Metabolites of a Tobacco-Specific Lung Carcinogen in Nonsmoking Women Exposed to Environmental Tobacco Smoke

Kristin E. Anderson, Steven G. Carmella, Ming Ye, Robin L. Bliss, Chap Le, Lois Murphy, Stephen S. Hecht

Background: Environmental tobacco smoke (ETS) is associated with lung cancer in nonsmokers. Most epidemiologic studies find a higher risk for lung cancer in nonsmoking women married to smokers than in those married to nonsmokers. We measured metabolites of a tobacco-specific lung carcinogen in urine from healthy, nonsmoking women exposed to ETS. Methods: We recruited women and their partners through advertisements. Couples completed questionnaires on smoking history and demographics, and both partners provided 100 mL of urine; 23 women had male partners who smoked in the home (i.e., exposed women), and 22 women had male partners who did not smoke (i.e., unexposed women). Urine samples were analyzed for nicotine, for cotinine, for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide (NNAL-Gluc), as well as for creatinine. NNAL and NNAL-Gluc are metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane (NNK). Unpaired Student’s t tests were conducted on log-transformed values. All statistical tests are two-sided. Results: Urinary levels of nicotine, cotinine, NNAL, and NNAL-Gluc were statistically significantly higher in exposed women than in unexposed women. Geometric means for these compounds in exposed versus unexposed women, respectively, were as follows: nicotine, 0.050 nmol/mg of creatinine (95% confidence interval [CI] = 0.033 to 0.076) versus 0.008 nmol/mg of creatinine (95% CI = 0.004 to 0.014); cotinine, 0.037 nmol/mg of creatinine (95% CI = 0.022 to 0.061) versus 0.007 nmol/mg of creatinine (95% CI = 0.004 to 0.011); NNAL, 0.013 pmol/mg of creatinine (95% CI = 0.007 to 0.024) versus 0.004 pmol/mg of creatinine (95% CI = 0.002 to 0.007); and NNAL-Gluc, 0.027 pmol/mg of creatinine (95% CI = 0.016 to 0.045) versus 0.004 pmol/mg of creatinine (95% CI = 0.003 to 0.006). Conclusions: Nonsmoking women exposed to ETS take up and metabolize the tobacco-specific lung carcinogen NNK, which could increase their risk of lung cancer. Within couples, the NNAL plus NNAL-Gluc level in exposed women compared with that of their smoking partners averaged 5.6%. Notably, epidemiologic studies have estimated the excess risk for lung cancer in nonsmoking women exposed to ETS as 1%–2% of that in smokers. [J Natl Cancer Inst 2001;93:378–81]

Epidemiologic data (1–6) have shown an increased risk of lung cancer in nonsmokers exposed to environmental tobacco smoke (ETS). The consensus in the scientific community is that ETS is a carcinogen and increases the risk of lung cancer in nonsmoking women (2,7). Approximately 4000 compounds, including known and suspected human carcinogens, are present in cigarette smoke. The concentrations of many of these compounds are higher in sidestream smoke, the major constituent of ETS, than in mainstream smoke (1). However, ETS dissipates quickly; consequently, exposures to nonsmokers are much lower than to smokers (1). Therefore, biomarkers of human ETS exposure, ideally the agents implicated in the disease or outcome of interest, are important in the assessment of potential health risks (1).

Many studies (1,8,9) have shown that ETS biomarkers, such as urinary nicotine and cotinine, are higher in nonsmokers exposed to tobacco smoke than in unexposed nonsmokers. However, nicotine and cotinine are not carcinogenic. There are few reports on the presence of tobacco-specific carcinogens in nonsmokers exposed to ETS (10,11). The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane (NNK) is a powerful pulmonary carcinogen in rodents, inducing primarily adenocarcinoma of the lung, the most common type of lung cancer observed in nonsmokers. NNK is believed to have a major role in lung cancer in smokers (12). Urinary metabolites of NNK [4-(methylnitrosamino)-1- (3-pyridyl)-1-butanol (NNAL) and its glucuronide (NNAL-Gluc)] are excellent biomarkers of human uptake of this tobacco-specific lung carcinogen (12,13). NNAL, like NNK, is a potent pulmonary carcinogen. There are no sources of NNAL and NNAL-Gluc in human urine other than exposure to tobacco products. Thus, urinary NNAL and NNAL-Gluc are ideal biomarkers for recent lung carcinogen uptake by exposure to ETS.

