Short Communication

Metabolism of the Tobacco-Specific Carcinogen 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanone to Its Biomarker Total NNAL in Smokeless Tobacco Users

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Abstract

The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is one of the most abundant carcinogens in smokeless tobacco products. NNK uptake by measurement of the urinary metabolites 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides (total NNAL) has been reported in many studies, but there are no data in the literature on the percentage of the NNK dose that is converted to NNAL in smokeless tobacco users. In this study, 15 male subjects abstained from tobacco use for 3 weeks before placing 2 g smokeless tobacco between their cheeks and gums for 30 min. They then continued abstinence and collected three consecutive 24-h urine samples. The amount of NNK in the tobacco before and after use was determined along with the amount in expectorated saliva. The NNK dose thus calculated was compared with the amount of total NNAL excreted in the next 72 h. These data, taken together with previous pharmacokinetic data, show that the percent conversion of NNK to total NNAL in smokeless tobacco users is ~14% to 17%. This figure can be used to calculate daily exposure to NNK in smokeless tobacco users (~6 μg). The results of this study also indicate that metabolic activation of NNK to intermediates that can react with DNA is its major pathway of metabolism in smokeless tobacco users. (Cancer Epidemiol Biomarkers Prev 2008; 17(3):732–5)

Introduction

Tobacco-specific nitrosamines are the most abundant strong carcinogens in smokeless tobacco products and are also found in cigarette smoke (1, 2). The most important tobacco-specific nitrosamines are 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*′-nitrosonornicotine because of their levels in tobacco products and their carcinogenic activities in rodents (1, 3, 4). NNK and *N*′-nitrosonornicotine are believed to be among the causative agents for cancer induction by smokeless tobacco and combusted tobacco products (5). NNK and *N*′-nitrosonornicotine are human carcinogens according to the IARC (1).

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides are urinary metabolites of NNK in humans (6). The sum of NNAL and its glucuronides, termed total NNAL, has been used extensively to estimate NNK uptake in smokeless tobacco users, smokers, and nonsmokers exposed to secondhand smoke (7, 8). These studies have shown that total NNAL levels vary predictably with dose, as determined by the amount of NNK in smokeless tobacco, or the number of cigarettes smoked per day (9, 10). Total NNAL levels decrease on cessation of tobacco use, and are significantly higher in tobacco users than in nonusers, in whom total NNAL is generally not detected unless there has been exposure to secondhand smoke, in which case levels are only 1% to 5% as high as those in tobacco users (11).

In spite of the reasonably large amount of data in the literature on total NNAL in human urine, no studies have reported the percent conversion of NNK to total NNAL in people who use tobacco products. Knowing the percent conversion of NNK to total NNAL is important because it would allow an estimate of human exposure to NNK when total NNAL is used as a biomarker. In this study, we estimated the dose of NNK in smokeless tobacco users, by measuring its amounts in smokeless tobacco before and after use, and were thus able to derive an estimate for metabolic conversion of NNK to total NNAL.

Materials and Methods

Subjects. This study was approved by the University of Minnesota Research Subjects' Protection Programs

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Institutional Review Board Human Subjects Committee. Smokeless tobacco users were recruited by advertisements on radio and in local newspapers. Subjects were enrolled in the study if they were occasional or current users of Copenhagen smokeless tobacco (manufactured by U.S. Smokeless Tobacco Co.) and did not smoke cigarettes. Subjects were asked to abstain from any tobacco products for a 3-week period. They attended weekly clinic visits to submit a urine sample to assess for cotinine, using NicCheck, as a confirmation of abstinence. At the end of the 3-week abstinence period, they reported to the clinic and completed a questionnaire regarding tobacco use patterns and provided a baseline morning urine sample. Breath carbon monoxide was measured to confirm recent abstinence from smoking. At this visit, they were given a single portion of Copenhagen smokeless tobacco. A 2-g portion of the smokeless tobacco was put into a tea bag–like sealed pouch. Each subject placed the pouch between his cheek and gum for 30 min and collected any excess saliva in a sample cup. At the end of the 30-min period, the pouch was removed from the mouth and saved for analysis. Then, subjects collected three consecutive 24-h urine samples. Saliva, the used tobacco pouch, and urine samples were stored at -20°C until analysis. Subjects were paid $200 for their participation and compliance to study procedures.

Analyses. Analysis of tobacco and saliva for NNK (12) and urine for total NNAL were done essentially as described (12, 13).

Results

Following the ''single use'' of smokeless tobacco, each subject collected three consecutive 24-h urine samples that were analyzed for total NNAL. The distribution half-life of total NNAL in smokeless tobacco users is ~1.5 days (14). Therefore, in the 3-day period, an average of ~75% of the NNAL formed from NNK in the smokeless tobacco would be excreted.

There were 15 subjects. They were all White male, and their mean age ± SD was 32.2 ± 6.1. They used 1.1 ± 0.8 (range, 0.3-3.5) tins of smokeless tobacco per week, and their duration of use was 11.7 ± 5.0 (range, 2-19 years). The results are summarized in Table 1. The percentage (mean ± SD) of NNK extracted from 2 g tobacco during chewing was 59 ± 23% (column A - column B / column A × 100). The dose (mean ± SD) of NNK from each 2 g portion of tobacco was 14,700 ± 7,660 pmol (3.04 ± 1.58 μg). The percentage (mean ± SD) of the dose excreted as total NNAL in the 3 days of urine collection was 12.9 ± 10.4%. Taking into account the distribution half-life of NNAL, this can be corrected to 17%.

