Analysis of *N*- and *O*-Glucuronides of 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in Human Urine

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4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is a tobacco-specific lung carcinogen which may play an important role as a cause of lung cancer in smokers. NNK is extensively metabolized to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), which like NNK is a potent pulmonary carcinogen. NNAL in turn is glucuronidated, and both NNAL and its glucuronides are excreted in human urine. Previous studies have clearly demonstrated the presence in human urine of 4-(methylnitrosamino)-1-(3-pyridyl)-1-(*O*-β-D-glucopyranuronosyl)-butane (NNAL-*O*-Gluc), but did not exclude the presence of 4-(methylnitrosamino)-1-(3-pyridyl-*N*-β-D-glucopyranuronosyl)-1-butanolonium inner salt (NNAL-*N*-Gluc). In this study, we quantified NNAL, NNAL-*N*-Gluc, and NNAL-*O*-Gluc in the urine of smokers, snuff-dippers, and people who used the oral tobacco product "toombak". The presence of NNAL-*N*-Gluc in the urine of toombak users was confirmed by LC-ESI-MS/MS. In smokers' urine, NNAL-*N*-Gluc, NNAL-*O*-Gluc, and NNAL comprised (mean ± SD) 26.5 ± 6.2, 32.1 ± 17.6, and 41.4 ± 16.6%, respectively, of total NNAL. In snuff-dippers' urine, the corresponding figures were 13.6 ± 5.1, 46.6 ± 11.7, and 36.6 ± 9.3%. NNAL-*N*-Gluc comprised 50 ± 25% of total glucuronidated NNAL in smokers and 24 ± 12% in snuff-dippers. This difference was significant ($P = 0.01$), suggesting that smoking induces glucuronidation of NNAL. The results of this study demonstrate that NNAL-*N*-Gluc contributes substantially to NNAL-glucuronides in human urine. These results are important for a clearer understanding of mechanisms of detoxification of NNK in humans.

Introduction

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK,[1] Figure 1) is a systemic lung carcinogen which induces pulmonary tumors in rats, mice, and hamsters independent of the route of administration (*1, 2*). Its lung tumorigenicity is particularly strong in rats, in which total doses as low as 6 mg/kg, administered by sc injection, or 35 mg/kg, administered in the drinking water, produced statistically significant incidences of lung tumors (*3, 4*). Even lower doses induced lung tumors when considered in dose−response trend analyses. NNK is the most prevalent systemic lung carcinogen in tobacco products (*5, 6*). Its presence has been repeatedly demonstrated and confirmed in multiple international studies (*7*). There is now considerable evidence that NNK plays a significant role as a cause of lung cancer in smokers (*5, 8*). NNK and the related nitrosamine *N*′-nitrosonornicotine (NNN) are likely causative agents for oral cancer in people who use smokeless tobacco products (*1, 8, 9*).

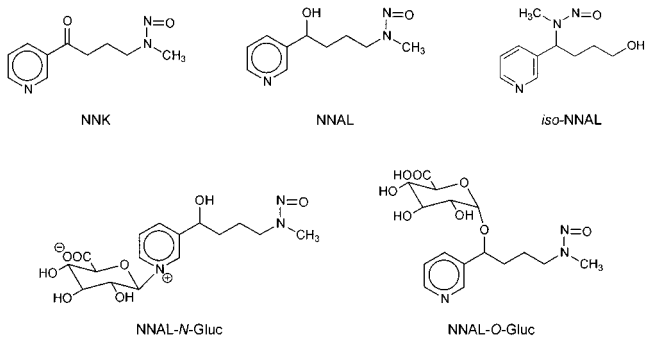

Figure 1. Structures of the compounds discussed in this paper.

Metabolism of NNK to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL, Figure 1) is quantitatively important in rodents and humans (*1*). NNAL is also a potent pulmonary carcinogen and can be regarded as a transport form of NNK (*1*). NNAL can be metabolized to NNAL-*O*-Gluc (Figure 1), which has been conclusively identified in the urine of rodents, monkeys, and humans exposed to NNK (*10*−*14*). We have developed an assay for quantitation of NNAL, both free and glucuronidated, in human urine (*13, 15, 16*). This assay has been applied in numerous studies to demonstrate and quantify the uptake of NNK in smokers, smokeless tobacco users, and nonsmokers exposed to environmental tobacco smoke (*1, 16*−*21*).