In this study, we sought biochemical evidence that might explain the more than 30 epidemiologic studies (1–4) on lung cancer and passive smoking that have shown that spousal smoking increased risk of lung cancer in female nonsmokers. We conducted a study in nonsmoking women to test the hypothesis that urinary levels of nicotine, cotinine, and the tobacco-specific nitrosamine metabolites NNAL and NNAL-Gluc would be higher in women exposed to tobacco smoke from their male partner than in unexposed women. Because the epidemiologic studies of ETS-associated lung cancer have focused on women and because there may be some sex differences in lung cancer etiology, we restricted our study to women. These data were presented previously in an abstract (14).

Subjects and Methods

Study Design

The Institutional Review Board of the University of Minnesota, Minneapolis, approved this study protocol. We sought nonsmoking women with male partners who smoked in the home (i.e., exposed women) and nonsmoking women with male partners who did not smoke (i.e., unexposed women). Potential participants were recruited through local advertisements and screened for eligibility through an interview to ensure that women were not exposed to occupational ETS and that tobacco-specific ETS biomarkers were measured in urine.
Forty-five couples completed questionnaires on smoking history and demographics, and both partners provided 100 mL of urine; 23 women had male partners who smoked in the home, and 22 women had male partners who did not smoke. Urine samples from smokers and nonsmokers were segregated for laboratory analyses to eliminate the possibility of carryover (false-positives) that may occur when samples with high and low levels are analyzed in the same assay set. Samples within these two groups were assayed in a blinded manner.

**Analysis for Nicotine, Cotinine, and Creatinine**

Aliquots of urine (0.1–0.5 mL) were treated with 0.15 N NaOH for 30 minutes at 80°C and then analyzed for nicotine plus nicotine-N-glucuronide and cotinine plus cotinine-N-glucuronide as described previously (15). Creatinine was determined by Fairview-University Medical Center Diagnostic Laboratories (Minneapolis, MN) using Vitros CREA slides (Johnson & Johnson Clinical Diagnostics, Rochester, NY).

**Analysis for NNAL and NNAL-Gluc**

These analyses were carried out essentially as described previously (10,11,16). Previous analyses have indicated that NNAL and NNAL-Gluc levels are substantially lower in nonsmokers than in smokers; thus, 20-mL samples from nonsmokers and 5-mL samples from smokers were analyzed. The normal-phase high-pressure liquid chromatography purification step for the urine samples from the non-smoking women was carried out with a Luna silica normal-phase high-pressure liquid chromatography column (Phenomenex, Torrance CA), 5-μm particle size, 4.6 x 250-mm internal diameter, instead of that described previously by Carmella et al. (16). For the internal standard, we used 1 ng of 4-(methylnitrosamine)-4-(3-pyridyl)-1-butanol (iso-NNAL).

**Statistical Methods**

For individuals with levels below the limit of detections, half of the minimal detectable level was added to calculate mean values. The metabolite levels in the four groups of subjects (males: smokers and nonsmokers; females: exposed and unexposed) were not normally distributed. The data were log-transformed to satisfy the assumptions of normality and equality of variance. Mean values and 95% confidence intervals (CIs) back-transformed to the original scale are reported in Table 1. Untransformed mean values and standard deviations (SDs) are included as well to facilitate comparison with other studies. Unpaired Student’s t statistics and corresponding P values were calculated from the analysis of log-transformed values. Analysis of covariance on transformed values was used to compare metabolite measures between women with and without exposure, adjusting for the age of the subject.

