Daily Use of Smokeless Tobacco: Systemic Effects

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Study Objective: To compare exposure to nicotine and related cardiovascular effects as well as urinary mutagenicity (a potential marker of systemic absorption of carcinogenic compounds) during use of oral snuff, chewing tobacco, and cigarettes, as desired.

Design: Crossover sequential treatments, balanced-order experimental study.

Setting: Clinical research center.

Participants: Eight healthy men who regularly smoked cigarettes and had previous experience with the use of both oral snuff and chewing tobacco.

Interventions: Four 3- or 4-day blocks during which participants used oral snuff, chewing tobacco, and cigarettes as desired, or abstained from all tobacco. Concentrations of nicotine and cotinine (the primary metabolite of nicotine), cardiovascular effects, and urine sodium, catecholamine and mutagenicity were measured over 24 hours at the end of each treatment block.

Measurements and Main Results: Circadian exposure to nicotine and cardiovascular effects, including urinary catecholamine excretion, were similar for all forms of tobacco use. Urine sodium excretion was greater while using smokeless tobacco than while smoking, probably due to absorption of sodium from the smokeless tobacco. Urine mutagenicity was markedly increased while smoking cigarettes and tended to be increased ($P < 0.10$) while chewing tobacco but not while using oral snuff.

Conclusions: Systemic absorption of nicotine, sodium, and carcinogenic chemicals from smokeless tobacco may cause or aggravate human illness in addition to the known adverse effects on the oral cavity.

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The use of smokeless tobacco is widespread among young persons in the United States, particularly in rural areas, and in some groups of adults, such as baseball players and miners (1-4). Lesions of the oral cavity have been clearly linked to smokeless tobacco use. There is also concern that habitual, long-term smokeless tobacco use produces systemic effects that might adversely affect health. Nicotine is well absorbed from smokeless tobacco and could contribute to adverse health consequences of smokeless tobacco use (4). We found that peak concentrations of nicotine in the plasma after single doses of oral snuff or chewing tobacco are similar to but are more sustained than those seen after smoking a cigarette (5). Likewise, the cardiovascular effects of a single dose of smokeless tobacco and of smoking a cigarette are similar.

We examined exposure to nicotine and related cardiovascular effects including catecholamine excretion, as well as urinary mutagenicity (a potential marker of systemic absorption of carcinogenic compounds) during free use of oral snuff, chewing tobacco, and cigarettes.

Methods

The subjects were eight healthy men, 27 to 61 years of age (mean, 49 years) who were paid volunteers recruited from newspaper advertisements. All subjects were habitual cigarette smokers and had previous experience with the use of both oral snuff and chewing tobacco. The subjects reported an average daily consumption of 32 cigarettes (range, 20 to 50). Consent was obtained from the subjects after the nature of the procedures had been fully explained.

Subjects were admitted to the Clinical Study Center at San Francisco General Hospital for 15 to 17 days. Subjects ate a regular hospital diet with no added salt, containing approximately 2 mmol sodium per kg body weight per day. The use of caffeinated beverages or alcohol was prohibited. Subjects were studied in four 3- or 4-day treatment blocks. These blocks included use as desired of their usual brand of cigarettes, oral snuff, or chewing tobacco, or abstinence from tobacco. The cigarettes smoked by our subjects had an average ($\pm$ SD) United States Federal Trade Commission machine delivery of 1.1 $\pm$ 0.2 mg nicotine, 17.9 $\pm$ 5.0 mg tar, and 14.3 $\pm$ 1.7 mg carbon monoxide. All subjects selected one of three popular brands of American snuff: Copenhagen (U.S. Tobacco Co., Franklin Park, Illinois; five subjects); Skoal Bandits-Wintergreen (U.S. Tobacco Co., Franklin Park, Illinois, two subjects); Hawken-Wintergreen (Conwood Corp., Memphis, Tennessee, one subject). The chewing tobacco was either Redman loose leaf (The Pinkerton Tobacco Co., Owensboro, Kentucky, five subjects) or Redman plug (Pinkerton, three subjects). Abstinence was verified by measuring expired carbon monoxide concentrations (Ecolyzer 2000 Series, Energetics Science, Inc., Hawthorne, New York) every 4 hours (expired carbon monoxide should be < 8 ppm) and by measuring urinary excretion of nico-

