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Genotoxicity of Occupational Exposure to Wood Dust: Micronucleus Frequency and Nuclear Changes in Exfoliated Buccal Mucosa Cells

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Occupational exposure to wood dust is associated with the occurrence of nasal cancer. In this study, we investigated micronuclei and nuclear changes (NCs: binucleates, karyorrhexis, karyolysis, and the "broken egg" effect) in exfoliated buccal cells of 20 workers exposed to wood dust and 20 age- and sex-matched controls. Micronucleus frequency and the frequency of each of the NCs were significantly higher for wood workers than controls ($P < 0.01$). Cigarette smoking was associated with increased frequencies of micronuclei and NCs in the buccal mucosa epithelium cells of both the control and exposed groups. Our findings indicate that buccal cells of wood workers display increased levels of genotoxicity and toxicity, and that these biomarker responses may be related to the increased cancer risk among wood workers. Environ. Mol. Mutagen. 47:693–698, 2006.

Key words: wood dust; genotoxicity; cytotoxicity; apoptosis; exfoliated buccal cells

INTRODUCTION

Wood is one of the world's most important renewable resources: at least 1,700 million m^3 are harvested for industrial use each year [Palus et al., 1999]. Wood dust, generated in the processing of wood for a wide range of uses, is a complex substance whose composition varies considerably according to the species of tree. Fifty-eight percent of all wood harvested worldwide is from hardwood species; however, much of this is used for fuel. Most wood used for industrial purposes is from softwood species, although the particular species varies greatly from region to region [IARC, 1995]. Wood dust is a complex mixture composed mainly of cellulose, polyoses, and lignin, and a large and variable number of substances of lower relative molecular mass, which may significantly affect the properties of the wood. These include nonpolar organic compounds (fatty acids, resin acids, waxes, alcohols, terpenes, sterols, steryl esters, and glycerols), polar organic compounds (tannins, flavonoids, quinones, and lignans), and water-soluble compounds [Palus et al., 1999].

Wood dust exposure occurs when individuals use machinery or tools to cut or shape wood. Breathing in the dust causes it to deposit in the nose, throat, and other airways. The amount of dust deposited within the airways depends on the size, shape, and density of the dust particles, and the strength (turbulence and velocity) of the airflow [IARC, 1981]. Particles with a diameter >5 μm ("inspirable" particles) are deposited almost completely in the nose. Particles 0.5–5 μm ("respirable" particles) are deposited in the lower airways [IARC, 1981, 1995].

Exposure to wood dust and the synthetic chemical products used to confer resistance and durability to wood are risk factors for cancer among wood workers [Bahia et al., 2005]. Wood dust is known to be a human carcinogen, based on sufficient evidence of carcinogenicity from studies in humans [Demers et al., 1995; IARC, 1995]. Several case reports, cohort studies, and case control studies that specifically addressed nasal cancer have observed an association between wood dust exposure and cancer of the nose. Strong and consistent associations with cancer of the nasal cavities and paranasal sinuses were observed both in studies of people whose occupations are associated with wood dust exposure and in studies that directly estimated wood dust exposure. Risks were highest for adenocarcinoma, particularly among European populations. Studies of U.S. populations showed similar significant positive associations. A pooled analysis of 12 case control studies showed that the estimated relative

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risk of adenocarcinoma was very high among men with the greatest exposure (45.5), and that the risk increased with the length of exposure [Demers et al., 1995].

Besides its carcinogenic effects, occupational exposure to various dust particles by wood-processing factory employees, as well as grain workers and feed-mill employees, is a common cause of respiratory disorders like wheezing and acute and chronic bronchitis [Esson, 1977]. Several independent groups have suggested that health risks are associated with chronic occupational exposure to wood dust [Vaughan, 2000]. These studies reported the occurrence of lower and upper respiratory diseases such as alveolitis allergica, bronchialasthma, aspergillomycosis, and rhinitis [Kuper et al., 1997]. Extensive studies have been performed indicating that the sensitization to wood dust can cause asthma, the symptoms typically occurring some time after exposure [Elavarasi et al., 2002].

So far, there are very limited data on the genotoxicity of wood dust. The genotoxicity related to an occupational exposure can be evaluated using different genetic endpoints, e.g., DNA damage, chromosomal aberrations, and micronuclei [Celik et al., 2003; Faust et al., 2004; Celik and Akbaş, 2005]. The frequency of micronuclei is considered a quantitative measure of chromosome damage, which is correlated with the incidence of cancer in human populations [Tolbert et al., 1992].

