Published in final edited form as:

Tob Control. 2008 April ; 17(2): 128–131. doi:10.1136/tc.2007.023242.

Exposure to Nicotine and a Tobacco-Specific Carcinogen Increase with Duration of Use of Smokeless Tobacco

Stephen S. Hecht*, Steven G. Carmella, Amanda Edmonds, Sharon E. Murphy, Irina Stepanov, Xianghua Luo, and Dorothy K. Hatsukami

Cancer Center and Transdisciplinary Tobacco Use Research Center University of Minnesota, Minneapolis, MN 55455, USA.

Abstract

Background—Smokeless tobacco is an efficient delivery vehicle for nicotine and can contain significant amounts of carcinogens. However, few studies have examined factors that might moderate levels of nicotine or carcinogen exposure.

Aims—To determine the effect of duration of smokeless tobacco use on the uptake of nicotine and a tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).

Design—Questionnaires on use of smokeless tobacco were administered, and urine samples from 212 smokeless tobacco users were analyzed for biomarkers of uptake of nicotine and NNK. The biomarkers were cotinine and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL).

Participants—Male smokeless tobacco users were recruited for studies designed to investigate methods of reducing smokeless tobacco use. The questionnaire and biomarker data were obtained at baseline, prior to reduction.

Results—Levels of cotinine ($p < 0.0001$) and total NNAL ($p = 0.0003$) were significantly correlated with duration (in years) of use of smokeless tobacco products. Median cotinine and total NNAL were 2.4 and 2.1 times higher, respectively, in the 21 + year than in the 0-5 year category of use.

Conclusions—Smokeless tobacco users adjust their intensity of use with experience in order to increase their nicotine dose, resulting in a corresponding increase in exposure to NNK, a powerful carcinogen. These results indicate the importance of educating smokeless tobacco users about the effects of prolonged use of these products.

INTRODUCTION

Smokeless tobacco, the predominant form of tobacco use at the beginning of the 20th century before the dramatic ascendance of cigarettes, re-emerged as a formidable product in the U.S. in the 1970s with the strong promotion of oral snuff products, and has been used for decades in other locations of the world such as Scandinavia and southeast Asia (1).

Smokeless tobacco products continue to increase in popularity in Western countries, and some tobacco control experts have proposed that “low nitrosamine” products be substituted for cigarettes by people who cannot quit tobacco, foreshadowing a potential second re-emergence of these products (2;3). While smokeless tobacco lacks the combustion products

* To whom correspondence should be addressed: The Cancer Center, University of Minnesota, Mayo Mail Code 806, 420 Delaware St SE, Minneapolis, MN 55455, USA. ph: 612-624-7604 fax: 612-626-5135 hecht002@umn.edu.

COMPETING INTEREST DECLARATION. I have had no financial involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated therein.

of cigarette smoke, and is certainly less harmful, it is not without harm. Smokeless tobacco is an efficient delivery vehicle for the addictive agent nicotine, contains multiple carcinogens – most notably tobacco-specific nitrosamines- and is considered by the International Agency for Research on Cancer as “carcinogenic to humans”, causing cancers of the oral cavity and pancreas (1).

There have been no previous large studies examining the long-term effects of smokeless tobacco use on the uptake of nicotine and carcinogens. In this study, we demonstrate that biomarkers of exposure to nicotine and a powerful tobacco-specific nitrosamine carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (4), increase significantly with duration of smokeless tobacco use. The biomarkers are urinary cotinine, an accepted measure of nicotine uptake, and urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (total NNAL), an established metric of NNK uptake (5). Only one small preliminary study from our group (N = 54) which included some of the subjects in the larger study reported here, previously examined the relationship of these biomarkers to duration of smokeless tobacco use, and reported a relationship to cotinine levels but not to total NNAL (6).

METHODS

Subjects

This research was approved by the University of Minnesota Research Subjects' Protection Programs Institutional Review Board Human Subjects Committee, and involved smokeless tobacco users who were seeking treatment for smokeless tobacco reduction. Baseline data from 3 studies were used. They examined the effects of tobacco free snuff use (an herbal snuff-like product; study 1) (7); brand switching (study 2) (8); or use of a nicotine lozenge (study 3) compared to control groups on reduction of smokeless tobacco use. Subjects were recruited from the Minneapolis, St. Paul, MN metropolitan area through newspaper and television advertisements, and were screened over the telephone to determine interest and eligibility. During this screening, they were informed that the study compared different interventions for smokeless tobacco use reduction. Interested participants were asked to attend a meeting for orientation and screening and to obtain informed consent. Potential subjects were eligible for enrollment if they were: a) between the ages of 18 and 70 years; b) interested in reducing smokeless tobacco use but not quitting (i.e., having an established quit date within the next 90 days); c) using smokeless tobacco daily (≥ 6 dips/day) for the past six months but do not smoke more than 10 cigarettes per month, d) in good physical health (i.e., absence of an unstable medical condition or use of a medication that might affect tobacco use or be affected by tobacco use reduction); and e) in good mental health (i.e., not taking psychotropic medications or manifesting a psychiatric co-morbidity within the past 6 months). If subjects met these criteria, they were scheduled for two baseline clinic visits during which tobacco use histories and first morning urine samples were obtained. Only the urine samples obtained at the second baseline visit were analyzed. Tobacco use histories included inquiries on brand of smokeless tobacco used, date of first daily use, and amount of use (dips per day, minutes per dip, tins per week).