Nicotine, its metabolite cotinine, and other pyridine-containing compounds can undergo glucuronidation at

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[1] Abbreviations: GC-TEA, gas chromatography with nitrosamine-selective detection; *iso*-NNAL, 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL-*O*-Gluc, 4-(methylnitrosamino)-1-(3-pyridyl)-1-(*O*−β-D-glucopyranuronosyl)butane; NNAL-*N*-Gluc, 4-(methylnitrosamino)-1-(3-pyridyl-*N*-β-D-glucopyranuronosyl)-1-butanolonium inner salt; NNAL-TMS, NNAL-trimethylsilyl ether; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*′-nitrosonornicotine.

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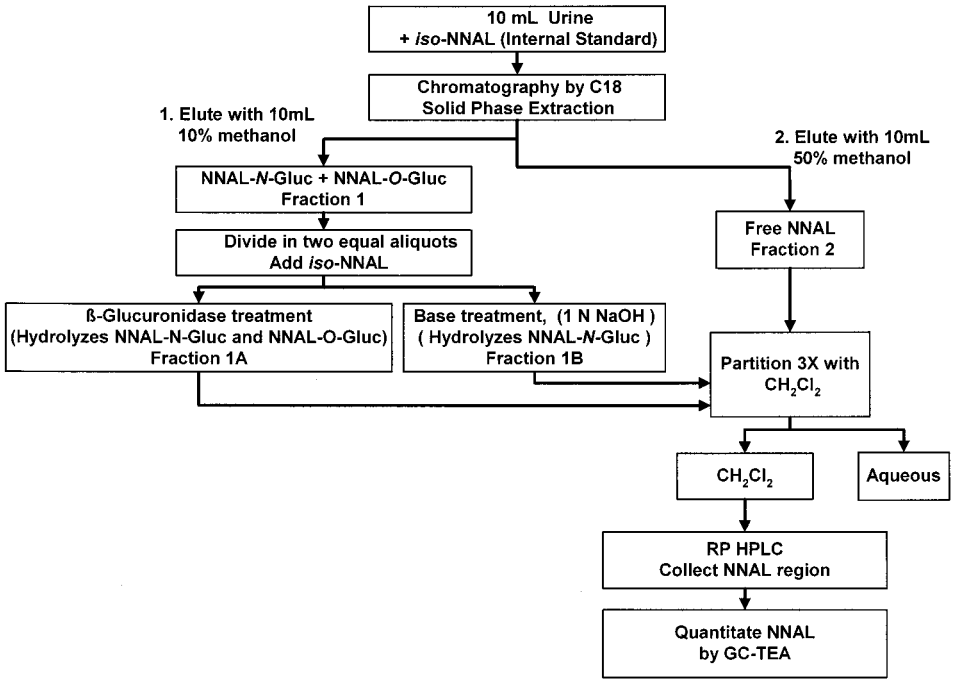
Scheme 1. Method for Analysis of NNAL, NNAL-*N*-Gluc, and NNAL-*O*-Gluc in Human Urine



the pyridine nitrogen (*22*−*24*). Therefore, it appeared likely that NNAL could be metabolized to NNAL-*N*-Gluc (Figure 1). Indeed, Murphy and co-workers have identified NNAL-*N*-Gluc as a metabolite of NNAL in human liver microsomes (*25*). Our previous analyses of NNAL glucuronides in urine would not have distinguished between NNAL-*O*-Gluc and NNAL-*N*-Gluc, because β-glucuronidase was used to release free NNAL. In this study, we have analyzed the urine of tobacco users for NNAL, NNAL-*N*-Gluc, and NNAL-*O*-Gluc.

Experimental Procedures

Apparatus. Gas chromatography with nitrosamine-selective detection (GC-TEA) was carried out with an HP 6890 gas chromatograph (Agilent Technologies, Wilmington, DE) interfaced to a model 543 Thermal Energy Analyzer (Orion Research, Beverly, MA). LC-ESI-MS/MS was performed with a TSQ 7000 instrument (Thermo Finnigan, San Jose, CA).

Chemicals and Enzymes. NNAL, (*R*)-NNAL-*O*-Gluc, NNAL-*N*-Gluc, and *iso*-NNAL were prepared as described previously (*26*−*29*). β-Glucuronidase, type IXA, was obtained from Sigma Chemical Co. (St. Louis, MO).

Analytical Procedures. Urine samples from smokers and snuff-dippers were obtained from ongoing tobacco reduction and cessation studies that were approved by the University of Minnesota Research Subjects' Protection Programs Institutional Review Board Human Subjects Committee. Urine samples from toombak users were the same ones as collected previously (*14*).