The mechanism underlying the formation of micronuclei has been firmly established [Heddle et al., 1991]. Micronuclei result from chromosomal fragments or whole chromosomes lagging during cell division. The micronucleus (MN) assay has been used in a variety of laboratory experiments and in biomonitoring studies for screening populations at risk of exposure to mutagenic agents. For instance, the MN assay detects genotoxic damage induced by environmental pollutants, such as pesticides and heavy metals, in plant cells [Ünyayar et al., 2006], in laboratory animal cells, such as rat bone marrow cells [Celik et al., 2005a,b], and in human cells, such as human peripheral blood lymphocytes [Fenech, 2000] and exfoliated buccal cells [Celik et al., 2003]. In the case of epithelial tissue, effective techniques have not yet been developed for preparing metaphase chromosomes for conventional aberration analysis. The MN assay, however, is an effective method for detecting chromosomal aberrations in exfoliated epithelial cells [Tolbert et al., 1992; Kayal et al., 1993; Pastor et al., 2001; Huvinen et al., 2002; Guzman et al., 2003].

Micronuclei that are detected in buccal mucosa cells reflect genotoxic events that occurred in basal cells, and these events can be observed in exfoliated cells over an approximate 3 week period [Livingston et al., 1990; Ogden et al., 1997; Junqueira et al., 1998]. One advantage of the MN assay in buccal mucosa is that cell culture is not required [Stich et al., 1983; Ogden et al., 1997]. In addition, the most common alterations in the morphology of neoplastic epithelial cells occur in the nucleus, which are characterized by modifications in size, density, and in the distribution of the chromatin. These phenomena include binucleated (BN) cells, the "broken-egg" (BE) effect, karyorrhexis (KR), and karyolysis (KL) or nuclear dissolution.

Exfoliated buccal cells have been widely used in cytological evaluations to detect abnormal morphology, premalignant changes, and cancer. Since most cancers arise in epithelial tissue, exfoliated epithelial cells may be particularly useful for monitoring human populations exposed to various hazardous agents [Cairns, 1975; Guzman et al., 2003]. The use of the MN assay on exfoliated cells has been described as an approach to identify genotoxic damage in human tissues that are targets for organ-specific carcinogens [Stich and Rosin, 1984].

In the present study, we have used the MN assay conducted with buccal epithelial cells to investigate the genotoxicity associated with occupational exposure to wood dust at a wooden furniture plant. In addition, measures of toxicity, BN, KR, KL, and BE were analyzed in addition to micronuclei. BN is a biomarker of toxicity [Celik et al., 2005a,b]; KR and KL are biomarkers of apoptosis [Celik et al., 2003]; and BE is a marker of genotoxicity [Ramirez and Saldanha, 2002].

MATERIALS AND METHODS

Subjects

The study was carried out on 20 male furniture workers (10 smokers and 10 nonsmokers). All worked in a single, poorly ventilated woodworking shop in the city of Mersin. The workers were exposed to a mixture of dust from soft wood and hard wood, but were not occupationally exposed to other potentially genotoxic chemicals, such as formaldehyde and solvents. The control group consisted of 20 healthy men (10 smokers and 10 nonsmokers) who had no history of exposure to chemicals or other potentially genotoxic substances. The purpose of the study was carefully explained to the participants before they were asked to sign an informed consent form and to complete a standardized questionnaire to obtain data on lifestyle and personal factors (age, employment history, health, smoking habits, etc.). This study was conducted with the approval of our institutional human subjects committee. Smoking history recorded the use of cigarettes, cigarillas, cigars, and pipe tobacco over the lifetime. Neither of the study groups differed with regard to any lifestyle or personal factor. The demographic characteristics of the study groups are summarized in Table I.

Measurement of Wood-Dust Levels

To estimate the wood dust level at the workplace, we measured the amount of wood dust accumulated on surfaces during a single work day. In order that the measurements could be related to the biomarker endpoints, the exposure measurements were performed 12 days before the buccal cell sampling.

