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Unusually high levels of carcinogenic tobacco-specific nitrosamines in Sudan snuff (toombak)

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The aim of these studies was to determine the levels of carcinogenic tobacco specific nitrosamines (TSNA) in Sudanese oral snuff (toombak) as recent retrospective epidemiological studies suggested an association between the use of toombak and subsequent development of oral cancer. We have analyzed the TSNA levels in 20 samples of Sudanese toombak, of four different quality levels, collected from five different vendors. Using GC coupled with thermal energy analysis, four TSNA were quantified in snuff extracts: *N'*-nitrosonornicotine (NNN), *N'*-nitrosoanatabine (NAT), *N'*-nitrosoanabasine (NAB) and 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Unusually high levels of these TSNA (mean; range, mg/g snuff, dry wt) were detected: NNN (1.13; 0.50–3.08); NAT (0.08; 0.02–0.29); NAB (0.22; 0.02–2.37); and NNK (2.31; 0.62–7.87). Previously, the highest levels of NNN and NNK reported in any snuff were 0.154 and 0.014 mg/g dry wt respectively. In comparison, the levels in Sudanese toombak were up to 20 and 560 times higher respectively. As the public health implications of these findings are significant, attempts should be made to reduce exposure to TSNA in oral snuff users in Sudan.

Introduction

The oral use of smokeless tobacco either in the form of snuff (as used in North America and western Europe) or mixed with lime and areca nut in the form of a betel quid (as used in Asia) has been unequivocally associated with human cancers, mainly of the oral cavity (1). However, little has been published about smokeless tobacco as it is used in Sudan and other African countries and any possible association with neoplastic diseases. In Sudan, the prevalent oral use of smokeless tobacco is in the form of snuff (saffa, 'toombak' in the local language), prepared from sun-dried tobacco leaves. The main tobacco species is *Nicotiana rustica*. The leaves are mixed with an aqueous solution of 'natron' (sodium carbonate decahydrate, an alternative base to slaked lime used in other parts of the world by tobacco chewers) until saturated. The product is then left in a closed container for ~24 h before use. The moist snuff is kept in the labial or buccal vestibule for a period ranging from a few minutes to several hours until it becomes bland. Clinicians have observed squamous cell carcinoma in snuff users more commonly than in non-users and the site of the tumor is related to the site where snuff is placed (1). Preliminary prevalence studies estimated that 23% of the Sudanese population uses toombak (A.M.Idris, in preparation).

In the snuff used in western Europe and North America, several established and probable human carcinogens have been quantified, including *N*-nitrosamines (volatile and non-volatile), polycyclic aromatic hydrocarbons and polonium 210. Of these, *N*-nitrosamines have been reported to be present at the highest concentrations (μg/g) (2,3) and are considered to be among the likely causative agents for cancer of the upper alimentary tract, lung and urinary bladder in tobacco users (3–5). Of the 22 *N*-nitroso compounds that have been identified in smokeless tobacco products, tobacco-specific nitrosamines (TSNA*) derived from *Nicotiana* alkaloids contribute 70–90% of the total amount (6). Among the TSNA, *N*-nitrosonornicotine (NNN) and 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are potent carcinogens in various rodent species; *N*-nitrosoanabasine (NAB) has been reported to be a weak carcinogen (3) with limited evidence for its carcinogenicity in experimental animals (1). To investigate the exposure to TSNA of oral snuff users in the Sudan, 20 samples of toombak were collected and analyzed for TSNA and nicotine.

*Abbreviations: TSNA, tobacco-specific nitrosamines; NNN, *N*-nitrosonornicotine; NNK, 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone; NAB, *N*-nitrosoanabasine; NAT, *N'*-nitrosoanatabine; NBuBzA, *N*-nitroso-*N*-butylbenzylamine; TEA, thermal energy analyzer.

Materials and methods

Chemicals

Both NNN and NNK were purchased from Chemsyn Laboratories, Lenexa, KS, USA. *N'*-nitrosoanatabine (NAT) and NAB were donated by Dr D.Hoffmann, Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY, USA. *N*-nitroso-*N*-butylbenzylamine (NBuBzA), used as an internal standard, was synthesized as reported (7). Nicotine was obtained from Sigma, St Louis, MO, USA and isoquinoline was purchased from Fluka AG, Buchs, Switzerland. The purity of the chemicals used as standards was verified by TLC and GC with flame ionization detection.

