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Mini-review

Tobacco smoke biomarkers and cancer risk among male smokers in the Shanghai Cohort Study

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ABSTRACT

Metabolites of tobacco smoke constituents can be quantified in urine and other body fluids providing a realistic measure of carcinogen and toxicant dose in a smoker. Many previous studies have demonstrated that these metabolites – referred to as biomarkers in this paper – are related to tobacco smoke exposure. The studies reviewed here were designed to answer another question: are these substances also biomarkers of cancer risk? Using a prospective study design comparing biomarker levels in cancer cases and controls, all of whom were smokers, the results demonstrate that several of these biomarkers – total cotinine, total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), *r*-1-,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT), and total *N*′-nitrosonornicotine (NNN) – are biomarkers of cancer risk. Therefore, these biomarkers have the potential to become part of a cancer risk prediction algorithm for smokers.

1. Introduction

Tobacco products are responsible for 22% of all cancer death worldwide and 30% of cancer mortality in the United States http://www.who.int/mediacentre/factsheets/fs297/en/index.html [1]. At least 18 different types of cancer are caused to varying extents by tobacco products [2]. This paper focuses on lung and esophageal cancer. Approximately 90% of lung cancer mortality in populations with prolonged use is attributed to cigarette smoking [3]. More than 3000 people in the world succumb to this deadly and relatively incurable disease daily [4]. Cigarette smoking is also an important cause of esophageal cancer, both squamous cell carcinoma and adenocarcinoma [3].

In addition to the addictive but non-carcinogenic compound nicotine, cigarette smoke contains over 70 established carcinogens, including tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons (PAHs), and volatile carcinogens such as 1,3-butadiene and benzene [5]. There is no doubt that daily exposure to these compounds is a major cause of cancer in smokers. Professor Ramesh Gupta has made important contributions to our understanding of the relevant DNA damage mechanisms [6–9]. However, we have little ability to predict which of the 1.3 billion smokers in the world will actually get cancer. It is axiomatic in toxicology that "the dose makes the poison" and therefore it is logical that susceptibility to cancer in smokers is a function of carcinogen dose. Tobacco carcinogen and toxicant biomarkers – quantitative measures of tobacco constituents or their metabolites in body fluids – can inform us about dose much more accurately than counting numbers of cigarettes smoked, even if that information could be reliably obtained [10]. This is because there are wide differences in carcinogen and toxicant delivery among different tobacco products depending on their characteristics and the ways in which they are smoked. In the studies reviewed here, we focused on a panel of tobacco carcinogen and toxicant biomarkers representing some of the most important compounds in cigarette smoke.

The structures of the tobacco carcinogen and toxicant biomarkers considered here are shown in Fig. 1 [10]. Cotinine and cotinine-*N*-glucuronide are metabolites of nicotine; their sum is "total cotinine." Total cotinine is a reliable measure of uptake of the addictive compound nicotine. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides are metabolites of the potent tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK); their sum is "total NNAL." Total NNAL is a well established biomarker of NNK uptake. NNN-*N*-glucuronide is a metabolite of the tobacco-specific esophageal and oral cavity carcinogen *N*′-nitrosonornicotine (NNN); values for each of these are reported. *r*-1,*t*-2,3,*c*-4-Tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT) is a metabolite of phenanthrene, structurally related to carcinogenic PAH [11]. This metabolite is formed by the diol epoxide metabolism pathway, believed to be important in the metabolic activation to DNA binding intermediates of carcinogenic

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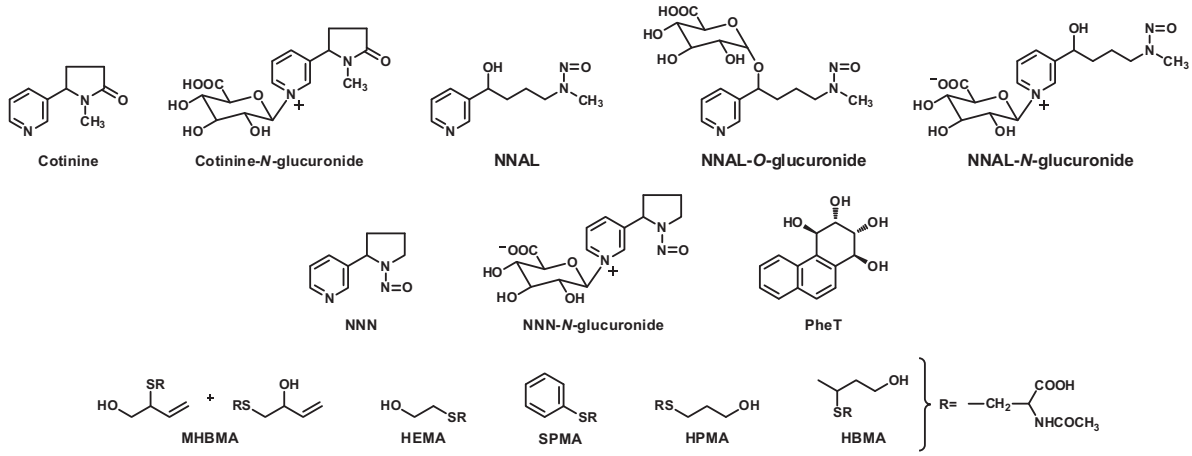

Fig. 1. Chemical structures of the tobacco carcinogen and toxicant biomarkers discussed in the text.

