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Biomarkers of Carcinogen Exposure and Cancer Risk in a Coke Plant

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To evaluate the association between an indicator of carcinogen exposure (peripheral blood leukocyte DNA adducts of polycyclic aromatic hydrocarbons) and an early indicator of neoplastic transformation (sputum epithelial cell membrane antigens binding by monoclonal antibodies against small cell lung cancer and against nonsmall cell lung cancer), a survey of 350 coke-oven workers and 100 unexposed workers was planned. This paper reports a pilot investigation on a subgroup of 23 coke-oven workers and 8 unexposed controls. A "gas regulator" worker with positive tumor antigen binding was identified. Results show that smokers, subjects with decreased pulmonary function (forced expiratory volume in 1 sec/forced vital capacity% <80), and those with morphological dysplasia of sputum cells have higher levels of DNA adducts. The gas regulators showed the highest values for adducts; however, no significant difference of adduct levels was found between the coke-oven group and unexposed controls.

Introduction

Coke oven workers are known to have an increased lung cancer risk (*1*). A lung cancer-oriented monitoring program among 350 coke-oven workers in a steel mill in Southern Italy was initiated with the following aims: *a*) to assess the frequency of positive immunostaining by monoclonal antibodies that bind to cell surface lung cancer (small cell lung cancer [SCLC] and nonsmall cell lung cancer [NSCLC]) differentiation antigens in sputum cells of coke oven workers with and without morphological atypia; *b*) to detect DNA adducts of polycyclic aromatic hydrocarbons (PAH) in lymphocytes using an enzyme-linked immunosorbent assay (ELISA); *c*) to examine the association between PAH-DNA adducts and positive tumor-specific immunostaining among coke-oven workers (after adjustment for tobacco smoking and charcoal-broiled food consumption); and *d*) to examine the associations by job titles. This paper is a report of a pilot investigation conducted in October 1988 on a subgroup of 23 coke-oven workers who showed squamous metaplasia or light/-moderate atypia of sputum cells in a previous survey (*2*). This subgroup was chosen because subjects with morphologically altered sputum cells may be more likely to show positive immunostaining.

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Materials and Methods

The cokery under study is located in the steel plant of Taranto (Italy) and consists of 11 batteries built between 1964 and 1976. In the first survey (*2*), 35 workers with squamous metaplasia or light/moderate dysplasia of sputum cells were identified among 524 workers screened; of these, 12 were retired and 23 (19 with squamous metaplasia and 4 with light/moderate dysplasia) were still employed in October 1988. Every worker underwent blood drawing (35 mL), sputum induction, spirometry, and a questionnaire–interview. All data related to the demographic, occupational, smoking habits, history of respiratory symptoms and diseases were collected by standardized questionnaire and reviewed by an occupational physician.

PAH–DNA Adducts Measurements

After centrifugation of whole blood, nucleated blood cells were removed from the serum-hemoglobin interface and DNA was extracted by high-salt fractionation (*3*) followed by chloroform-isoamyl alcohol extraction. Measurement of PAH–DNA adducts was performed by ELISA as previously described (*4*) with anti-benzo[*a*]pyrene-modified DNA (BaP-DNA) antiserum, a standard curve (BaP-DNA) modified in the same range as the biological samples, and a fluorescent methylumbelliferyl-phosphate substrate. Because the antiserum cross-reacts with DNA modified with multiple PAHs (*5*), the values generated reflect the many PAHs to which humans are exposed and are termed "PAH–DNA adducts." The lower limit of sensitivity, by comparison with the (above) BaP-DNA standard curve, is in the range of 0.04 fmole adducts/μg DNA. Levels of

Table 1. Age, levels of PAH–DNA adducts, severity of atypia by Papanicolaou method, smoking habits, pulmonary function (FVC, FEV_1), and job title of 23 coke-oven workers.

