Regulatory Toxicology and Pharmacology 68 (2014) 269–274

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

TSNA exposure from cigarette smoking: 18 Years of urinary NNAL excretion data

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ARTICLE INFO

Article history:
Received 29 April 2013
Available online 3 August 2013

Keywords:
Cigarettes
NNAL
NNN
NNK
Tobacco products
Tobacco specific nitrosamines (TSNA)

ABSTRACT

The objective of this work was to characterize trends over time in urinary excretion of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) among cigarette smokers in the US. We identified 35 studies presenting data that either reported, or could be converted to, common units of total urinary NNAL excretion as pmol/mg creatinine. The studies spanned 18 years, reported urinary NNAL excretion estimates for 61 defined populations, and included a combined total of 3941 study participants. Analyses show that urinary NNAL excretion trends downward with study publication year, and the trend is statistically significant. The trend does not appear to be accounted for by a reduction in cigarettes smoked per day by study participants over the same time period. This trend is consistent with reductions in tobacco specific nitrosamine (TSNA) levels in both cigarette tobacco filler and mainstream cigarette smoke observed over the past decade and with efforts by the tobacco industry and the agricultural community to reduce levels of TSNAs in tobacco and cigarette smoke.

1. Introduction

Tobacco specific nitrosamines (TSNAs) are a class of cigarette smoke constituents believed to play a potential role in smoking-related carcinogenesis (IARC, 2007). The TSNAs, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*-nitrosonornicotine (NNN), have received the greatest attention due to their carcinogenic activity in animal studies. Both are classified by the International Agency for Research on Cancer as carcinogenic to humans (Group 1), and both are on the US Food and Drug Administration list of harmful and potentially harmful constituents in tobacco products and tobacco smoke (IARC, 2007; USDHHS, 2012).

We previously reported that TSNA levels have declined significantly in mainstream cigarette smoke over at least the last decade (Appleton et al., 2013). The objective of this work was to characterize trends in urinary excretion of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) among cigarette smokers to determine possible changes in exposure to TSNAs among cigarette smokers over time. We gathered and analyzed available published data on urinary NNAL excretion among cigarette smokers over an 18-year period. Total urinary NNAL (sum of NNAL and NNAL-glucuronide) has been reported to be a specific biomarker for exposure to NNK (Hecht, 2002) and has been widely used as a biomarker indicative of TSNA exposure from cigarette smoking. It has also been reported to be a biomarker of risk for lung cancer (IARC, 2009).

2. Methods

We searched PubMed Central® (US National Institutes of Health, National Library of Medicine; http://www.ncbi.nlm.nih.gov/pubmed) for published papers reporting urinary NNAL excretion data in cigarette smokers. Studies were also identified as referenced in reports identified in the PubMed search and from other sources.

We used the following criteria for inclusion of studies identified in the literature:

- Study was original research.
- Study reported urinary excretion of total NNAL (sum of NNAL and NNAL-glucuronide).
- Study participants were adult cigarette smokers.
- Study conducted in the US of participants smoking US commercial cigarettes.
- Cigarettes were smoked under natural conditions (no forced smoking regimens).

Abbreviations: NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*-nitrosonornicotine; CPD, cigarettes per day; TSNA, tobacco specific nitrosamine.

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0273-2300
http://dx.doi.org/10.1016/j.yrtph.2013.07.013

- Only baseline data was used from intervention studies.

Published studies were examined to determine if a data set for one or more defined populations had been previously published. In cases where a data set was clearly identified as having been previously published, the default approach was to use only the data from the earliest publication. Because our objective was to compare levels of urinary NNAL excretion from different studies over time, the analysis was confined to NNAL data that were either reported as, or could be converted to, common units suitable for comparison.

Urinary NNAL excretion estimates for different defined populations (e.g., male vs. female, Black vs. White) that were reported separately in the original publications were handled as separate population estimates with no attempt to average or merge individual estimates. If both individual population estimates and an overall population estimate were presented, only the highest-order population estimate was used in the analysis. In cases where only individual subject data were presented, we calculated group means. Likewise, in cases where values for NNAL and NNAL-glucuronide were only presented separately as molar units, we calculated the sum of the two to obtain total NNAL.

The data were analyzed for trend with year of publication using a general linear model in a standard statistical analysis software package (IBM SPSS Statistics, Armonk, NY). In the primary analysis the dependent variable of the model was NNAL level in pmol/mg creatinine, and the covariates were study publication year and cigarettes per day (CPD). The data points were weighted by the number of study participants.

3. Results

Our literature search identified 52 potentially relevant study publications. Among these publications, a wide variety of units were used to express urinary NNAL excretion. Twenty-nine of the 52 studies expressed urinary NNAL excretion as pmol/mg creatinine (Anderson et al., 2001; Benowitz et al., 2005, 2010b, 2012a,b; Carmella et al., 1995, 1997, 2003; Church et al., 2010; Hatsukami et al., 2004, 2010; Hecht et al., 1995, 1999, 2007; Hughes et al., 2004; Hurt et al., 2000; Joseph et al., 2005; Khariwala et al., 2012; Le Marchand et al., 2008; Melikian et al., 2007; Mendoza-Baumgart et al., 2007; Muscat et al., 2005, 2009; Sellers et al., 2003; St. Helen et al., 2012; Stepanov and Hecht, 2005; Strasser et al., 2011; Taioli et al., 1997; Ter-Minassian et al., 2012).