Table 1. *Tobacco Consumption and Nicotine Exposure with Smokeless Tobacco Use and Cigarette Smoking**

	Oral Snuff	Chewing Tobacco	Cigarettes
Grams or cigarettes per day	15.6 ± 5.9† (6.8-22.0)‡	72.9 ± 21.6 (33.7-103.7)	36.4 ± 10.4 (25.0-54.0)
Maximal plasma nicotine concentration, $\mu mol/L$	0.20 ± 0.10 (0.07-0.38)	0.17 ± 0.07 (0.07-0.29)	0.19 ± 0.03 (0.14-0.22)
AUC_{nic}, $\mu mol \cdot L^{-1} \cdot h^{-1}$	2.48 ± 1.13 (0.97-4.67)	2.06 ± 0.84 (0.83-3.69)	3.04 ± 0.69 (2.45-4.37)
Urine nicotine, $\mu mol/24\ h$	5.58 ± 5.06 (1.04-15.84)	6.45 ± 4.65 (1.29-13.68)	6.97 ± 2.87 (3.58-10.75)
AUC_{cot}, $\mu mol \cdot L^{-1} \cdot h^{-1}$	48.50 ± 30.77 (24.79-118.26)	48.25 ± 29.66 (13.67-113.87)	46.17 ± 13.29 (29.19-64.87)
AUC_{cot}/AUC_{nic}	21.4 ± 6.2§ (9.7-29.6)	23.9 ± 4.6‡ (17.8-33.6)	16.4 ± 3.0 (11.6-21.4)

* AUC = area under the plasma concentration-time curve; cot = cotinine; nic = nicotine.
† Mean ± SD.
‡ Range.
§ $P < 0.05$, compared with cigarette condition.

time. The sequence of treatment blocks was balanced across subjects using 4 × 4 Latin squares.

The number of cigarettes smoked and the weight of smokeless tobacco consumed were recorded each day. All urine was collected for measurement of nicotine and catecholamine concentrations and mutagenic activity. Each day recumbent resting blood pressure and heart rate were measured using a Dynamap automated blood pressure machine (Critikon, Inc., Raritan, New Jersey) by nurses every 4 hours. On the last day of each treatment block, an indwelling butterfly catheter was inserted into a forearm vein to collect samples to measure plasma concentrations of nicotine and cotinine (the major metabolite of nicotine) and blood carboxyhemoglobin. Samples were measured every 2 hours, independent of when smokeless tobacco or cigarettes were consumed.

Plasma concentrations of nicotine and cotinine were measured by gas chromatography modified for use with a capillary column (6). Carboxyhemoglobin was measured with an Instrumentation Laboratory 280 Co-oximeter (Instrumentation Laboratory, Lexington, Massachusetts). Urinary catecholamines were measured by high performance liquid chromatography using a coulometric electrochemical detector (7, 8). Mutagenic activity of the urine was measured by the *Salmonella*-histidine auxotroph-reversion assay (Ames *Salmonella* strain TA98). Details of the preparation of samples and controls as modified by our laboratory have been published previously (9). Mutagenicity in aliquots of the extracts equivalent to 5 mL urine was determined by the method of Kado and colleagues (10). After cessation of smoking, urinary mutagenicity rapidly declines, returning to abstinence values within 8 hours (11), so there is no carryover from previous treatments to confound measurements made on day 3 or 4 of a new treatment block.

Plasma nicotine and cotinine and blood carboxyhemoglobin concentrations over the day were expressed as area under the blood concentration-time curve (AUC), estimated using the trapezoidal rule. Treatment groups were compared by repeated measures analysis of variance, with Tukey post-hoc tests. Significant differences indicates $P < 0.05$.

