chloroform extraction.

The DNA adducts were measured by ^{32}P-postlabeling analysis with the nuclease P_1 modification as described previously (8). This procedure detects aromatic and/or hydrophobic DNA adducts, predominantly those formed by PAHs (7). Thin layer chromatography maps showed the diagonal band of radioactivity characteristic of DNA adduct formation by a complex mixture of compounds. All samples were analyzed in triplicate on separate days. The adduct maps were qualitatively reproducible and the quantitative variability between assays was <15%.

The *GSTM1* genotype was determined by polymerase chain reaction amplification as described previously (11). The control group in the genotype study comprised 177 males with the same ethnic background as the patients. The mean age and smoking dose for the controls was 54 ± (SD) 13 years and 17.1 ± 10.2 pack years, respectively. Data for the cases are given in Table 1.

Results and Discussion

DNA was isolated from normal lung tissue from 63 patients with primary non-small cell lung carcinoma and analyzed for the presence of DNA adducts using ^{32}P-postlabeling with nuclease P_1 digestion modification. The adduct levels were significantly higher (Wilcoxon test, $P < 0.0001$) among smokers (10.52 ± 7.88 adducts/10^8 nucleotides) than among a group consisting of never-smokers and exsmokers of 2 or more years of abstinence (2.24 ± 1.14; Table 1). These values should be regarded as minimum values because of inherent uncertainties in the efficiency of labeling, and hence the absolute quantitation, of adducts formed by complex mixtures. A tendency towards higher adduct levels in female than in male smokers was also observed (Table 1). When the smokers were divided into quartiles according to adduct levels, there was a significant trend in the direction of more females in the high adduct quartiles (linear rank trend test: 2-sided $P = 0.043$, data not shown), even without taking account of the fact that the women had, on average, smoked significantly less (Table 1). When the data are adjusted for smoking dose and the individuals are divided into quartiles according to total adducts per pack year, there was a highly significant difference in the distribution of males and females (Fig. 1) (trend test: 2-sided $P = 0.005$). Similar results were

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[3] The abbreviations used are: AHH, aryl hydrocarbon hydroxylase; GST, glutathione *S*-transferase; PAH, polycyclic aromatic hydrocarbon; GSTM1, glutathione *S*-transferase M1.

Table 1 *PAH adducts and smoking-related parameters for males and females*

Variables	Males (mean ± SD)		Females (mean ± SD)		*P* (Wilcoxon rank test)
Adducts/10^8 nucleotides					
Smokers	(*N* = 38)	9.75 ± 7.86	(*N* = 11)	13.55 ± 7.89	0.058
Nonsmokers[a]	(*N* = 7)	2.72 ± 1.23	(*N* = 7)	1.70 ± 0.80	
Cigarettes/day[b]		18.6 ± 8.4		15.6 ± 5.2	0.264
Smoking years[b]		45.0 ± 9.5		32.9 ± 9.1	0.0001
Pack years[b]		42.0 ± 20.2		26.4 ± 12.8	0.009
Age[b]		62.8 ± 9.3		56.7 ± 11.0	0.093

[a] Never-smokers and exsmokers of 2 or more years of abstinence.
[b] Data for smokers only.

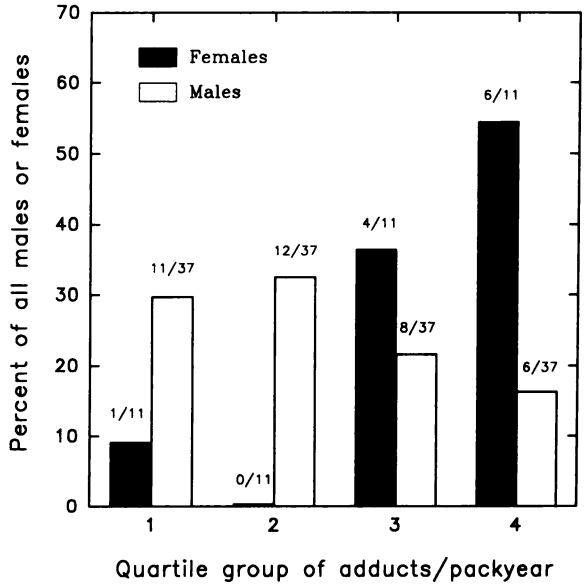

Fig. 1. Distribution of males and females in quartiles according to adducts per pack year. The parameter "adducts per pack year" was calculated for all smokers and ranked according to its size. After the group was divided into 4 equal parts, the percentage of males and females in each group was determined. The range of adducts per pack year were 0.033–0.130, 0.131–0.220, 0.221–0.440, and 0.441–2.300 for groups 1 to 4, respectively.

obtained when the variable tested was adducts per smoking year. Thus, among patients suffering from non-small cell lung cancer, disposition towards high levels of DNA adducts is more pronounced in females than in males.