PAH. The mercapturic acids shown in Fig. 1 are metabolites of the tobacco smoke volatiles 1,3-butadiene, ethylene oxide, benzene, acrolein, and crotonaldehyde, respectively [12]. 1,3-Butadiene, ethylene oxide, and benzene are considered carcinogenic to humans by the International Agency for Research on Cancer, based mainly on occupational studies and mechanistic data related to hematopoietic malignancies [13,14]. Acrolein is a highly toxic but marginally carcinogenic compound while crotonaldehyde is a relatively weak hepatocarcinogen [15]. Many studies have shown that concentrations of all of the biomarkers discussed here are higher in the urine and other body fluids of smokers than non-smokers and that in most cases their levels significantly decrease upon cessation of smoking [10]. Therefore, these substances are clearly biomarkers of tobacco smoke exposure and dose. But are they also biomarkers of cancer risk? We addressed this question in the studies reviewed here by quantifying tobacco carcinogen and toxicant biomarkers in pre-diagnostic urine samples from the Shanghai Cohort Study [16–20].

2. Approach

The Shanghai Cohort Study enrolled 18,244 men between January 1, 1986 and September 30, 1989 [20,21]. They were between 45 and 64 years old and lived in one of four geographically defined communities in Shanghai, China. In-person interviews were conducted and a urine sample was obtained from each subject upon enrollment. Cases of lung cancer or esophageal cancer were identified annually by in-person re-interviews of all surviving cohort members and review of reports from the Shanghai Cancer Registry and the Shanghai Municipal Vital Statistics Office. For the lung cancer studies, we identified cases who smoked cigarettes at baseline and then randomly selected one control subject for each case from all cohort members who were current smokers at enrollment, free of cancer, and alive at the time of cancer diagnosis of the index case. Controls were matched to the index case by age at enrollment, date of urine collection, and neighborhood of residence at recruitment. The selection of controls was the same for the esophageal cancer study, except that three control subjects were randomly selected for each case in order to increase statistical power due to the smaller number of esophageal cancer than lung cancer cases.

Urine samples were retrieved from the biospecimen bank and analyzed for the following tobacco smoke carcinogen and toxicant biomarkers (Fig. 1): total cotinine (the sum of cotinine and cotinine-*N*-glucuronide); total NNAL (the sum of NNAL, NNAL-*O*-glucuronide and NNAL-*N*-glucuronide); PheT; free NNN and total NNN (the sum of free NNN and NNN-*N*-glucuronide); 1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene and 1-(*N*-acetylcysteinyl)-2-hydroxy-3-butene (collectively called MHBMA for monohydroxybutyl mercapturic acid, metabolites of 1,3-butadiene); 2-hydroxyethyl mercapturic acid (HEMA), a metabolite of ethylene oxide; *S*-phenyl mercapturic acid (SPMA), a metabolite of benzene; 3-hydroxypropyl mercapturic acid (HPMA), a metabolite of acrolein; and 4-hydroxybut-2-yl mercapturic acid (HBMA), a metabolite of crotonaldehyde. The analyses were carried out as previously described [10,16–19].

For total cotinine, total NNAL, and PheT there were urine samples from 476 lung cancer cases and 476 controls. Due to sample depletion, there were 392 urine samples from lung cancer cases and 343 from controls for measurement of mercapturic acids. For total NNN, there were urine samples from 74 esophageal cancer cases and 218 from controls.

3. Results

Urinary levels of all biomarkers increased significantly with numbers of cigarettes smoked per day. Furthermore, levels of all urinary biomarkers were significantly higher in cancer cases than in controls.

The results of the lung cancer study are summarized in Table 1. Total cotinine, total NNAL, and PheT were all independently and significantly associated with lung cancer risk, even after adjustment for number of cigarettes smoked per day, number of years of smoking, and the other two biomarkers (e.g., results for total cotinine adjusted for total NNAL and PheT). The odds ratios in the highest versus the lowest tertile in the adjusted models were 2.97 for total cotinine, 1.67 for total NNAL, and 1.69 for PheT. The trend for increasing odds ratios with increasing tertile of biomarker were statistically significant in all cases, $P < 0.0001$ for total cotinine, $P = 0.0436$ for total NNAL, and $P = 0.0157$ for PheT.

All of the mercapturic acids were significantly associated with lung cancer risk after correction for number of cigarettes smoked per day and number of years of smoking. The odds ratios in the highest versus the lowest tertile were 1.44 for MHBMA, 1.81 for HEMA, 1.59 for SPMA, 1.67 for HPMA, and 1.87 for HBMA. However, adjustment for cotinine resulted in a null association for all of the mercapturic acids with lung cancer risk.

The results of the esophageal cancer study are summarized in Table 2. Total NNN and free NNN were significantly associated with esophageal cancer risk, even after adjustment for number of ciga-