Results

Tobacco consumption and resultant exposure to nicotine and cotinine on the last day of each study block are shown in Table 1. Tobacco consumption and urine nicotine excretion were similar on the last and next to last days of study blocks, indicating no effects of the blood drawing procedure. Plasma concentrations of nicotine throughout the day (Figure 1), analyzed as maximal concentrations or area under the plasma concentration-time curve (AUC) (Table 1), were similar while smoking or using snuff or chewing tobacco. Likewise, plasma cotinine AUC was similar across conditions. However, the ratio of AUC-cotinine to AUC-nicotine was significantly greater while using snuff or chewing tobacco compared with smoking cigarettes. The correlation between cigarettes per day and AUC-nicotine was 0.54, between grams of tobacco and AUC-nicotine for snuff was 0.24, and for chewing tobacco was 0.39 (none was significant). During abstinence from tobacco, plasma nicotine levels were undetectable. Plasma cotinine levels on the last day of abstinence averaged 0.20 μmol/L. Carryover of cotinine from one treatment to the next did not affect plasma cotinine measurement for the second treatment as evidenced by the observation that trough (0800 hours) concentrations at the beginning and end of the 24-hour sampling period were similar, consistent with achieving steady state.

The average heart rate was higher while using smokeless tobacco than while abstaining (Table 2). The magnitude of heart rate acceleration was similar for cigarette and chewing tobacco blocks and slightly less while using oral snuff. Heart rate was greater while using tobacco at all times of day except at 0700 hours compared with abstinence (Figure 1).

Systolic and diastolic blood pressure were similar during cigarette smoking and smokeless tobacco use and tended to be higher than during abstinence, although the difference was not significant (Table 2). Rate-pressure product, the product of systolic blood pressure and heart rate (an index of myocardial work and oxygen demand), was significantly increased during cigarette smoking and tobacco chewing and increased to a lesser extent while using oral snuff compared with tobacco abstinence.

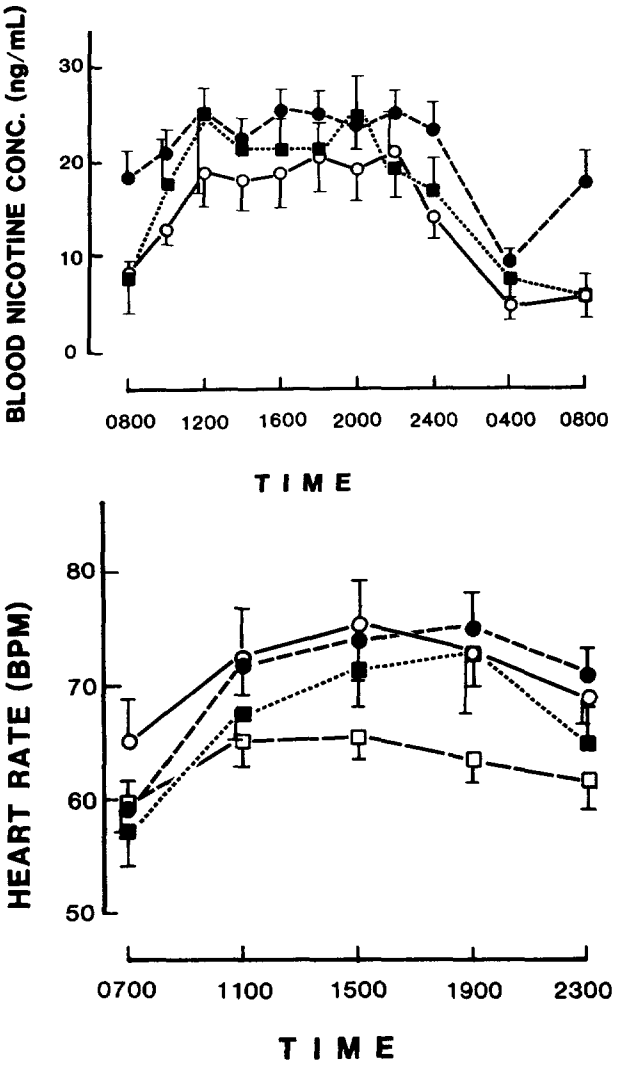

Figure 1. Comparison of blood nicotine concentrations (*top*) and heart rate (*bottom*) in subjects who smoked cigarettes (*closed circles*), used chewing tobacco (*open circles*), used oral snuff (*closed squares*), or abstained from tobacco (*open squares*). Blood concentrations of nicotine were nearly zero in the tobacco abstinence condition and are not shown. Data are shown as mean ± SE for eight subjects. To convert to μmol/L, divide ng/mL by 162.2.