Biomarker analyses

Total NNAL and total cotinine were determined essentially as described (9-11).

Analysis of tobacco

Copenhagen long cuts, Skoal straight long cuts, and Kodiak premium wintergreen were purchased at retail stores in three locations chosen randomly in the Minneapolis, St. Paul, MN area. Tobacco from 3 tins of each product purchased at each location was thoroughly mixed. Thus, for each product, three representative samples were obtained. NNK, nicotine,

and pH were analyzed essentially as described previously and mean values calculated (12-14).

Statistical analyses

In exploratory data analyses, cotinine and total NNAL were summarized using geometric means, medians, and ranges because of the skewness of their distributions. The non-parametric Spearman correlation coefficient was used for describing the relationship of duration of daily use to biomarker levels and amount of use. Scatter plots with Loess smooth curves were used to illustrate the relationship between biomarker levels and duration. Multiple regressions were applied to assess the contribution of duration of daily use, amount of use, and brand to the interindividual variability of cotinine and total NNAL. Biomarker levels were transformed to the natural log scale in both scatter plots and regression analyses.

RESULTS

The 212 smokeless tobacco users in this study were all male, 98% white, and had a mean age of 33.8 (95% CI 32.8 – 34.8) years. They consumed an average of 4.2 (95% CI 3.9 – 4.4) tins of smokeless tobacco per week and took 9.9 (95% CI 9.1 – 10.6) dips per day. Their mean duration of daily use of smokeless tobacco was 14.1 (95% CI 13.3 – 15.0) years. They currently used Copenhagen fine and long cuts (31%), Skoal long cuts, mint and straight (14%), Kodiak premium wintergreen (42%), and other brands (14%). The highest brand prevalence amounts in each category of duration of smokeless use were: 0-10 years of use, Kodiak, 63%; 11-20 and 21 plus years of use, Copenhagen, 39% and 49%, respectively.

The relationship of cotinine and total NNAL to duration of daily smokeless tobacco use is illustrated in Figure 1A,B. Cotinine (Spearman correlation 0.27, $p < 0.0001$) and total NNAL (Spearman correlation, 0.23, $p = 0.0003$) were both correlated with duration of use. Geometric means and median amounts of cotinine and total NNAL for five categories of years of daily use are summarized in Table 1. Median cotinine and total NNAL were 2.4 and 2.1 times higher, respectively, in the 21+ year than in the 0-5 year category.

Dips of smokeless tobacco per day, minutes per day, and dips per tin of smokeless tobacco were correlated with duration of daily smokeless tobacco use (Table 2). Minutes per dip and tins per week were not correlated with duration. Minutes per dip of smokeless tobacco, dips per day, minutes per day, and dips per tin, but not tins per week, were significantly correlated with cotinine and total NNAL (Table 2).

The relationship of duration of use to dependence, as indicated by reported time to first dip of the day, was examined. These data were available for 188 of the 212 subjects. Duration was longer in subjects ($N = 135$) who reported using smokeless tobacco within 30 min of waking than in subjects ($N = 53$) who did not: 14.9 ± 6.09 vs 12.4 ± 5.79 years ($p = 0.01$).

Duration of use was significantly greater for Copenhagen than Skoal or Kodiak ($P < 0.0001$). Brand was not related to the biomarker levels after adjustment for duration and amount of use in multiple regression models. Levels of NNK in recent analyses of the most common smokeless tobacco products used here were similar: ($\mu\text{g/g}$ wet weight) Copenhagen long cuts (0.47); Skoal straight long cuts (0.63); and Kodiak premium wintergreen (0.55). Levels of nicotine and unprotonated nicotine (mg/g wet weight) were: Copenhagen (11.6, 3.1); Skoal (11.4, 2.7); Kodiak (8.9, 5.5).

DISCUSSION

The results of this study demonstrate a significant increase in levels of urinary cotinine and total NNAL with duration of daily smokeless tobacco use. This increase can be explained mainly by corresponding increases in minutes per day, dips per day, and dips per tin of smokeless tobacco use with duration of use, and was not related to brand, or to levels of NNK or nicotine in different brands. The results strongly suggest that smokeless tobacco users adjust their intensity of use with experience in order to increase their nicotine dose, resulting in a corresponding increase in exposure to NNK, and presumably other constituents as well.