Subject	Age	Job title	Cigarettes smoked/day	Cytomorphology	FVC, %[a]	FEV_1, %[a]	Adducts,[b] fmole/μg DNA
A	47	Top side	Ex-smoker	Squamous metaplasia	110	96	0.15
B	32	Bench	0	Squamous metaplasia	112	105	—
C	39	Maintenance	0	Squamous metaplasia	116	101	—
D	45	Supervisor	20	Squamous metaplasia	85	83	0.20
E	41	Maintenance	30	Squamous metaplasia	91	86	—
F[c]	48	Gas regulator	5	Moderate dysplasia	125	61	0.19
G	49	Bench	Ex-smoker	Moderate dysplasia	95	88	0.25
H	40	Bench	Ex-smoker	Moderate dysplasia	92	83	0.07
I	46	Gas regulator	15	Normal	98	88	0.09
J	42	Supervisor	Ex-smoker	Normal	90	87	0.20
K	37	Maintenance	20	Squamous metaplasia	129	111	0.21
L	39	Bench	Ex-smoker	Normal	98	84	0.10
M	32	Bench	0	Squamous metaplasia	110	109	0.11
N	32	Bench	20	Squamous metaplasia	94	91	0.17
O	55	Maintenance	0	Squamous metaplasia	95	87	0.19
P	35	Bench	15	Squamous metaplasia	107	106	0.12
Q	44	Gas regulator	30	Moderate dysplasia	91	87	0.44
R	42	Bench	Ex-smoker	Normal	107	92	—
S	35	Bench	20	Squamous metaplasia	91	89	0.23
T	41	Maintenance	20	Normal	104	79	—
U	39	Bench	30	Squamous metaplasia	84	78	—
V	40	Supervisor	0	Normal	95	89	—
W	39	Top side	20	Normal	114%	109	—

Abbreviations: FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec.
[a]Percent of predicted.
[b]Mean of two determinations; DNA adducts measurements were not available for eight workers.
[c]Immunostaining positive, all others negative.

DNA adducts of coke oven workers were compared with those of eight unexposed workers. Among the coke-oven workers, levels of adducts were compared by cigarette smoking status (smoker, nonsmoker, ex-smoker), by pulmonary function, by severity of sputum cells atypia, and by job title. All comparisons were performed using rank-sum Wilcoxon test.

Cytomorphology and Immunostaining of Sputum Cells

Sputum induction was performed by a 25-min inhalation of sterile Hank's balanced salt solution following a standard procedure. For each subject eight glass slides were prepared and fixed at the field center and then studied by Papanicolaou method. The remaining material of sputum was homogenized and placed in Saccomanno's preservative solution (2% polyethylene glycol in 50% ethanol). Multiple slides of each sputum specimen were stained separately to minimize the effect of staining variability. A complete description of the staining protocol used in this study can be found in previous reports (*7,8*). Briefly, slides were washed in phosphate-buffered saline (PBS), 0.01 M at pH 7.4, and incubated with SCLC or NSCLC antibodies in a sealed, humidified chamber at room temperature (25°C). Diaminobenzidine immunostaining was enhanced by a biotinylated secondary antibody solution (directed against the species/subclass of immunoglobulin in which the primary antibody was raised), and a biotinylated tertiary antibody solution (directed against the secondary antibody) (*7*). Specimens were then counterstained with 0.125% methylene blue and mounted by routine methods. Monoclonal primary antibodies (IgM with specificity for a glycolipid antigen of SCLC [code number 624H12] and IgG to a protein antigen of NSCLC [code number 703D4]) were provided by the Navy Oncology Branch of the National Cancer Institute.

Pulmonary Function Tests

Pulmonary function tests were performed using protocols and equipment (Collins Survey II Spirometer interfaced with a IBM/XT using an S&M Instrument Co. interface) suggested by Atherosclerosis Risk in Communities Study (ARIC) and based on the recommended procedures of American Thoracic Society.

Results

The mean age of the 23 coke-oven workers was 40.83 (± 5.82) years. A 48-year-old subject with positive immunostaining, morphological atypia (moderate dysplasia), and severe airways obstruction was identified. He was a current smoker and he had worked 14 years as a gas regulator.