Tobacco use had no effect on daily urine volume. Sodium excretion was significantly higher and potassium excretion tended to be higher while using smokeless tobacco compared with cigarettes (Table 2). Daily excretion of epinephrine was higher with all forms of tobacco use than during abstinence. Norepinephrine excretion was higher with cigarette smoking and chewing tobacco compared with abstinence. Dopamine excretion was similar for all treatments. Urine mutagenicity was greater while smoking cigarettes and chewing tobacco compared with abstinence, although the chewing tobacco effect did not reach statistical significance ($P < 0.10$).

Discussion

Our study is the first to describe concentrations of nicotine and resultant cardiovascular effects throughout the day in persons using smokeless tobacco. Exposure to nicotine was similar in peak levels and circadian pattern while smoking cigarettes or while consuming oral snuff or chewing tobacco, although the average exposure (AUC) tended to be less with the use of smokeless tobacco. However, it should be noted that our subjects were primarily heavy cigarette smokers and had only occasionally used smokeless tobacco. Average concentrations of nicotine and cotinine with smokeless tobacco use in subjects in this study as well as in subjects in a previous study (12) in which nicotine and cotinine concentrations measured once in the morning and once in the afternoon during smokeless tobacco use revealed levels of nicotine and cotinine that were comparable to those observed in large populations of cigarette smokers (13). We conclude that daily exposure of nicotine in smokeless tobacco users is in general similar to that of cigarette smokers.

Although nicotine levels tended to be lower, cotinine levels tended to be higher during smokeless tobacco use compared with cigarette smoking. We have observed a similar pattern comparing chewing nicotine gum with cigarette smoking (14). The likely explanation is that some of the nicotine from the smokeless tobacco is swallowed and subsequently undergoes presystemic (first pass) metabolism to cotinine before reaching the systemic circulation. Assuming a typical plasma clearance of nicotine of 1300 mL/min and an oral bioavailability of 30%, the percentage of nicotine that is swallowed can be estimated from the AUC data for nicotine and cotinine in our subjects (14). On average, while using snuff our subjects absorbed 27.6 mg nicotine through the buccal mucosa and swallowed 12.9 mg nicotine; while chewing tobacco, they absorbed 20.1 mg through the buccal mucosa and swallowed 20.2 mg. The finding that considerable nicotine from smokeless tobacco is swallowed and absorbed with some delay from the gastrointestinal tract is consistent with observations that nicotine absorption persists for 30 minutes or longer after smokeless tobacco is removed from the mouth (5).

Single-dose studies of cigarette smoking, oral snuff, chewing tobacco, and intravenous nicotine have shown increases in heart rate and blood pressure (15-17). In our circadian study, cigarette smoking increased heart rate an average of 7 beats per minute, which is similar to that observed when heart rate is measured continuously over 24 hours by ambulatory electrocardiographic monitoring in cigarette smokers (18). Heart rate was increased to a similar magnitude with chewing tobacco and to a lesser extent with the use of snuff. Of note is that the heart rate increase persisted throughout the whole day while using tobacco. The average blood pressure tended to be higher with all tobacco use compared with abstinence, as has been reported in previous circadian studies of cigarette smoking (18). The rate-pressure product is correlated to myocardial oxygen consumption and the myocardial requirement for nutrient blood flow. The increment in rate-pressure product above the abstinence condition reflects the additional burden imposed by tobacco use on the heart. Both cigarette smoking and chew-

Table 2. *Cardiovascular and Other Effects of Smokeless Tobacco Use and Cigarette Smoking*

	Oral Snuff	Chewing Tobacco	Cigarettes	Abstinence
Heart rate, *min*$^{-1}$	66.7 ± 10.0	70.9* ± 9.5	69.9* ± 6.8	62.7 ± 5.3
Systolic blood pressure, *mm Hg*	118.9 ± 9.1	119.2 ± 8.1	121.4 ± 12.6	116.1 ± 10.7
Diastolic blood pressure, *mm Hg*	66.7 ± 6.3	67.6 ± 6.8	67.7 ± 9.8	64.7 ± 7.5
Rate pressure product, *mm Hg* · *min*$^{-1}$	7965 ± 1437	8456* ± 1211	8480* ± 972	7285 ± 843
Urine sodium, *mmol/24 h*	136.9 ± 63.1	151.7* ± 72.2	106.9 ± 48.6	110.9 ± 24.3
Urine potassium, *mmol/24 h*	39.8 ± 23.2	44.0 ± 25.3	32.8 ± 14.7	34.6 ± 10.1
Urine volume, *mL/24 h*	1990 ± 884	2142 ± 737	2121 ± 1016	1836 ± 689
Urine norepinephrine, *μmol/24 h*	0.23 ± 0.06	0.27* ± 0.08	0.27* ± 0.08	0.20 ± 0.06
Urine epinephrine, *μmol/24 h*	0.038* ± 0.021	0.034* ± 0.008	0.038* ± 0.014	0.022 ± 0.009
Urine dopamine, *μmol/24 h*	1.71 ± 0.44	1.76 ± 0.66	1.82 ± 0.70	1.98 ± 0.90
Urine mutagenicity, *colonies/5 mL*	13.5 ± 2.8	25.4† ± 6.8	76.5* ± 25.4	15.6 ± 4.1