Quantitation of NNAL, NNAL-N-Gluc, and NNAL-O-Gluc in Urine. The pH of a 10 mL urine sample was adjusted to 6−7, if necessary, and *iso*-NNAL, 5 ng, was added. *iso*-NNAL, a geometric isomer of NNAL, was used as internal standard; *iso*-NNAL glucuronides were not available. A 2 g Sep-Pak Vac C18 solid-phase extraction cartridge (Waters Corp., Milford, MA) was equilibrated by elution with 5 mL of methanol, then 15 mL of H_2O. Then, the 10 mL urine sample containing *iso*-NNAL was applied to the cartridge. Collection of eluant began as soon as the sample was applied. After the 10 mL had almost completely eluted, the cartridge was then eluted with 10 mL of 10% methanol. Fraction 1 (Scheme 1), containing NNAL-*N*-Gluc and NNAL-*O*-Gluc, consisted of the initial eluant and the 10% methanol eluant. Then the cartridge was eluted with 10 mL of 50% methanol, giving Fraction 2, containing NNAL plus *iso*-NNAL. *iso*-NNAL (5 ng) was added to Fraction 1, which was then concentrated to dryness on a Speedvac concentrator (Thermo Savant, Farmingdale, NY). The residue was redissolved in 10 mL of H_2O and split into 2 aliquots, one for β-glucuronidase treatment (Fraction 1A) and one for base treatment (Fraction 1B). Fraction 1A (5 mL) was adjusted to pH 6−7, if necessary, and 25 000 units of β-glucuronidase were added. This amount of β-glucuronidase was sufficient for complete hydrolysis (*15*). The mixture was shaken on an orbital shaker for 16 h at 37 °C, and then extracted 3 times with CH_2Cl_2. The CH_2Cl_2 extracts containing NNAL released from NNAL-*N*-Gluc and NNAL-*O*-Gluc were combined and brought to dryness. The residue was redissolved by first adding 50 μL of methanol, then 900 μL of 50 mM NaH_2PO_4 buffer, pH 7. It was purified further by reverse-phase HPLC, and the NNAL was quantified by GC-TEA as described previously (*16*). Fraction 1B (5 mL) was treated with 1 N NaOH, 1 h, 80 °C, to release NNAL from NNAL-*N*-Gluc. The mixture was neutralized with 1.0 N HCl, and then processed in a manner identical to that used for Fraction 1A. Fraction 2 was concentrated to dryness, redissolved in 5 mL of H_2O, the pH adjusted to 6−7, if necessary, and extracted with CH_2Cl_2 as above. It was subjected to HPLC and GC-TEA as described for Fractions 1A,B.

Identification of NNAL-N-Gluc in the Urine of Toombak Users. Urine (1 mL) was applied to a solid-phase extraction cartridge. The cartridge was washed with 4 mL of H_2O. The eluants were discarded. It was then eluted with 5 mL of 10% methanol in H_2O. The eluants were concentrated to dryness and reconstituted in H_2O, and a portion was analyzed by LC-ESI-MS/MS using a 100 × 3.0 mm Hypercarb 5 μm column (Phenomenex) with elution by a 60 min gradient from 100% 10 mM ammonium acetate, pH 6.5, to 100% methanol at a flow rate of 0.36 mL/min. The MS was operated with source current 9.0 μA, source voltage 4.0 kV, and capillary temperature 270 °C. The transition *m*/*z* 386−210 was monitored.

NNAL Chirality. Fraction 1B was treated with base, and the released NNAL was analyzed by chiral stationary phase GC-TEA using a Cyclosil-B column as previously described (*19*).

Results

The method for analysis of NNAL-*N*-Gluc, NNAL-*O*-Gluc, and NNAL in human urine is summarized in

Table 1. NNAL, NNAL-*N*-Gluc, and NNAL-*O*-Gluc in Urine

		pmol/mL		
subject	gender	NNAL	NNAL-*N*-Gluc	NNAL-*O*-Gluc
		(A) Smokers		
1	M	0.853	0.470	ND[a]
2	F	0.417	0.534	0.624
3	M	0.256	0.318	0.510
4	F	0.673	0.328	1.06
5	M	0.611	0.332	0.362
6	M	0.491	0.358	0.526
7	M	0.554	0.522	0.824
8	F	0.316	0.126	0.226
9	M	0.177	0.150	0.194
10	F	0.267	0.084	0.016
mean ± SD		0.462 ± 0.214	0.322 ± 0.161	0.434 ± 0.343
		(B) Snuff-Dippers		
1	M	1.63	0.218	2.23
2	M	3.97	1.98	8.69
3	M	1.63	0.572	2.10
4	M	2.62	1.12	0.980
5	M	0.715	0.278	0.680
6	M	0.342	0.170	0.698
7	M	0.564	NA[b]	NA
8	M	0.541	0.112	0.464
9	M	1.79	0.426	2.02
10	M	1.02	0.446	1.34
mean ± SD		1.48 ± 1.13	0.59 ± 0.60	2.13 ± 2.55
		(C) Toombak Users		
1	M	206.9	28.2	153.8
2	M	526.7	55.5	624.4
3	M	186.2	32.8	75.1
4	M	99.2	13.7	74.0
mean ± SD		254.8 ± 187.2	32.6 ± 17.4	231.8 ± 264.4

[a] ND: not detected. [b] NA: not analyzed due to technical problems.