A wide range of adduct levels was also evident among men (Fig. 1; Table 1). Since there was more cases available in this group, a multivariate analysis was performed. Surprisingly, the number of cigarettes smoked per day had only low significance for the variation in adduct levels. The nonsmoking individuals were excluded from the analysis, since this group may overestimate the importance of daily smoking dose. We found that the number of smoking years was the single smoking-related parameter which could best explain the variation in adducts. Linear regression analysis revealed that the adduct levels were inversely related to number of smoking years ($r = 0.52$, $P = 0.001$) (Fig. 2). When one outlier was excluded, 42% of the variation in adduct levels could be attributed to variation in years of smoking. This correlation was highly significant ($P = 2.0 \times 10^{-5}$). We also found a significant inverse correlation between adducts and age and pack years ($r = 0.47$, $P = 0.003$ and $r = 0.38$, $P = 0.021$, respectively). Therefore, male lung cancer patients with high adduct levels showed trends similar to those for females; the duration of smoking is generally shorter before the clinical onset of the disease. Although a number of factors may influence the amount of hydrophobic DNA adducts formed, the results above seem to indicate that genetic factors are either directly or indirectly involved in the disposition to high levels. This is also illustrated by the fact that 50% of our male patients smoked between 15 and 20 cigarettes per day but the variation in adduct levels in this group was 25-fold. Furthermore, for male patients with a similar daily smoking dose and years of age at smoking start, there was up to 35 years difference in the clinical onset of the disease. Some of the previous studies of lung cancer patients have found a correlation of adduct levels in lung tissue and daily smoking dose (7–9). However, this correlation was not strong, especially when non-smokers were excluded. Generally, it is probable that the range of distribution of age and smoking in the study group may affect which factors are best correlated to the variation of adduct levels, either tobacco smoke exposure or genetic factors.

Previous studies have indicated that GSTM1 may protect from DNA damage caused by reactive electrophilic metabolites of PAHs (12, 13). About 40–50% of the Caucasian population have deletions in the *GSTM1* gene which makes the enzyme inactive (null genotype) (14). We therefore amplified, by polymerase chain reaction, DNA from each patient in order to detect this deletion polymorphism and to examine its influence on adduct levels. Higher adducts were found among male patients homozygous for the deletion (*GSTM1* null) than in the group with at least one allele intact (*GSTM1* positive), but the difference was not statistically significant (Table 2). We also found a higher proportion of patients with the *GSTM1*-null genotype in the high adduct group (higher than the median 7.63 per 10^8 nucleotides) compared to a group of healthy controls (Table 2). Most of the females had the *GSTM1*-positive genotype (9 of 11; data not shown). In a recent study of 38 autopsy lung samples from cancer-free subjects, Shields *et al.* (15) reported an association of *GSTM1*-null

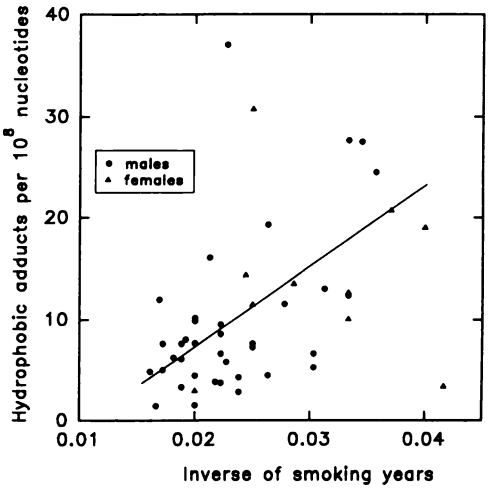

Fig. 2. Scatterplot of adduct level against the inverse of smoking years. Males and females are indicated with different symbols. The regression line is for male patients only ($n = 37$, $r = 0.52$, $P = 0.001$).