Our results suggest growing dependence on smokeless tobacco use with duration of use. Our users used more dips per tin, and presumably smaller dips, with longer duration. It is possible that nicotine may be extracted more readily in the mouth from these smaller dips. Increased nicotine dependence with years of use followed by a plateau has been observed in smokers and probably occurs in smokeless tobacco users as well (15;16). This pattern seems consistent with our observations. Our finding that time to first dip in the morning was related to duration is also consistent with increased nicotine dependence among those who used smokeless tobacco for longer periods of time. In smokers, higher nicotine dependence is associated with decreased likelihood of quitting and the same phenomenon may have influenced our results (17).

We observed a greater increase in cotinine levels than in total NNAL levels with duration of use. While the reason for this is not clear, a related phenomenon has been observed in smokers, in whom levels of NNAL plateau at higher levels of cotinine (18). This could be due to induction of alternate pathways of NNK metabolism at higher doses of nicotine and other cigarette smoke constituents.

While the driving force for the results observed here is undoubtedly nicotine, exposure to NNK also increases with duration. NNK is a powerful carcinogen, inducing tumors of the lung, pancreas, and nasal cavity in rodents at relatively low doses (4;19). Oral cavity tumors were observed when NNK and the related tobacco-specific nitrosamine, *N*²-nitrosonornicotine (NNN), also present in relatively substantial quantities in smokeless tobacco, were co-administered to rats (20). NNK and NNN are considered carcinogenic to humans by the International Agency for Research on Cancer (1).

A limitation of this study was that our smokeless tobacco users were seeking to reduce their use. These individuals are dependent and may have a different pattern of use than smokeless tobacco users who are not seeking treatment. A cross-sectional population-based investigation might be a more appropriate design.

In summary, the results of this study clearly demonstrate that levels of urinary cotinine and total NNAL in smokeless tobacco users increase with duration of use. Smokeless tobacco users need to be educated about the effects of prolonged use. These findings should be considered when recommending smokeless tobacco use as a harm reduction strategy as increased duration of use may have unintended consequences due to higher than expected toxicant exposure.

Acknowledgments

This study was supported by grants DA-13333, DA-14404, and CA-77598 from the U.S. National Institutes of Health and RP-00-138 from the American Cancer Society.

References

1. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 89. IARC; Lyon, FR: 2007. Smokeless tobacco and tobacco-specific nitrosamines.. in press
2. Levy DT, Mumford EA, Cummings KM, Gilpin EA, Giovino G, Hyland A, et al. The relative risks of a low-nitrosamine smokeless tobacco product compared with smoking cigarettes: estimates of a panel of experts. *Cancer Epidemiol.Biomarkers & Prev.* 2004; 13:2035–42.
3. Savitz DA, Meyer RE, Tanzer JM, Mirvish SS, Lewin F. Public health implications of smokeless tobacco use as a harm reduction strategy. *Am.J.Public Health.* 2006; 96:1934–9. [PubMed: 17018821]
4. Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. *Chem.Res.Toxicol.* 1998; 11:559–603. [PubMed: 9625726]
5. Hatsukami DK, Benowitz NL, Rennard SI, Oncken C, Hecht SS. Biomarkers to assess the utility of potential reduced exposure tobacco products. *Nicotine and Tob.Res.* 2006; 8:600–22. [PubMed: 16920658]
6. Lemmonds CA, Hecht SS, Jensen JA, Murphy SE, Carmella SG, Zhang Y, et al. Smokeless tobacco topography and toxin exposure. *Nicotine Tob.Res.* 2005; 7:469–74. [PubMed: 16085515]
7. Hatsukami DK, Ebbert JO, Edmonds A, Le C, Hecht SS. Smokeless tobacco reduction: tobacco free snuff vs. no snuff. *Nicotine Tob.Res.* 2007 in press.
8. Hatsukami DK, Ebbert JO, Anderson A, Lin H, Le C, Hecht SS. Smokeless tobacco brand switching: a means to reduce toxicant exposure? *Drug and Alcohol Dependence.* 2006; 87:217–24. [PubMed: 16996230]
9. Carmella SG, Han S, Villalta PW, Hecht SS. Analysis of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in smokers' blood. *Cancer Epidemiol.Biomarkers & Prev.* 2005; 14:2669–72.
10. Carmella SG, Han S, Fristad A, Yang Y, Hecht SS. Analysis of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in human urine. *Cancer Epidemiol.Biomarkers & Prev.* 2003; 12:1257–61.
11. Hecht SS, Carmella SG, Chen M, Koch JFD, Miller AT, Murphy SE, et al. Quantitation of urinary metabolites of a tobacco-specific lung carcinogen after smoking cessation. *Cancer Res.* 1999; 59:590–6. [PubMed: 9973205]
12. Stepanov I, Jensen J, Hatsukami D, Hecht SS. Tobacco-specific nitrosamines in new tobacco products. *Nicotine Tob.Res.* 2006; 8:309–13. [PubMed: 16766423]
13. Stepanov I, Hecht SS, Mirvish SS, Gonta M. Comparative analysis of tobacco-specific nitrosamines and total N-nitroso compounds in Moldovan cigarette tobacco. *Journal of Agricultural and Food Chemistry.* 2005; 53:8082–6. [PubMed: 16190674]
14. Richter P, Spierto FW. Surveillance of smokeless tobacco nicotine, pH, moisture, and unprotonated nicotine content. *Nicotine Tob.Res.* 2003; 5:885–9. [PubMed: 14668072]
15. Breslau N, Johnson EO, Hiripi E, Kessler R. Nicotine dependence in the United States: prevalence, trends, and smoking persistence. *Archives of General Psychiatry.* 2001; 58:810–6. [PubMed: 11545662]
16. Hatsukami DK, Severson HH. Oral spit tobacco: addiction, prevention and treatment. *Nicotine.Tob.Res.* 1999; 1:21–44. [PubMed: 11072386]
17. Hyland A, Borland R, Li Q, Yong HH, McNeill A, Fong GT, et al. Individual-level predictors of cessation behaviours among participants in the International Tobacco Control (ITC) Four Country Survey. *Tob.Control.* 2006; 15(Suppl 3):iii83–iii94. [PubMed: 16754952]
18. Lubin JH, Caporaso N, Hatsukami DK, Joseph AM, Hecht SS. The association of a tobacco-specific biomarker and cigarette consumption and its dependence on host characteristics. *Cancer Epidemiol.Biomarkers & Prev.* 2007; 16:1852–7.
19. Hecht SS, Hoffmann D. Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. *Carcinogenesis.* 1988; 9:875–84. [PubMed: 3286030]