Evaluation of Micronuclei and Nuclear Changes in Buccal Cells

Buccal epithelium cell samples were collected from workers during the work day. The subjects were asked to rinse their mouth with water before sampling. A wooden spatula was used to obtain buccal cell sam-

TABLE I. General Characteristics of the Exposed and Control Groups

Study group	*N*	Age (mean ± SD)	Years of cigarette smoking (mean ± SD)	Years of employment (mean ± SD)	Cigarettes/day (mean ± SD)
Controls					
Smokers	10	26.7 ± 5.91	7.70 ± 4.83	—	22.5 ± 7.45
Nonsmokers	10	22.4 ± 2.55	—	—	—
Total	*20*	*24.6 ± 4.95*	*7.70 ± 4.83*	—	*22.5 ± 2.63*
Workers					
Smokers	10	32.5 ± 7.38	13.8 ± 7.80	17.2 ± 8.50	27.0 ± 3.49
Nonsmokers	10	29.5 ± 5.97	—	16.0 ± 6.83	—
Total	*20*	*31.0 ± 6.71*	*13.8 ± 7.80*	*16.6 ± 7.51*	*27.0 ± 3.49*

N, number of subjects; SD, standard deviation.

TABLE II. Micronuclei and Nuclear Changes in Buccal Epithelial Cells From Control Subjects

		NC				
ID no.	MN	BN	KR	KL	BE	TNC
Nonsmokers						
1	3	2	4	4	1	11
2	2	3	4	3	1	11
3	1	1	2	4	1	8
4	1	2	2	4	1	9
5	1	1	4	3	—	8
6	5	2	4	2	1	9
7	4	4	4	3	1	12
8	4	3	4	4	—	11
9	1	2	4	4	—	10
10	5	2	3	3	1	9
Smokers						
11	3	7	8	7	2	24
12	3	8	8	8	3	27
13	7	8	8	9	4	29
14	3	8	8	5	8	29
15	5	12	8	8	4	32
16	4	15	9	9	6	39
17	14	16	12	8	8	44
18	11	16	10	10	7	43
19	10	16	12	11	8	47
20	3	12	6	8	8	34

All data are the number counted in 3,000 cells.
ID, identification number; MNC, micronucleated cells; MN, micronuclei; NC, nuclear changes; BN, binucleated cells; KR, cells with karyorrhexis; KL, cells with karyolysis; BE, cells having the broken egg effect; TNC, total nuclear changes.

TABLE III. Micronuclei and Nuclear Changes in Buccal Epithelial Cells From Exposed Subjects

		NC				
ID no.	MN	BN	KR	KL	BE	TNC
Nonsmokers						
1	19	26	21	27	6	80
2	14	25	21	18	13	77
3	15	19	19	18	12	68
4	15	19	17	16	9	61
5	17	34	31	21	17	103
6	18	32	32	23	20	107
7	24	32	31	30	22	115
8	15	32	32	29	18	111
9	13	31	31	28	18	108
10	15	33	27	19	17	96
Smokers						
11	16	31	25	26	15	97
12	22	34	25	19	14	92
13	24	37	33	27	15	112
14	18	32	28	22	13	95
15	28	42	27	21	15	105
16	25	36	26	23	19	104
17	29	41	35	33	31	140
18	23	32	35	27	21	115
19	25	41	41	24	18	124
20	23	33	21	21	16	91

All data are the number counted in 3,000 cells.
ID, identification number; MNC, micronucleated cells; MN, micronuclei; NC, nuclear changes; BN, binucleated cells; KR, cells with karyorrhexis; KL, cells with karyolysis; BE, cells with broken egg appearance; TNC, total nuclear changes.

ples by gently scraping the roof of the mouth. The samples were then smeared onto clean microscope slides; three slides were prepared for each subject. The smears were air-dried and fixed in 3:1 (v/v) methanol: acetic acid. The slides were stained by the Feulgen reaction technique according to Stich and Rosin [1984], with slight modifications. Feulgen was preferred over Giemsa staining since Giemsa is less specific for DNA-containing structures.

One of the authors (A.C.) evaluated 1,000 cells per slide to determine MN, KR, KL, BE, and BN frequencies. Nuclear changes (NCs) were classified according to Tolbert et al. [1992]. Briefly, cells with two nuclei were considered BN cells. Nuclei fragmented into irregular pieces were scored as KR. Nuclear dissolution, in which a Feulgen-negative, ghost-like figure of the nucleus remains, was evaluated as KL. For MN analysis, a MN must (1) be less than one-third the diameter of the main nucleus, (2) be on the same plane of focus as the nucleus, (3) have a chromatin structure similar to that of the main nucleus, (4) have a smooth, oval, or round shape, and (5) be clearly separated from the main nucleus.