PAH–DNA adducts levels were assessed for 15 coke-oven workers (Table 1). The adduct level of the subject with positive immunostaining was similar to the mean of immunostaining negative subjects. Means of PAH–DNA adducts in 15 coke-oven workers (mean age: 41.7 ± 6.8) and controls ($n = 8$; mean age: 38.2 ± 8.9; 1 smoker, 7 nonsmokers) were, respectively, 0.188 (± 0.12) and 0.146 (± 0.05) fmole/μg DNA. No significant difference was detected between groups. The means of PAH-DNA adducts by cigarette smoking habits, by pulmonary function forced expiratory volume in 1 sec/forced vital capacity (FEV_1/FVC), by cytomorphology and by job title are reported in Table 2. Although no significant differences were observed, smokers, workers with FEV_1/FVC $\leq$80%, and workers with morphological dysplasia of sputum cells had the highest level of adducts. The job title of "gas regulator" showed the highest values of adducts.

Table 2. Means of PAH–DNA adducts of 15 coke-oven workers by cigarette smoking habits, pulmonary function, cytomorphology, and job title.

Parameter	N^a	Mean (SD)
Cigarette smoking habits		
Smokers	8	0.205 (±0.106)
Ex-smokers	5	0.151 (±0.075)
Nonsmokers	2	0.147 (±0.055)
Pulmonary function		
$FEV_1/FVC \leq 80\%$	9	0.186 (±0.114)
$FEV_1/FVC > 80\%$	6	0.170 (±0.049)
Cytomorphology		
Normal	3	0.128 (±0.063)
Squamous metaplasia	8	0.171 (±0.044)
Moderate dysplasia	4	0.235 (±0.156)
Job title		
Bench	7	0.148 (±0.070)
Maintenance	2	0.200 (±0.205)
Supervisor	2	0.198 (±0.003)
Gas regulator	3	0.238 (±0.182)
Top side	1	0.146 —
Unexposed controls	8	0.141 (±0.050)

Abbreviations: FEV_1, forced expiratory volume in 1 sec; FVC, forced vital capacity.

[a]DNA adducts measurements were not available for eight workers.

Discussion

Recognition of biologic changes associated with carcinogen exposure and with lung cancer risk is important for lung cancer prevention and for successful early intervention (*6,11,12*). In this survey two types of biologic indices have been measured: *a*) a biologic index of lung cancer risk assessed by frequency and intensity of avidin-biotin complex immunostaining of cell surface lung cancer differentiation antigens (*8*); *b*) a biologic index of exposure assessed by frequency and level of PAH–DNA adducts (*13,14*). Among 23 coke-oven workers, 1 subject was found to have cell-surface lung-cancer differentiation antigens detected by immunostaining. It is interesting that this subject was a smoker, had a severe airways obstruction, and had moderate dysplasia of bronchial epithelium cells. The role of cigarette smoking and cellular atypia as factors associated with lung cancer is well established. Tockman et al. (*15*) and Kuller et al. (*16*) have independently found that airways obstruction can be considered an independent risk factor for lung cancer after adjusting for cigarette smoking. PAH–DNA adducts have been measured in the peripheral white blood cells of coke-oven workers and controls by ELISA. All the coke-oven workers and all the presumed unexposed noncoke-oven workers had detectable adducts levels. Other sources of exposure (for example, diet, smoking, air pollution) may explain the presence of adducts of PAH in DNA of lymphocytes of controls. No information about charcoal-broiled food was obtained in this preliminary survey. The mean level of adducts in smokers and in subjects with $FEV_1/FVC \leq 80\%$ was marginally higher than in ex-smokers or nonsmokers and in workers with $FEV_1/FVC > 80\%$, respectively; however, the differences were not significant. Among the job titles, the "gas regulators" exhibited the highest adduct level, while the job title of "top side," considered the most exposed to PAH, was represented by only one worker with adduct level below the means of the other job titles. However, a large amount of interindividual variation in adduct levels was observed among workers with the same job title and the same smoking habits. The planned survey provides the opportunity to evaluate the association of these two biologic monitoring methods, one of carcinogen exposure (DNA adducts) and one of lung cancer risk (immunostained cytology), in order to examine the association between effective genomic carcinogen dose and target cell transformation. This study is the first to evaluate the characteristics of monoclonal antibody recognition of cell-surface differentiation antigens in the sputum cells of a high-risk employment group.

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