Table 2 *GSTM1 genotypes in relation to adduct level in male lung cancer patients compared to healthy controls*

GSTM1 genotype	Adducts[a] (mean ± SD)	Adduct level[b] Low	Adduct level[b] High[c]	Healthy controls
Null	10.58 ± 8.52	9	14	89
Positive	8.48 ± 6.80	10	5	88

[a] Adducts/10^8 nucleotides. Wilcoxon test of null *versus* positive genotype, $P = 0.289$.
[b] Males with adduct levels lower and higher than the median (7.63/10^8 nucleotides) are grouped. The number of patients with each genotype are indicated.
[c] Fisher exact test of patients with high adducts versus healthy controls: $P = 0.043$; odds ratio, 2.77; 95% confidence interval, 0.96–8.02.

genotype and detectable DNA adduct levels. The *GSTM1* genotype may therefore have some effect on the disposition for high adducts at least for male lung cancer patients, but its penetrance is probably not high. It is also interesting that our results indicated an excess of GSTM1-deficient individuals only among patients with high adduct levels.

It has been hypothesized that cancer risks may be related to metabolic imbalance between activating and detoxifying pathways for carcinogens (16). Several PAHs are converted to reactive epoxides by AHH particularly associated with the *CYP1A1* gene. Higher activity of AHH has been reported among recent smoking lung cancer patients than in cancer-free controls with a similar smoking history (17, 18). A positive, linear correlation between DNA adduct levels and AHH activity has also been described (19, 20). The involvement of genetic factors in DNA adduct formation is further supported by familial studies of adduct variation (21).

In studying lung cancer patients, there may be a selection of individuals with high sensitivity to both PAH and other carcinogenic compounds in the tobacco smoke. The current study indicates that patients with high DNA adduct levels developed lung cancer after lower smoking dose and/or shorter duration than patients with low adduct levels. This fits the expected behavior of susceptible individuals. Our results suggest that susceptibility to DNA damage caused by PAH-like compounds may be higher among women than among men. This may be related to (unknown) differences in metabolism of PAH and other carcinogens in tobacco smoke and/or different accumulation of these carcinogens in the body. Our results provide biochemical evidence to support recent epidemiological data indicating that women are at higher risk (1.5–2-fold) than men of developing smoking-induced lung cancer (22, 23). This may be significant for predictions of future lung cancer incidence in women, based on current smoking trends.