The rest of the studies expressed urinary NNAL excretion using a variety of units. Six studies expressed urinary NNAL excretion as ng/24 h: Frost-Pineda et al. (2008), Heck (2009), Kinser et al. (2002), Roethig et al. (2008, 2009) and Sarkar et al. (2010). Five studies expressed urinary NNAL excretion as pg/mL urine: Benowitz et al. (2010a), Bernert et al. (2010), Blank and Eissenberg (2010), Breland et al. (2003) and Heck (2009). Four studies expressed urinary NNAL excretion as pmol/mL urine: Derby et al. (2009), Hatsukami et al. (2007), Murphy et al. (2004) and Stepanov et al. (2009). Four studies expressed urinary NNAL excretion as nmol/24 h: Carmella et al. (1993, 2009), Hecht et al. (1995) and Stepanov et al. (2008). Four studies expressed urinary NNAL excretion as pg/mg creatinine: Benowitz et al. (2010a), Goniewicz et al. (2009), Heck (2009) and Rostron (2013). Two studies expressed urinary NNAL excretion as ng/mL urine: Jones et al. (2013) and Stepanov et al. (2007). One study expressed urinary NNAL excretion as ng/mg creatinine: Ashley et al. (2010); and one study expressed urinary NNAL excretion as ng/g creatinine: Sarkar et al. (2008).

To make valid comparisons of NNAL excretion data over time, we wanted to use data expressed in common units. The most frequently used unit of NNAL expression was pmol/mg creatinine. However, almost half of the studies used a variety of other units to express urinary NNAL excretion. Therefore, in order to bring more data into the primary analysis, we converted data from the studies reporting NNAL excretion in units other than pmol/mg creatinine. This resulted in the consolidation of data from nine different units of NNAL expression into three: pmol/mg creatinine, nmol/24 h, and pmol/mL urine. The vast majority of data spanning the longest possible time frame were data normalized to creatinine, namely, pmol/mg creatinine. Therefore, NNAL excretion data expressed as pmol/mg creatinine formed the basis of our primary analysis.

Table 1 presents characteristics of the 35 studies used in this analysis. The publication of these studies covered a time span of 18 years from 1995 to 2012, inclusive. These studies collectively reported urinary NNAL excretion estimates for 61 defined study populations involving a total of 3941 study participants. Fig. 1 is a scatter plot of data for total NNAL excretion over time from the 61 study populations in which the data are expressed as pmol/mg creatinine.

We initially analyzed the data without adjusting for CPD or weighting the data by number of study participants, thus allowing the use of all 61 study populations. The results show a statistically significant decline in urinary NNAL excretion with study publication year. The trend line is shown in Fig. 1. We conducted the primary analysis of data from the 51 study populations that also reported CPD and number of study participants. The primary analysis shows that urinary NNAL excretion trends downward with study publication year by 0.137 pmol/mg creatinine/year, and the trend is statistically significant ($p < 0.00001$). NNAL excretion increased by 0.049 pmol/mg creatinine for each additional CPD, and the increase was statistically significant ($p = 0.0002$). The model explained much of the variance in the NNAL excretion levels (adjusted $R^2 = 0.61$).

4. Discussion

The U. S. Surgeon General and others have stated that the role of specific smoke constituents in smoking-related disease is currently not known (Burns et al., 2008; USDHHS, 2010). Likewise, it is not known whether reduction of TSNA exposure from cigarette smoking would result in a reduction in the risk of smoking-related disease.

Based on the analysis presented here, urinary NNAL excretion among cigarette smokers appears to have been declining over the period from 1995 to 2012. Given the utility of urinary NNAL excretion as a specific biomarker for NNK exposure and a general marker for TSNA exposure, the observed trend in urinary NNAL excretion provides evidence of a downward trend in TSNA exposure from cigarette smoking.

The downward trend in urinary NNAL excretion does not appear to be explained by reductions over time in CPD among study participants. Although NNAL excretion is significantly associated with CPD, our primary analysis shows that the downward trend of NNAL is statistically significant over time independent of CPD. Furthermore, we analyzed the data from all the study populations and did not find a statistically significant downward trend of CPD with study publication year. At first glance, this may seem surprising given reports of a significant decline in mean CPD among daily smokers in recent years (MMWR, 2012). However, it must be kept in mind that participant eligibility criteria for most of the studies reviewed here included a requirement that a minimum number of CPD were smoked (usually 10–15). Therefore, these studies are not necessarily designed to be representative of contemporary population smoking patterns. Nevertheless, they are still useful for identifying trends in TSNA exposure over time that may be reflective of changes in TSNA levels in cigarette smoke.