Statistical Analysis

Frequencies are expressed as the number per 1,000 cells (‰), and given as means and standard deviations. A two-factorial experimental design was used to evaluate the data. Factor 1 was the group (level 1 = control, level 2 = workers) and Factor 2 was smoking status (level 1 = smokers, level 2 = nonsmokers). Each combination included 10 subjects. Statistical analysis was performed using SPSS 12.0 for Windows (SPSS,

TABLE IV. Frequencies (‰) of Micronuclei and Other Nuclear Changes in Exfoliated Buccal Cells of Control and Exposed Subjects

Group	*N*	MN (‰)	BE (‰)	KL (‰)	KR (‰)	BN (‰)
Controls						
Smokers	10	2.10 ± 1.34	1.93 ± 0.78	2.77 ± 0.55	2.97 ± 0.64	3.67 ± 1.22
Nonsmokers	10	0.90 ± 0.57	0.30 ± 0.11	1.07 ± 0.26	1.13 ± 0.28	0.73 ± 0.31
Total	*20*	*1.50 ± 1.18*	*1.12 ± 1.00*	*1.92 ± 0.97*	*2.05 ± 1.06*	*2.20 ± 1.74*
Workers						
Smokers	10	7.77 ± 1.33	5.90 ± 1.76	8.10 ± 1.36	9.87 ± 2.04	11.97 ± 1.39
Nonsmokers	10	5.50 ± 1.07	5.07 ± 1.68	7.63 ± 1.74	8.73 ± 2.01	9.43 ± 1.90
Total	*20*	*6.63 ± 1.65**	*5.48 ± 1.73**	*7.87 ± 1.54**	*9.30 ± 2.06**	*10.70 ± 2.08**

All values are given as mean ± SD.MN, micronuclei; BE, broken egg; KL, karyolysis; KR, karyorhexis; BN, binucleated cells.
*$P < 0.01$ compared to control, for totals only.

Chicago, IL). $P < 0.05$ was considered as the level of significance. Square root transformation was applied to the MN, BN, KR, KL, and BE frequency data before performing analysis of variance. Multivariate analysis of variance was used evaluate the MN, BN, KR, KL, and BE frequency data.

RESULTS

Measurements of dust levels in the wood-working shop in which the worker group was employed showed that total dust concentrations ranged from 4.7 to 28.9 mg/m^3 (estimates based on surface samples). These levels were all in excess of the 1-mg/m^3 limit recommended by the National Institute for Occupational Safety and Health [NIOSH, 1977]. No dust measurements were taken in the environments of the control subjects.

Tables II and III give the frequencies of micronuclei and NCs in the control and exposed groups, respectively. Micronucleated cells always contained only a single MN.

MN, BE, BR, KL, and BN frequencies were higher in exposed individuals than in controls (Table IV). Smoking had a significant effect on the MN, KR, and KL frequencies for both the control and the exposed group (Table IV) ($P < 0.01$).

DISCUSSION

The relationship between wood dust and cancer was first identified in England in 1960 [Macbeth, 1965]. There also is a recognized association between employment in the furniture industry (wood dust) and malignancies of the paranasal sinuses [Demers et al., 1995]. Recent epidemiological studies have demonstrated an increased occurrence of this neoplasm among wood workers in different countries. For instance, Bahia-Arias et al. [2005] reported that oral cavity cancer is the second most common cancer among Brazilian wood workers. They observed significant increases in the incidences of cancer of the lung and the oral cavity/pharynx. In addition, Persson [1996] reported a relationship between occupational exposure to wood dust and Hodgkin's disease.

In this study, we evaluated the genotoxic and toxic effects of occupational exposure to wood dust in exfoliated buccal cells. We found that wood dust exposure increased the frequency of micronuclei and NCs. Wood dust appeared to increase apoptosis as judged by increases in the frequency of KL and KR in exfoliated epithelial buccal cells and caused cytotoxicity by increasing the frequency of BN. In our study, we found a MN frequency of (6.63 ± 1.65)‰ in the exposed group and (1.50 ± 1.18)‰ in the control group, implying that damage in the basal cell layer of the buccal epithelium of the workers resulted in increased frequencies of cells missing whole chromosomes or fragments of chromosomes from the nucleus.

