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Tobacco-specific nitrosamines, hemoglobin adducts and exposure to environmental tobacco smoke

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Abstract

Hemoglobin (Hb) adducts of 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB), a metabolite of two tobacco-specific nitrosamines: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN), were measured in blood samples collected from 47 non-smokers, including 21 cases of lung cancer, enrolled in four centers. Exposure to environmental tobacco smoke (ETS) during the previous year from the spouse and at the workplace was assessed through questionnaire. Non-smokers exposed to ETS had a lower and non-significant level of HPB-Hb adducts than unexposed non-smokers (medians 15.8 and 20.1fmol/g Hb, p-value of the difference 0.32). Adjustment for age, sex, center and lung cancer case-control status had no effect on the results. Our results appear not to support the use of HPB-Hb adduct level as a marker of exposure to ETS in non-smokers. The conclusion drawn is however based on a small number of subjects and might suffer from exposure misclassification.

Key words: Environmental tobacco smoke, hemoglobin adducts, tobacco-specific nitrosamines, biomarkers

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Abbreviations: Hb, hemoglobin; ETS, environmental tobacco smoke; HPB, 4-hydroxy-1-(3-pyridyl)-1- butanone; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N'-nitrosonornicortine; OR, odds ratio; Cl, 95% confidence interval.

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INTRODUCTION

Exposure to environmental tobacco smoke (ETS) has been associated with increased risk of lung cancer (7). ETS contains measurable amounts of human carcinogens, many of which have been shown to covalently bind to DNA (4). A methodological problem in the study of exposure to ETS is the lack of a sensitive and reliable indicator for the biologically effective dose. The biologically effective dose reflects the amount of carcinogen that had interacted with DNA. A proposed solution to this problem is the application of analytical methods that measure the binding of tobacco-specific carcinogens to macromolecules such as DNA or proteins.

The analytical method applied in this study measures the level of adducts with hemoglobin (Hb) of 4-hydroxy-1- (3-pyridyl)-1-butanone (HPB) (1). HPB is a common metabolite of two nitrosamines found only in tobacco: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and nitrosonornicotine (NNN). NNK is a potent pulmonary carcinogen in rodents (9). Both nitrosamines might be important in human lung carcinogenesis (3). HPB has not been detected as a metabolite of other nitrosamines. Therefore, HPB should provide a specific marker for the metabolic activation of NNK and NNN (9). Although the persistence of HPB-Hb adducts has not been adequately determined in humans, studies have estimated that that Hb adducts of the aromatic amine 4-aminobiphenyl, also formed as a result of exposure to tobacco smoke, has a half-life of seven to nine weeks in humans (10,11). Thus the concentration of HPB released from Hb should give an indication of the amount of tobacco-specific nitrosamines, NNK and NNN, to which an individual has been exposed during the last few months.

Previous studies from the United States (3,6) and Germany (5,14) have shown that smokers have a higher level of HPB-Hb adducts than non-smokers. Although a transitional study was conducted (15) with the aim to assess the potential role of HPB-Hb adducts as a marker of the biologically effective dose of ETS in non-smokers, no data is currently available on the level of this biomarker in non- smokers exposed to ETS.

MATERIALS AND METHODS

For this analysis, 47 human subjects from four countries (Sweden, Russia, Romania and Germany) were selected within the framework of a multi-center, collaborative study on genetic alterations and susceptibility to lung cancer among non-smokers. Twenty-one had been diagnosed of having primary lung cancer, 12 were hospital patients with a disease not related to tobacco smoking, and the remaining 14 participants were apparently healthy subjects.

All participants were interviewed using a standard questionnaire designed to gather information on active smoking, ETS exposure during childhood and adulthood, as well as occupational exposures, residential history and family history of cancer. Non-smokers were designated as subjects not having smoked more than one cigarette per day during one year (or approximately 400 cigarettes during life), or the equivalent as cigars, cigarillos and pipe tobacco. The questionnaire on ETS was validated using measurement of urinary cotinine (13). We considered regular exposure to ETS during the previous year. We restricted the analysis to exposure from the spouse and at the workplace, the two main sources of ETS identified in a previous validation study (13). Quantitative information on exposure to ETS from the spouse, expressed as a number of cigarette smoked in the presence of the study subject were collected.

Details of the assay used in the analysis of HPB-Hb adducts from red blood cells (RBC) have been published previously (13). Briefly, a 30-ml blood sample was collected from study subjects; white blood cells, red blood cells and plasma were separated and frozen at -80^0C in each collaborating center. After thawing of the samples, Hb was purified by dialysis and HPB was released from Hb under alkaline conditions. After clean up with solid-phase extraction, the HPB-pentafluorobenzoate derivative was formed before quantification by gas chromatography/negative ion chemical ionization mass spectrometry. The amount of HPB released from a particular sample of Hb was expressed as the amount of HPB in fentimol (fmol) divided by the amount of Hb in the samples in grams. The detection limit for HPB

was 1 pg HPB/injection or about 9 fmol HPB/g Hb, depending on the amount of Hb in the particular sample analyzed. The coefficient of variation of reproducibility of paired aliquots from the same sample ranged between 7% and 25%, the within-sample reproducibility of 4 and 8 aliquots was 4% and16% (13).

For 25 subjects for whom duplicate samples were analyzed, the mean of two values was recorded as the subject whose adduct levels were at or below the method's detection limit (9 fmol HPB/g Hb), the detection limit value was used for statistical analysis. Mean and median HPB-Hb adduct values were calculated for groups of subjects defined according to ETS exposure, and presence of lung cancer. Differences between groups were determined using non-parametric (Kruskal-Wallis and Mann-Whitney) tests. In addition, multivariate linear regression analysis was conducted to assess the contribution of ETS exposure on (log transformed) HPB-Hb adducts level after controlling for other possible determinants of adducts. A statistical package (16) was used to analyze the data by the above named statistical models.