Some subjects had NNAL in their baseline urine sample. This might have come from inappropriate use of tobacco during the 3-week abstinence period, from persistence of NNAL in the body, or from exposure to secondhand smoke. The baseline urine samples were not 24-h samples. We used the average urine volume excreted per 24 h for each subject to estimate the amount of total NNAL per 24 h in the baseline sample, and this amount was subtracted from the amount of total NNAL in the 3-day urine sample following chewing. When the data were analyzed this way, the percentage of the NNK dose excreted as total NNAL was 10.3 ± 8.99% or 14% when the half-life is considered.

Discussion

Our results provide the first data on the percentage of NNK dose excreted in the urine of smokeless tobacco users as total NNAL. This 14% to 17% estimate should be very useful for our overall understanding of NNK exposure and metabolism in smokeless tobacco users,

Table 1. NNK in smokeless tobacco and saliva and total NNAL in the urine of smokeless tobacco users

Subject	NNK (pmol)*				Total NNAL (pmol)	
	(A) 2.0 g Tobacco before use*	(B) Tobacco after use	(C) Expectorated saliva	Dose = A - B - C	Excreted in urine in 3 d after chewing	Total NNAL excreted as % of NNK dose
1	42,600	6,230	1,880	34,500	878	2.54
2	42,600	21,600	3,620	17,400	3,100	17.8
3	42,600	5,020	9,080	28,500	2,100	7.37
4	42,600	23,900	4,300	14,400	1,490	10.3
5	42,600	35,400	1,400	5,800	1,730	29.8
6	42,600	32,100	821	9,680	1,320	13.6
7	42,600	21,800	5,070	15,700	6,350	40.4
8	42,600	29,200	6,520	6,880	1,280	18.6
9	42,600	17,900	7,540	17,200	1,070	6.22
10†	16,900	6,540	72	10,300	682	6.62
11	16,900	4,730	0	12,200	290	2.38
12	16,900	4,060	599	12,200	975	7.99
13	16,900	4,230	1,570	11,100	603	5.43
14	16,900	3,200	1,850	11,900	1,720	14.5
15	16,900	3,800	725	12,400	1,260	10.2
						12.9 ± 10.4

*To convert to microgram, multiply by 207×10^{-6}.
† Different tobacco batches were used for subjects 1-9 and 10-15.

as discussed below. Although it may be somewhat imprecise due to the known persistence of NNAL in the body and the presence of background levels of NNAL in some of our subjects, we note that the uncorrected data (last column of Table 1) consistently show that NNAL comprises <40% of the NNK dose and in 11 subjects <15%.

We have recently published data on levels of total NNAL in the urine of 182 smokeless tobacco users (16). The mean 24-h excretion of total NNAL was estimated as 4.3 nmol. Using our 14% to 17% of the dose figure, the daily NNK dose would average 25 to 31 nmol (5.2-6.4 μg). This dose can also be calculated based on NNK levels in tobacco. The 182 subjects used an average of 4.2 tins per week or ~20 g smokeless tobacco per day. Levels of NNK in the brands they used (mainly Copenhagen, Skoal, and Kodiak) were ~0.5 μg/g wet weight (17) or ~10 μg per 20 g. Because our data show that an average of 59% of NNK is extracted during chewing, the estimated daily dose of NNK based on the amount in tobacco is 6 μg/d. This agrees very well with the 5.2 to 6.4 μg estimate based on urinary total NNAL. This means that the dose of NNK to a daily user of smokeless tobacco will be ~44 mg in 20 years of use or ~0.6 mg/kg (0.003 mmol/kg). This is ~60 times less than the total dose of NNK that induced a significant incidence of lung and pancreatic tumors in rats when given chronically in the drinking water (18), a minimal safety margin when one considers that smokeless tobacco products also contain *N*′-nitrosonornicotine, an esophageal carcinogen, usually in amounts 3 to 10 times greater than NNK (12). Recent meta-analyses find that smokeless tobacco use is a risk factor for both esophageal and pancreatic cancer in addition to oral cancer.[2]

The major pathways of NNK metabolism in rodents and monkeys are NNAL formation and glucuronidation, α-hydroxylation, and pyridine-*N*-oxidation (4). α-Hydroxylation is firmly established as the major pathway of NNK metabolic activation to DNA adducts, which are critical in its carcinogenicity (4). Total NNAL comprised 19% to 22% of the urinary metabolites of NNK in the patas monkey (18). Total NNAL in urine increased with dose and comprised 0% to 51% of urinary metabolites in mice and 0% to 33% in rats (20). α-Hydroxylation was generally the most prevalent route of metabolism in patas monkeys, mice, and rats, and pyridine-*N*-oxidation levels were either comparable or less than those of total NNAL at low doses (19, 20). Our previous data show that total NNAL exceeds pyridine-*N*-oxidation by ~7-fold in smokeless tobacco users; thus, pyridine-*N*-oxidation should comprise only 2% to 2.5% of the NNK dose (21). Taken together, these data indicate that α-hydroxylation is the major NNK metabolic pathway in smokeless tobacco users. Although direct measurements are not available in smokeless tobacco users, our recent study showed that α-hydroxylation comprises ~86% of the NNK dose in smokers.[3] These results support the concept that the α-hydroxylation metabolic activation pathway is a major route of NNK metabolism in both smokers and smokeless tobacco users.

In summary, the results of this study provide the first estimate of percentage of NNK dose converted to total NNAL in smokeless tobacco users (14-17%). Taken together with other data, this figure allows one to estimate the daily dose of NNK in a typical snuff dipper (6 μg) and indicates that most of this NNK is metabolically activated in smokeless tobacco users. These data provide useful insights on NNK metabolism in humans and the consequent cancer risk.

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