Smoking also affected the genotoxicity and toxicity endpoints in both the exposed and control groups. Cigarette smoking is a known confounding factor that may influence the frequency of cytogenetic damage, such as chromosomal aberration, SCE, and MN formation in humans [Al-Sabti et al., 1992; Lakhanisky et al., 1993; Pitarque et al., 1997; Celik et al., 2005a,b]. Also, smoking is reported to increase the MN frequency in buccal cells [Sarto et al., 1987; Piyathilake et al., 1995; Celik et al., 2003]. Our findings indicate that cigarette smoking significantly increases the frequencies of micronuclei and NCs in both controls and exposed subjects. (Table IV). The size and morphological features of nuclei from the epithelial cells of a specific tissue tend to be uniform [Junqueira et al., 1998]. The buccal epithelium is composed of four strata, the basal layer, the prickle cell layer, the intermediate layer, and the superficial layer. Buccal cell epithelium maintains itself by continuous cell renewal in which new cells produced by mitosis in the basal layer migrate to the surface to replace those that are shed [Ünal et al., 2005].

Although the MN assay is widely used to investigate genotoxicity in various cells of occupationally exposed workers (e.g., [Yager et al., 1999; Gomez-Arroyo et al., 2000; Elavarasi et al., 2002; Celik et al., 2003]), there are few reports on the genotoxicity of occupational exposure to wood dust. Palus et al. [1999] studied DNA damage in the leukocytes of thirty-five wood workers and 41 con-

trols using the Comet assay, and detected increased levels of DNA damage in the wood workers. Elavarasi et al. [2002] found that occupational exposure to wood dust causes increases in both lymphocyte MN and SCE frequencies. Another study showed an increase in the frequency of chromatid breaks in peripheral blood lymphocytes from nonsmoking wood workers [Kurttio et al., 1993]. Our findings are in general agreement with these previous studies, and indicate that buccal epithelial cells can be used to monitor the genotoxic effects of occupational exposure to wood dust.

Analysis of buccal epithelium cells also provides information about NCs, such as KR (fragmented chromatin structure), KL (dissolution of the nucleus), BE structures, and BN (two nuclei within a cell). Our previous work [Celik et al., 2003; Ünal et al., 2005] indicates that KR and KL are indicators of apoptosis. Apoptosis is a fundamental biological process that is genetically controlled and required for both normal development and tissue homeostasis [Cerqueira et al., 2004]. Our results suggest that wood dust exposure induced the apoptotic response, which may have interfered with MN induction. In the present study, we determined that the frequencies of KL and KR in the exposed and control groups were (7.87 ± 1.54)‰ and (1.92 ± 0.97)‰, and (9.30 ± 2.06)‰ and (2.05 ± 1.06)‰, respectively. Both frequencies were significantly increased in the exposed group.

The BE frequency also was increased in the exposed group, and again, smoking induced increased BE formation in buccal epithelium tissue of both the exposed and control subjects. This metanucleated abnormality has been described by Tolbert et al. [1992] and may be an early morphological stage in epithelial cell MN formation. Cerqueira et al. [2004] reported that BEs are involved in the normal process of tissue differentiation, but indicated that their mechanism of formation is not understood.

Our results indicate that wood dust exposure significantly increases the frequency of BNs in buccal epithelium, which we interpret as indicating an increase in cytotoxicity among cells from the wood workers. Similarly, smoking increases the frequency of BN cells in both the exposed and control groups. Several studies demonstrate that occupational or habitual exposure to chemical agents, such as benzene, pesticides, alcohol, and cigarette smoke, increases the frequency of BN cells and causes cytotoxicity in the buccal epithelium [Torres-Bugarin et al., 1998; Gómez-Arroyo et al., 2000; Ramirez and Saldanha, 2002; Celik et al., 2003].

In conclusion, occupational exposure to wood dust was associated with increased frequencies of micronuclei and NCs. Our results indicate that the epithelial tissue of wood workers could be at increased risk of genetic damage and that the risk may be mitigated by increases in apoptosis. We all learn about risks at an early age—how to recognize them and how to avoid them. Some risks are obvious and immediate: proximity to hot stoves, use of chain saws, driving on the highway. But other risks (especially those associated with cancer), like tobacco use and chemical and radiation exposure, result in delayed effects that are often hard to recognize. Although the control of dust exposure is not a simple task, particularly in small workplaces with poor hygiene and little or no ventilation, our results indicate that disease risk may be reduced by controlling dust in wood-working shops.

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