RESULTS

The mean age of study subjects was 62.0 (range 34-84). The overall mean value of HPB-Hb adducts was 19.8±7.8 fmol/g Hb (median 19.5 fmol/g Hb). Table 1 shows the median adducts levels by age, gender, center and presence of lung cancer. There were small, non- significant differences in adduct level according to age, lung cancer, status and center. A total of 13 subjects reported regular exposure to ETS during the last year: 6 from the spouse and 7 at the workplace. Table 2 shows the results of the comparison between non-smokers exposed to ETS and unexposed non-smokers. HPB-Hb adduct levels were non-significantly lower in ex-smokers and never smokers than in current smokers. The results of multiple linear regression analysis, which included adjustment for age, sex, center, and presence of lung cancer were similar to those reported in Table 2; the regression coefficient for ETS exposure was –0.15 (standard error 0.14, p=0.29). We had quantitative information on ETS exposure among subjects exposed to ETS from the spouse (N=6): there was no correlation between ETS exposure and HPB-Hb adduct level (p-value of Spearman correlation coefficient 0.50).

Table 1: Median level of HPB-Hb adducts (fmol/g Hb) according to age, gender, center and presence of lung cancer.

	No. of subjects	**HPB-HB adduct level**	**p-value of difference∗**
Age group			
<60	18	15.3	
60-69	18	17.8	
70+	11	23.4	0.20
Gender			
Male	9	16.0	
Female	38	19.9	0.90
Center			
Sweden	11	23.0	
Germany	9	24.8	
Russia	12	19.9	
Romania	15	15.9	0.22
Presence of lung cancer			
No	26	15.9	
Yes	21	21.2	0.38

∗ Two-sided p-value based on Mann-Whitney or Kruskal-Wallis test.

Table 2: Median and mean of HPB-Hb adducts (fmol/g Hb) according to exposure to environmental tobacco smoke∗

	No. of subjects	Median	Mean	Standard deviation	p-value of difference∗∗
No exposure	34	20.1	20.6	8.1	
Exposure	13	15.8	17.8	7.0	0.32

∗ Exposure from the spouse or at the workplace during the previous year. ∗∗ Two-sided p-value based on Mann-Whitney test

DISCUSSION

This transitional study addresses the possible role of Hb adducts formed by tobacco-specific nitrosamines as a biological marker of exposure to ETS. Since HPB is a metabolite of two nitrosamines found only in tobacco smoke, NNK and NNN we hypothesized that HPB-Hb adducts might be useful to integrate low-level of exposure to tobacco smoke. Our results indicate that HPB-Hb levels are slightly lower in non-smokers exposed to ETS than in unexposed non-smokers.

Previous studies on HPB-Hb adducts as a marker of tobacco smoking revealed a contrast between smokers and non-smokers. Foiles and co-workers (6) found higher mean levels of HPB-Hb adducts in 100 smokers (163 fmol/g Hb) and 37 non-smokers (68±26 fmol/g Hb) in the United States. Two additional studies conducted in volunteers and pregnant women in Germany showed mean levels in smokers and non-smokers similar to those of the American study (5,14). A direct comparison with our results is complicated due to lack of a standard definition of non-smoker, and of the lack of data on the age distribution and source of enrollment of the subjects in these previous studies. It is unlikely however that these factors explain completely the differences between our results and those of previous studies. During method validation, we measured HPB-Hb adduct levels in blood samples taken at random from the general population to validate our method (1), and the results were comparable to those reported in previous studies. We think therefore that the lower level among non-smokers in this study as compared to previous studies are not explained by differences in the assay used to quantify HPB-Hb adduct levels. The more stringent definition of non-smokers in this study might be an explanation. In addition, it is possible that the lower level reflects decay of adducts during storage of samples. The samples analyzed in this study were stored for approximately one year at -80^0C, while samples analyzed in previous studies have been mainly stored for a short period.

An innovative part of our study was the attempts to correlate HPB-Hb adduct level with ETS exposures in non-smokers. We found a non-significant decrease in adduct level in non-smokers exposed to ETS as compared to unexposed non-smokers. We assessed ETS exposure via a standardized questionnaire which was validated against urinary cotinine levels (13) and was used in a study in which an association was shown between ETS exposure and lung cancer risk (2). In this study, attention was concentrated on ETS exposure from the spouse and at the workplace, which are the main predictors on urinary or serum cotinine in non-smokers (12,13). However, we did not have information on very recent exposure to tobacco and ETS and the analysis was based on reported yearly exposure, which might have resulted in non-differential misclassification of exposure. Lack of sensitivity of the assay to detect the small effect, if any, of ETS on HPB-Hb adducts and limited statistical power are additional possible explanations for our findings.

It could imply that a higher level of HPB-Hb adducts is a marker of susceptibility to tobacco carcinogenesis. NNK and NNN are compounds strongly suspected to be responsible for at least part of the carcinogenic properties of tobacco smoke. NNK is highly carcinogenic to the rodent lung, and causes cancer in rats, hamsters and mice independent on the route of administration, and NNN is an important esophageal carcinogen in rodents (8). It should also be considered that estimation of DNA adduct levels formed by tobacco-specific nitrosamines from Hb adduct levels is complex (10), as Hb adduct levels do not exactly reflect DNA adduct levels and, consequently, possible DNA damage. Such misclassification, however, would result in an underestimation of the association between DNA adducts, as measured by Hb adducts, and lung cancer risk.

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