* $P < 0.05$, compared with abstinence.
† $P < 0.10$, compared with abstinence.

ing tobacco substantially increase myocardial work, whereas oral snuff does so to a lesser extent. Cigarette smoking also results in carbon monoxide exposure, of course, which further impairs oxygen delivery to the heart. Consequently, the use of smokeless tobacco as well as cigarette smoking would be expected to adversely affect the myocardial oxygen supply and demand relationship and impair exercise capacity or otherwise produce ischemia in patients with severe coronary heart disease.

Consistent with the known effects of nicotine to activate the sympathetic nervous system (19) and the cardiovascular effects described here, all forms of tobacco use substantially increased excretion of epinephrine and norepinephrine compared with abstinence. We conclude that absorption of nicotine from smokeless tobacco produces a similar level and temporal pattern of sympathetic nervous system activation as does cigarette smoking. Because inappropriate sympathetic neural arousal may contribute to the development of vascular disease (20), smokeless tobacco use would be expected to present a risk similar to that of cigarette smoking. Of interest in this regard is a recent report (21) of thromboangiitis obliterans in a heavy user of chewing tobacco, which resolved after cessation of tobacco use.

Smokeless tobacco contains sodium (average, 1.75% by weight) (22) which may be added for flavor and as part of an alkaline buffer to facilitate buccal absorption of nicotine. Sodium absorption from smokeless tobacco in our subjects was substantial, averaging 26 mmol/d and 41 mmol/d while using oral snuff and chewing tobacco, respectively, compared with the abstinence condition. A sodium level of this magnitude could contribute to blood pressure elevation or aggravation of cardiac failure or other sodium-retaining conditions in users.

Urinary mutagenicity was markedly increased while smoking cigarettes, consistent with the absorption of mutagenic chemicals from tar, which has been reported in many other studies (23, 24). Tobacco snuff itself and the saliva of snuff users contain carcinogenic *N*-nitrosoamines (25). Snuff extracts are mutagenic in the Ames system (26). The increase in mutagenic activity in the urine of our subjects while chewing tobacco suggests some systemic exposure to potentially carcinogenic chemicals, possibly a result of swallowing tobacco or the salivary extract of tobacco, which are known to contain carcinogenic chemicals. Increased urine mutagenicity with use of smokeless tobacco has been reported previously (27). These findings suggest that chewing tobacco might increase the risk of cancers other than those of the oral cavity; further epidemiologic studies should examine this possibility.

Conclusions

Our study showed that use of smokeless tobacco results in levels of nicotine and cardiovascular effects throughout the day that are similar to those observed with daily cigarette smoking. Because nicotine is the cause of addiction to cigarettes (28), we expect that smokeless tobacco use has a similar addiction liability. This theory is supported by the observation that cessation of smokeless tobacco use is difficult for many people and that a nicotine withdrawal syndrome follows the cessation of regular smokeless tobacco use (29). Our data on the level of nicotine exposure support the proposed use of nicotine replacement strategies as an adjunct to tobacco cessation therapy in treating smokeless tobacco users.

Chronic systemic exposure to nicotine may contribute to accelerated coronary and peripheral vascular disease, delayed wound healing, reproductive disturbances, peptic ulcer disease, and esophageal reflux (27). Insofar as nicotine contributes to adverse health effects of cigarette smoking, the nicotine in smokeless tobacco would be expected to present similar hazards. Illness due to systemic absorption of nicotine and other toxins from smokeless tobacco should be considered as a potential sequel to long-term smokeless tobacco use.

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