Samples

Snuff samples prepared from *N.rustica* were obtained from local shops in Khartoum, Sudan. Samples of up to five different quality levels (Table I), based essentially on price, were obtained from suppliers.

Extraction and analysis of samples

N-nitrosamines and nicotine were extracted from snuff by a procedure described earlier (7,8). NBuBzA and isoquinoline were added as internal standards to the samples prior to the extraction. The extracts were analyzed for TSNA on a Perkin-Elmer (Model Sigma 2B) GC coupled to a thermal energy analyzer (TEA, Model 543, Thermedics Inc., Woburn, MA, USA) (Figure 1). Separation was achieved on a 2 m × 3 mm i.d. glass column packed with 10% UCW-982 on chromosorb WHP 80/100 mesh (Supelco, Inc., Bellefonte, PA, USA). After 12 min at an initial temperature of 220°C the column temperature was raised at 10°C min^{-1} to 250°C where it was held for 5 min. Nicotine was analyzed by a method published previously involving capillary GC separation and nitrogen–phosphorous detection (7).

Results

A typical chromatogram for the GC/TEA analysis of the four TSNA is shown in Figure 1. The order of elution was the internal standard and the TSNAs NNN, NAT, NAB and NNK. Table I reports the levels for four TSNA in ready-to-use oral snuff

Table I. Levels of TSNA and nicotine in Sudanese snuff samples

Sample[a]	Quality	Moisture content (%)	Nicotine content (mg/g dry wt)	NNN (mg/g dry wt)	NAT (mg/g dry wt)	NAB (mg/g dry wt)	NNK (mg/g dry wt)	Total TSNA content (mg/g dry wt)
A1	I	59.6	32 5	1.15	0.07	0.12	2 35	3 69
A2	I	7.2	102.4	3.08	0.29	2.37	7.87	13.61
A3	II	6.6	73.3	1 52	0.14	0.04	3.75	5.45
A4	III	5.6	32.2	0.83	0.06	0.07	0.63	1.59
A5	III	60 3	48.3	1.26	0.08	0.08	2.24	3.66
B6	I	57.3	26.0	1.55	0.14	0.21	2 79	4 69
B7	I	5.4	25.2	1 33	0.14	0.16	2.41	4.04
B8	II	5.6	14.1	1.20	0.12	0.15	1.79	3.26
B9	IV	5.6	10.6	0.63	0.04	0.06	1.63	2.36
B10	III	6.0	8.4	0.42	0.03	0.06	1.14	1.65
C11	super	5.9	19.8	0.94	0 03	0.02	2.13	3.12
C12	super	62.6	18.6	0.96	0.05	0.11	2.27	3.39
C13	I	6.2	22.8	0.92	0.08	0.22	2.08	3.30
C14	II	6.1	16.5	0.49	0.02	0.02	1.17	1.70
D15	I	6.5	24.7	1.80	0.13	0.23	3.83	5.99
D16	I	59.6	17.9	1.16	0.07	0.12	1.88	3 23
D17	II	6.7	40.6	0.85	0.05	0.09	0 62	1.61
D18	III	5.9	29.9	0.85	0.06	0.13	2.30	3.34
F19	I	23.6	26.6	0.97	0 04	0.08	1.68	2 77
F20	II	6.1	26.4	0.78	0.02	0 08	1.61	2.49
Mean ±SD		20.4 ±23 7	30.8 ± 22.2	1.13 ±0.58	0.08 ±0.06	0.22 ±0.51	2 31 ±1.55	3.75 ± 2.70

[a]Samples with the same letter are from the same vendor.

obtained from the Sudan. Moisture content ranged from ~6 to 60% and nicotine content from ~8 to 102 mg/g dry wt. Unexpectedly high levels of NNK (up to 7.87 mg/g dry wt) and NNN (up to 3.08 mg/kg dry wt) were found in these samples.

Figure 2 shows the levels of individual TSNA expressed as a percentage of the total TSNA. In this analysis, we found that NNK represented at least 50% of TSNA in all of the samples except A4 (39%) and D17 (39%). NNN represented 23−53% of all TSNA. In samples A4 and D17, samples where NNN was the highest of the TSNA examined, NNN represented 52 and 53% respectively. NAT and NAB represented a small percentage of all TSNA, ranging from 1 to 17%. Total TSNA levels fell in the range 1.59−13.61 mg/g dry wt (Table I).