Because males and females were selected as pairs, comparisons of cotinine and NNAL plus NNAL-Gluc in exposed women relative to their smoking partners were calculated as the average of the within-couple ratios. Analysis of variance on transformed values was used to compare metabolite measures among women with and without leisure-time exposure by their partner’s smoking status. Pearson’s correlation coefficients were calculated to determine the association between cotinine and NNAL plus NNAL-Gluc. All statistical tests are two-sided.

**RESULTS**

The mean age of the 23 exposed women was 48.3 years (range = 21–66 years), and the mean age of the 22 unexposed women was 33.4 years (range = 21–63 years). Male smokers had a mean age of 48.1 years (range = 21–73 years), and male nonsmokers had a mean age of 33.7 years (range = 22–60 years). The mean number of cigarettes smoked per day by the male smokers was 25 (range = 10–45), and the SD was 11.2. Exposed women were Caucasian (n = 21), Hispanic (n = 1), and American Indian.

**Table 1. Levels of nicotine, cotinine, and 4-(methylnitrosamine)-4-(3-pyridyl)-1-butanol (NNAL) plus NNAL-glucuronide (NNAL-Gluc) in the urine of nonsmoking women**

<table>
<thead>
<tr>
<th></th>
<th>Nicotine (nmol/mL)</th>
<th>Cotinine (nmol/mL)</th>
<th>NNAL + NNAL-Gluc (pmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exposed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 23</td>
<td>Mean (SD)</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td></td>
<td>0.046 (0.062)</td>
<td>(0.020 to 0.072)</td>
<td>(0.004 to 0.011)</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>0.029</td>
<td>0.012 (0.019)</td>
<td>0.006 (0.009)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(0.013 to 0.076)</td>
<td>(0.003 to 0.009)</td>
<td>(0.004 to 0.011)</td>
</tr>
<tr>
<td><strong>Unexposed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 22</td>
<td>Mean (SD)</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td></td>
<td>0.012 (0.010)</td>
<td>(0.004 to 0.014)</td>
<td>(0.004 to 0.010)</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>0.006</td>
<td>0.006</td>
<td>0.005</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(0.004 to 0.011)</td>
<td>(0.004 to 0.014)</td>
<td>(0.004 to 0.010)</td>
</tr>
</tbody>
</table>

*Mean (standard deviation [SD]) and geometric mean (95% confidence interval [CI]) of nicotine, cotinine, and NNAL plus NNAL-Gluc in the urine of women who live with smokers (n = 23, exposed) or women who live with nonsmokers (n = 22, unexposed). Differences in the levels of the compounds from tobacco smoke between exposed and unexposed women were statistically significant, as follows: nicotine (P < .001); cotinine (P = .002); and NNAL plus NNAL-Gluc (P < .001). Geometric means (95% CI) for NNAL were 0.013 pmol/mg of creatinine (0.007 to 0.024) in exposed women and 0.004 pmol/mg of creatinine (0.002 to 0.007) in unexposed women (P = .027); for NNAL-Gluc, they were 0.027 pmol/mg of creatinine (0.016 to 0.045) in exposed women and 0.004 pmol/mg of creatinine (0.003 to 0.006) in unexposed women (P = .001). These values are statistically significantly different. All statistical tests are two-sided. Nicotine, cotinine, and NNAL plus NNAL-Gluc are reported per milliliter of urine and per milligram of creatinine. The limit of detection for nicotine as well as for cotinine is 0.5 ng/mL of urine (0.003 nmol/mL of urine). The limit for NNAL and NNAL-Gluc is less than 0.005 pmol/mL of urine. NNAL plus NNAL-Gluc is the sum of the individual values for NNAL and NNAL-Gluc. A supplementary table containing individual levels of nicotine, cotinine, and NNAL plus NNAL-Gluc in the urine of all nonsmoking women in this study can be found on the Journal’s web site <http://jnci.oupjournals.org>.
dian (n = 1). Unexposed women were Caucasian (n = 18), Asian (n = 2), mixed ethnicity (n = 1), and missing ethnicity (n = 1).