Scheme 1. In preliminary experiments in which the analytes were added to nonsmokers' urine, we established that 5% methanol is required to elute NNAL-*N*-Gluc from the C18 solid-phase extraction cartridge while 10% methanol is needed to elute NNAL-*O*-Gluc. NNAL begins to elute when the methanol concentration is between 15 and 20% and is completely removed from the column with 50% methanol. Therefore, we used 10% methanol to separate NNAL-*N*-Gluc plus NNAL-*O*-Gluc from NNAL. We then used 50% methanol to elute NNAL. *iso*-NNAL, the internal standard, elutes from the cartridge in the NNAL fraction. The glucuronides of *iso*-NNAL were not available for use as internal standards. Therefore, we added *iso*-NNAL to the NNAL-*N*-Gluc plus NNAL-*O*-Gluc fraction after elution from the cartridge, for correction of any losses that might occur from that point forward.

For the analysis of NNAL-*N*-Gluc, we took advantage of its sensitivity to base. NNAL-*N*-Gluc is completely hydrolyzed to NNAL by treatment with 1.0 N NaOH, 80 °C, 1 h. This reaction does not occur at pH 7.0, 80 °C, 1 h. We also established that NNAL is not released from NNAL-*O*-Gluc by base treatment. However, both NNAL-*O*-Gluc and NNAL-*N*-Gluc are converted to NNAL by treatment with β-glucuronidase. Therefore, the material eluted from the solid-phase extraction cartridge with 10% methanol (Fraction 1) was split into two aliquots (see Scheme 1). One aliquot was treated with β-glucuronidase to release NNAL from both NNAL-*N*-Gluc and NNAL-*O*-Gluc, giving Fraction 1A. The second aliquot was treated with base to release NNAL from NNAL-*N*-Gluc, yielding Fraction 1B. The NNAL released from these fractions was quantified in the same manner as that in Fraction 2 containing NNAL eluted from the solid-phase extraction cartridge. This analysis required CH_2Cl_2 extraction and reverse-phase HPLC enrichment prior to conversion to NNAL-trimethylsilyl ether (NNAL-TMS) and quantitation by GC-TEA. NNAL in urine was determined directly by analysis of Fraction 2, while NNAL-*N*-Gluc was similarly determined by analysis of NNAL released upon base treatment (Fraction 1B). NNAL-*O*-Gluc was determined by the difference between NNAL released in Fraction 1A (from NNAL-*O*-Gluc plus NNAL-*N*-Gluc) and Fraction 1B (from NNAL-*N*-Gluc).

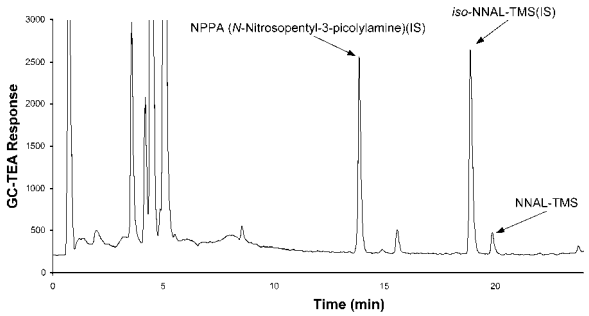


Figure 2. Chromatogram obtained upon GC-TEA analysis of NNAL released in Fraction 1B of Scheme 1 by base treatment.