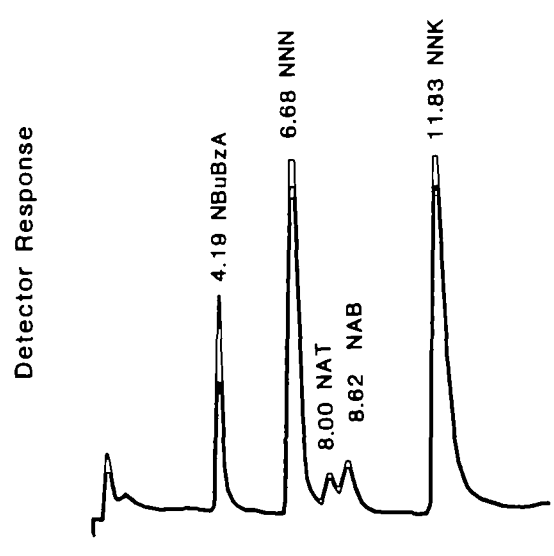

Fig. 1. Chromatogram for the GC/TEA analysis of the four TSNA and the internal standard in sample F19.

Discussion

This is the first report on levels of TSNA in Sudanese toombak. Among the types of smokeless tobacco analyzed from various regions of the world, snuff (used for dipping) has been reported to have the highest levels of carcinogenic TSNA with maximum levels of NNN and NNK reported to be 0.154 and 0.014 mg/g respectively (9). It has been noted that levels of TSNA in snuff exceed levels in any other consumer product by over two orders of magnitude (9). Sudanese toombak contains high concentrations of TSNA, ranging up to mg/g levels. Especially noteworthy are the high levels of NNK, the most potent carcinogenic nitrosamine among TSNA. Levels of NNK in snuff samples have been reported to be ~10−20% of those of NNN (6,10,11). However, in most Sudanese snuff samples, NNK levels were 150−270% of NNN and represented >50% of all TSNA (Figure 2).

The high levels of TSNA in Sudanese snuff could be partly due to the use of *N.rustica* in its preparation. NNN and NNK levels in *N.rustica* have been reported to be much higher than *Nicotiana tabaccum* (12). Other factors may be the treatment with natron and subsequent storage before use. Samples with highest nicotine levels, A2 (102.4 mg/g dry wt) and A3 (73.3 mg/g dry wt) also had high total TSNA levels (13.61 and 5.45 mg/g dry wt respectively). However, this association did not hold across all samples. This is in agreement with previous studies where