Cotinine levels measured in this study were consistent with the self-reported smoking status of all participants. Levels of both cotinine and nicotine were in line with other published data on smokers, nonsmokers, and nonsmokers exposed to ETS (1,2,8). The geometric mean level of cotinine in males who were smokers was 13.70 nmol/mg of creatinine (95% CI = 9.51 to 19.70) and in males who were nonsmokers was 0.007 nmol/mg of creatinine (95% CI = 0.004 to 0.013); the level of NNAL plus NNAL-Gluc in smokers was 1.70 pmol/mg of creatinine (95% CI = 1.31 to 2.21) and in nonsmokers was 0.007 pmol/mg of creatinine (95% CI = 0.004 to 0.011).

Women with smoking partners had statistically significantly higher mean levels than women with nonsmoking partners for all compounds measured except creatinine (Table 1). Adjustment for age did not substantively alter any of the findings, and all differences remained statistically significant (data not shown). The geometric mean levels of tobacco smoke-related compounds in exposed and unexposed women, respectively, were as follows: for cotinine, 0.037 nmol/mg of creatinine (95% CI = 0.022 to 0.061) and 0.007 nmol/mg of creatinine (95% CI = 0.004 to 0.011); for nicotine, 0.050 nmol/mg of creatinine (95% CI = 0.033 to 0.076) and 0.008 nmol/mg of creatinine (95% CI = 0.004 to 0.014); and for NNAL plus NNAL-Gluc, 0.045 pmol/mg of creatinine (95% CI = 0.027 to 0.073) and 0.007 pmol/mg of creatinine (95% CI = 0.004 to 0.010). When geometric means were compared, the levels of cotinine, nicotine, and NNAL plus NNAL-Gluc were fivefold to sixfold higher in exposed women than in unexposed women. Results for nitrosamine levels per milliliter of urine were comparable to the results standardized by creatinine. In male smokers, cotinine levels correlated with NNAL plus NNAL-Gluc levels (r = .53; P<.014), but these levels were not correlated in the ETS-exposed females (r = -.03; P = .90).

Consistent with previous reports (8), cotinine levels in exposed women were approximately 0.6% of the levels seen in their smoking male partners. Further comparison within couples revealed that NNAL plus NNAL-Gluc in ETS-exposed women was, on average, 5.6% of that in their male smoking partners.

Women with routine workplace exposure (e.g., exposure in bars, restaurants, or casinos) were not eligible for the study. Among the women included in the study, only three of the 23 exposed women and none of the 22 unexposed women reported any exposure to smoke at work; excluding these three women did not substantively alter the results (data not shown).

We excluded women from this study if they reported extensive leisure-time exposure to ETS away from home, but we examined possible differences between women who reported some leisure-time exposure and those who did not. Exposed women and unexposed women did not differ substantively with respect to the proportion that self-reported leisure-time exposure to tobacco smoke (data not shown).

We compared levels of the various metabolites in urine (standardized by creatinine) between women whose partner usually smoked when they were together in the same room and women whose partner usually smoked elsewhere in the home. Men who smoked in the same room as their partner tended to smoke more cigarettes per day than men who smoked elsewhere in the home. The number of cigarettes smoked per day by each group (mean ± SD) was 29.5 ± 11.1 and 21.2 ± 10.0 (P = .07), respectively. Levels of both cotinine and NNAL were statistically significantly higher in women whose partners smoked when they were together in the same room than levels in women whose husbands smoked elsewhere; however, the differences in levels of NNAL-Gluc and of NNAL plus NNAL-Gluc were not statistically significant. Importantly, both subgroups of exposed women had statistically significantly higher mean values than unexposed women on all measures, except for NNAL in women whose husbands smoked elsewhere.