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References

1. Nebert, D. W. Role of genetics and drug metabolism in human cancer risk. Mutat. Res., *247:* 267–281, 1991.
2. Idle, J. R. Is environmental carcinogenesis modulated by host polymorphism? Mutat. Res., *247:* 259–266, 1991.
3. Bartsch, H., Petruzzelli, S., De Flora, S., Hietanen, E., Camus, A-M., Castegnaro, M., Alexandrov, K., Rojas, M., Saracci, R., and Giuntini, C. Carcinogen metabolism in human lung tissues and the effect of tobacco smoking: results from a case-control multicenter study on lung cancer patients. Environ. Health Perspect., *98:* 119–124, 1992.
4. Ketterer, B., Harris, J. M., Talaska, G., Meyer, D. J., Pemble, S. E., Taylor, J. B., Lang, N. P., and Kadlubar, F. F. The human glutathione *S*-transferase supergene family, its polymorphism, and its effects on susceptibility to lung cancer. Environ. Health Perspect., *98:* 87–94, 1992.
5. Rüdiger, H. W., Schwartz, U., Serrand, E., Stief, M., Krause, T., Nowak, D., Doerjer, G., and Lehnert, G. Reduced O^6-methylguanine repair in fibroblast cultures from patients with lung cancer. Cancer Res., *49:* 5623–5626, 1989.
6. Hemminki, K. DNA adducts, mutations and cancer. Carcinogenesis (Lond.), *14:* 2007–2012, 1993.
7. Phillips, D. H., Hewer, A., Martin, C. N., Garner, R. C., and King, M. M. Correlation of DNA adduct levels in human lung with cigarette smoking. Nature (Lond.), *336:* 790–792, 1988.
8. Phillips, D. H., Schoket, B., Hewer, A., Bailey, E., Kostic, S., and Vincze, I. Influence of cigarette smoking on the levels of DNA adducts in human bronchial epithelium and white blood cells. Int. J. Cancer, *46:* 569–575, 1990.
9. Dunn, B. P., Vedal, S., Richard, S. H. C., Kwan, W-F., Nelems, B., Enarson, D. A., and Stich, H. F. DNA adducts in bronchial biopsies. Int. J. Cancer, *48:* 485–492, 1991.
10. Swenberg, J. A., Richardson, F. C., Boucheron, J. A., and Dryoff, M. C Relationships between DNA adduct formation and carcinogenesis. Environ. Health Perspect., *62:* 5–18, 1985.
11. Ryberg, D., Kure, E., Lystad, S., Skaug, V., Stangeland, L., Mercy, I., Borresen, A-L., and Haugen, A. *p53* mutations in lung tumors: relationship to putative susceptibility markers for cancer. Cancer Res., *54:* 1551–1555, 1994.
12. Wiencke, J. K., Kelsey, K. T., Lamela, R. A, and Toscano, W. A., Jr. Human glutathione *S*-transferase deficiency as a marker of susceptibility to epoxide-induced cytogenetic damage. Cancer Res., *50:* 1585–1590, 1990.
13. van Poppel, G., de Vogel, N., van Balderen, P. J., and Kok, F. J. Increased cytogenetic damage in smokers deficient in glutathione *S*-transferase isozyme μ. Carcinogenesis (Lond.), *13:* 303–305, 1992.
14. Seidegård, J., Vorachek, W. R., Pero, R. W., and Pearson, W. R. Hereditary differences in the expression of the human glutathione transferase active in *trans*-stilbene oxide are due to a gene deletion. Proc. Natl. Acad. Sci. USA, *85:* 7293–7297, 1988.
15. Shields, P. G., Bowman, E. D., Harrington, A. M., Doan, V. T., and Weston, A. Polycyclic aromatic hydrocarbon-DNA adducts in human lung and cancer susceptibility genes. Cancer Res., *53:* 3486–3492, 1993.
16. Nakachi, K., Imai, K., Hayashi, S., and Kawajiri, K. Polymorphisms of the *CYP1A1* and glutathione *S*-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. Cancer Res., *53:* 2994–2999, 1993.
17. Anttila, S., Hietanen, E., Vainio, H., Camus, A-M., Gelboin, H. V., Park, S. S., Heikkilä, L., Karjalainen, A., and Bartsch, H. Smoking and peripheral type of cancer are related to high levels of pulmonary cytochrome P4501A in lung cancer patients. Int. J. Cancer, *47:* 681–685, 1991.
18. Petruzzelli, S., Camus, A-M., Carrozzi, L., Ghelarducci, L., Rindi, M., Menconi, G., Angeletti, C. A., Ahotupa, M., Hietanen, E., Aitio, A., Saracci, R., Bartsch, H., and Giuntini, C. Long-lasting effects of tobacco smoking on pulmonary drug-metabolizing enzymes: A case-control study on lung cancer patients. Cancer Res., *48:* 4695–4700, 1988.
19. Geneste, O., Camus, A-M., Castegnaro, M., Petruzzelli, S., Macchiarini, P., Angeletti, C. A., Giuntini, C., and Bartsch, H. Comparison of pulmonary DNA adduct levels, measured by ^{32}P-postlabelling and aryl hydrocarbon hydroxylase activity in lung parenchyma of smokers and ex-smokers. Carcinogenesis (Lond.), *12:* 1301–1305, 1991.
20. Bartsch, H., Castegnaro, M., Rojas, M., Camus, A-M., Alexandrov, K., and Lang, M. Expression of pulmonary cytochrome P4501A1 and carcinogen DNA adduct formation in high risk subjects for tobacco-related lung cancer. Toxicol. Lett., *64/65:* 477–483, 1992.
21. Nowak, D., Schmidt-Preuss, U., Jörres, R., Liebke, F., and Rüdiger, H. W. Formation of DNA adducts and water-soluble metabolites of benzo(*a*)pyrene in human monocytes is genetically controlled. Int. J. Cancer, *41:* 169–173, 1988.
22. Risch, H. A., Howe, G. R., Jain, M., Burch, J. D., Holowaty, E. J., and Miller, A. B. Are female smokers at higher risk for lung cancer than male smokers? Am. J. Epidemiol., *138:* 281–293, 1993.
23. Zang, E. A., and Wynder, E. L. Cumulative tar exposure. A new index for estimating lung cancer risk among cigarette smokers. Cancer (Phila.), *70:* 69–76, 1992.

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