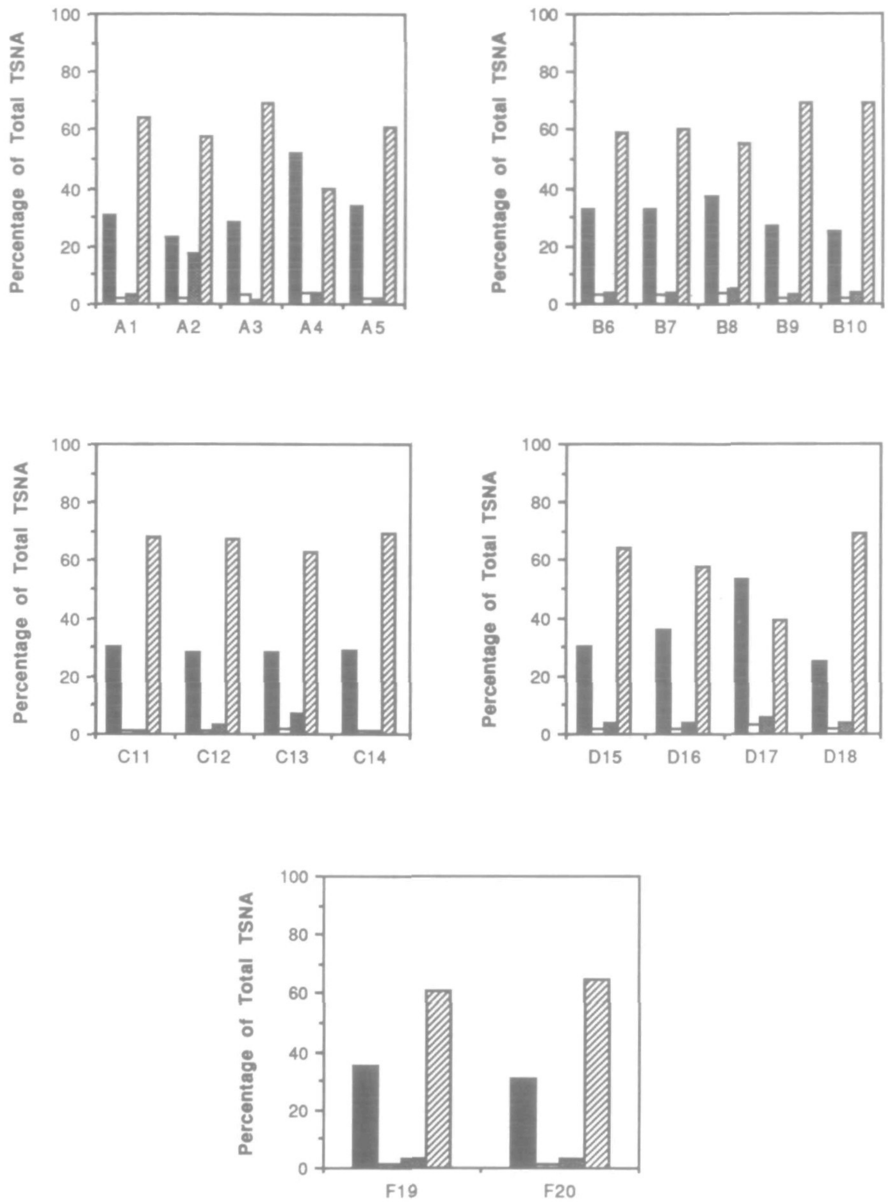

Fig. 2. Each bar represents a TSNA expressed as a percentage of total TSNA Each brand (A, B, C, D, F) had several levels of snuff quality (I, II, III, etc.) that were separately analyzed. Patterns of nitrosamine content, expressed as a percentage of total TSNA, were similar for all types, except brand A4 and D17, in which NNN, not NNK, was the TSNA observed at the highest percentage □, NNN; □, NAT; ▨, NAB, ■ NNK.

positive correlations of nicotine and TSNA content have not been seen (13,14).

The carcinogenicity of NNN and NNK in rodents is well established (3). NAB is reported to be a weak esophageal carcinogen in rats. On the basis of NNN and NNK content ($\sim$67 μg/g tobacco) it has been estimated that a snuff user in the USA is exposed to $\sim$0.7 nmol/kg body wt of NNN and NNK in 40 years of snuff dipping. This is very close to the dose (1.6 nmol/kg body wt) sufficient to induce a significant incidence of oral tumours in rats (5). If a similar extrapolation were done for a Sudanese snuff user, (s)he would be exposed to an equivalent amount (1.6 mmol/kg) of NNK alone in <6 years, even if (s)he consumed only half the amount of snuff of a USA counterpart (mean NNK level in Sudanese snuff = 2.3 mg/g).

Although extensive clinical or epidemiological data are not available for the Sudanese population, oral snuff use should be considered as a major risk factor for oral cancer and other tobacco-related neoplastic disease, as this form of smokeless tobacco had the highest reported TSNA levels and the use of oral snuff has been associated with cancer of oral cavity and pharynx (15,16). The results from a study conducted to measure the levels of TSNA in the saliva of Sudanese oral snuff users will be reported elsewhere (A.M.Idris, J.Nair, H.Ohshima, M.Friesen, I.Brouet, E.M.Faustman and H.Bartsch, in preparation). Although TSNA levels in snuff could be significantly reduced by choosing another tobacco species or by modifying the processing of tobacco, as shown in Denmark and Sweden (13,17), cessation of tobacco use will be the only way to totally prevent the exposure to carcinogenic TSNA. Nevertheless, epidemiological studies should be undertaken to investigate whether the unusually high TSNA levels in toombak are associated with a much higher risk for oral cancer in Sudan as compared to other countries where tobacco contaminated with lower levels of TSNA is used for the same habit.

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