**DISCUSSION**

In this study of nonsmoking women, we showed that urinary levels of NNAL and NNAL-Gluc, metabolites of the tobacco-specific lung carcinogen NNK, are statistically significantly higher in women who are exposed to their partner’s cigarette smoke than in unexposed women. The geometric mean level of NNAL plus NNAL-Gluc was approximately sixfold higher in the exposed women than in unexposed women. Like NNK, NNAL is a potent pulmonary carcinogen in animals (12,13) and a likely human lung carcinogen. NNK induces mainly adenocarcinoma of the lung in laboratory animals, and adenocarcinoma is the tumor type most frequently observed in female nonsmokers. Other than exposure to tobacco products and ETS, to our knowledge, there is no other source of urinary NNAL and NNAL-Gluc. Thus, these data provide biochemical evidence indicating that exposure to ETS increases the levels of NNK metabolites in exposed nonsmoking women, which may increase their risk of lung cancer.

Epidemiologic studies have estimated that the excess risk for lung cancer in women exposed to ETS is about 20% higher than the risk in unexposed women (2,7). This value is 1%–2% of the excess risk for lung cancer in smokers (1400%–1900%) compared with nonsmokers (2,7). Notably, the amount of NNAL plus NNAL-Gluc in the urine of the exposed women in this study was 5.6% of that in the urine of their partners who smoked.

 Urinary levels of cotinine and nicotine measured in this study were consistent with past reports (1,7). The geometric mean level of cotinine in the ETS-exposed women was approximately fivefold higher than in the unexposed women.

 Dietary sources can contribute to cotinine and nicotine levels. Plants of the Solanaceae family and tea contain nicotine. The average contribution of dietary nicotine to urinary cotinine is expected to be less than 1 ng/mL of urine with very high consumption of nicotine-containing foods resulting in maximum levels of 6 ng/mL of urine (1). Although we did not collect dietary data, we think it unlikely that the differences that we observed reflect high levels of tea and particular plant food consumption selectively in the exposed women. Moreover, such dietary considerations are moot with respect to NNK and its metabolites: They are not known to exist in plants other than tobacco and are considered to be unambiguous markers of tobacco exposure (12,13). The levels of NNK metabolites that we found in exposed women may underestimate their average exposure over a period of a year because 18 of the 23 samples were collected during spring or summer months when ventilation in Minnesota may be better than in fall or winter months.
The statistics do not indicate that leisure-time exposure is associated with higher levels of any measures in women exposed and unexposed to cigarette smoke at home, but these data were based on small numbers and women with substantial leisure-time exposures were excluded from this study. Women whose husbands usually smoked in the same room had higher cotinine levels than women whose husbands usually smoked elsewhere in the home. Although differences in NNAL plus NNAL-Gluc levels were not statistically significantly different between these subgroups of women, both subgroups showed statistically significant differences when compared with unexposed women.

The numbers of participants in any of these subgroup analyses are small, and although such considerations may be of interest for future studies, our primary hypothesis involved comparisons of women exposed and unexposed to ETS by their male partner in a domestic setting. Our findings demonstrate that urinary levels of all of the compounds analyzed, including NNAL and NNAL-Gluc, differ statistically significantly between these two groups of women.

The actual dose of ETS received by individuals is complex and dependent on many factors, including smoking behavior, room size, and ventilation. Even when conditions are identical, different people exposed to the same concentrations of ETS receive different doses related to such factors as age, activity level, and breathing rate. In addition to differences in ETS exposure, functional polymorphisms in enzymes that metabolize carcinogens may cause interindividual variation in the mutagenic dose. Particularly because of such complexity, NNAL and NNAL-Gluc may be useful biomarkers in studying exposure to ETS and associations with cancer in nonsmokers.

REFERENCES

(2) Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. Health effects of exposure to environmental tobacco smoke. Sacramento (CA): California Environmental Protection Agency; 1997.

NOTES

Supported by Public Health Service grant CA81301 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. S. S. Hecht is an American Cancer Society (ACS) Research Professor supported by an ACS grant (RP-00-138).

We are grateful to Carol Hansen and Gina Atwood for help in facilitating this project and preparing the manuscript.

Manuscript received June 16, 2000; revised December 19, 2000; accepted December 26, 2000.