20. Hecht SS, Rivenson A, Braley J, DiBello J, Adams JD, Hoffmann D. Induction of oral cavity tumors in F344 rats by tobacco-specific nitrosamines and snuff. *Cancer Res.* 1986; 46:4162–6. [PubMed: 3731083]

What this paper adds: This study shows for the first time that exposure to nicotine and the tobacco-specific carcinogen NNK, as measured by their biomarkers cotinine and total NNAL in urine, significantly increases with duration of smokeless tobacco use.

Figure 1.

Log-scaled levels of urinary cotinine (A) and total NNAL (B) plotted by years of daily use of smokeless tobacco in 212 men: Spearman correlations; cotinine, 0.27 ($p < 0.0001$); total NNAL, 0.23 ($p = 0.0003$).

Table 1

Levels of cotinine and total NNAL in the urine of 212 smokeless tobacco users stratified by categories of years of daily use.

Duration (years of daily use)	N	Cotinine (nmol/ml)		Total NNAL (pmol/ml)	
		Geometric mean (95% CI)	Median (range)	Geometric mean (95% CI)	Median (range)
0-5	14	23.6 (13.5-41.2)	29.0 (3.03-71.9)	2.47 (1.42-4.31)	2.12 (0.39-16.7)
6-10	54	37.3 (30.9-45.1)	36.9 (4.91-112)	3.21 (2.62-3.92)	3.11 (0.57-22.4)
11-15	57	43.9 (37.4-51.6)	46.3 (5.88-143)	4.26 (3.60-5.03)	4.06 (0.66-23.0)
16-20	52	44.3 (35.2-55.7)	56.9 (5.93-155)	4.07 (3.34-4.94)	4.00 (0.84-14.7)
21+	35	55.9 (40.4-77.3)	62.0 (4.16-232)	5.21 (4.06-6.70)	4.53 (1.09-25.8)

Table 2

Relationship of duration of smokeless tobacco use and levels of cotinine and total NNAL in urine to parameters of smokeless tobacco use in 212 users.

Parameter	Spearman correlation, <i>P</i> value		
	Duration of Use	Cotinine	Total NNAL
Minutes per dip	0.11, 0.12	0.20, 0.004	0.16, 0.02
Dips per day	0.26, <0.0001	0.15, 0.03	0.19, 0.005
Minutes per day	0.28, <0.0001	0.29, 0.0001	0.28, <0.0001
Tins per week	0.04, 0.54	0.08, 0.23	0.13, 0.055
Dips per tin	0.21, 0.003	0.27, <0.0001	0.23, 0.0007