In preliminary experiments, we analyzed smokers' urine for NNAL-*N*-Gluc by the method shown in Scheme 1. The results of these experiments were consistently positive. A typical GC-TEA trace of NNAL released from this fraction is shown in Figure 2. Further experiments in which NNAL, NNAL-*O*-Gluc, and NNAL-*N*-Gluc were added to urine samples demonstrated that the method illustrated in Scheme 1 gave satisfactory results (data not shown). We assessed assay precision by dividing a smoker's urine into six aliquots. Coefficients of variation

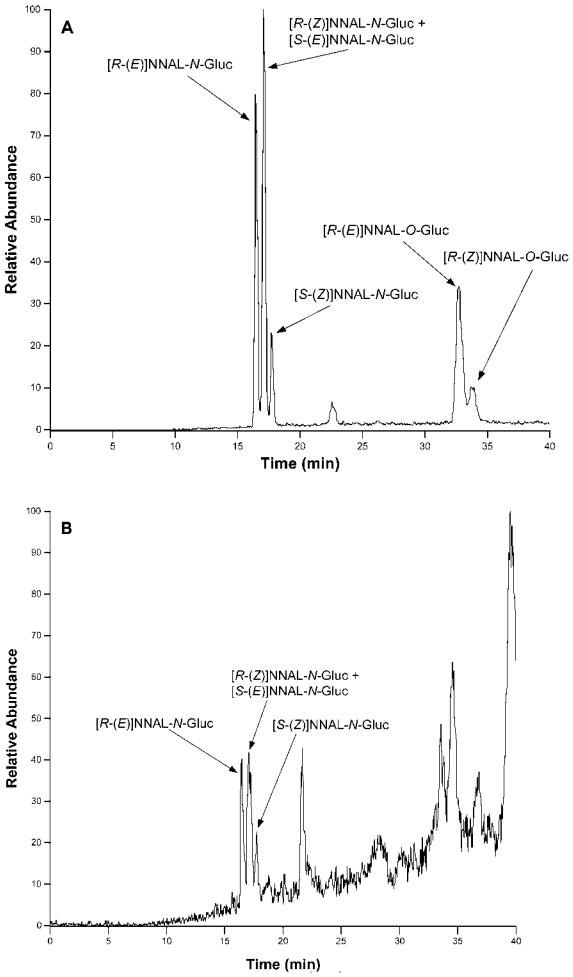


Figure 3. (A) Separation of standard NNAL-*N*-Gluc diastereomers and rotamers and NNAL-*O*-Gluc diastereomers on a graphite stationary phase HPLC column. Detection was by ESI-MS/MS (*m*/*z* 386−210). (B) LC-ESI-MS/MS analysis under the same conditions as used in (A) of a fraction from toombak users' urine, prepurified as described under Experimental Procedures. NNAL-*N*-Gluc identity was also confirmed by co-injection of the indicated peaks with standards.

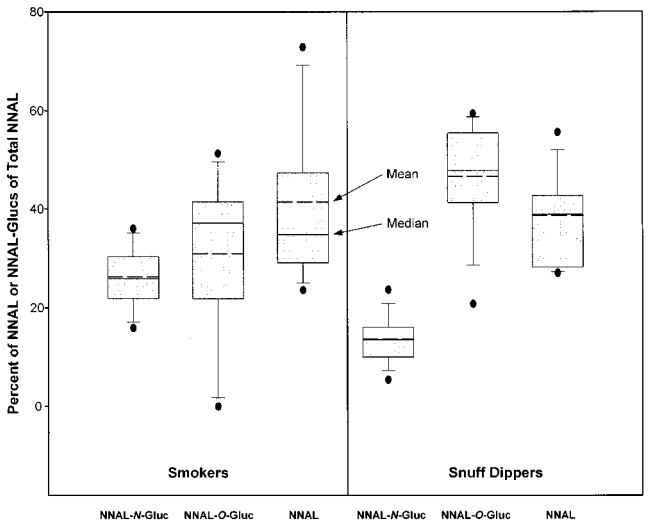


Figure 4. Percent contributions of NNAL-*N*-Gluc, NNAL-*O*-Gluc, and free NNAL to total NNAL in the urine of smokers and snuff-dippers. Boxes represent the 25th−75th percentile, outer lines represent the 10th−90th percentile, and dots represent outliers.

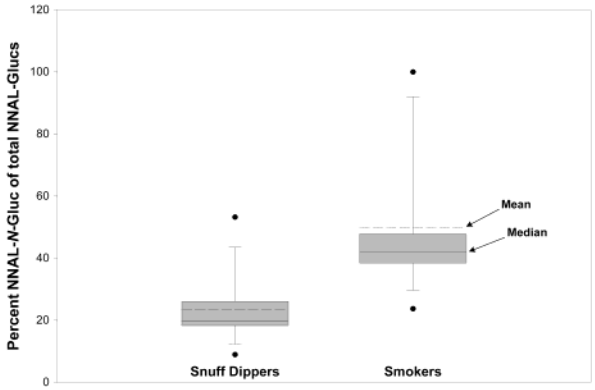


Figure 5. Percent contributions of NNAL-*N*-Gluc to total NNAL glucuronides in snuff-dippers and smokers. Boxes represent the 25th−75th percentile, outer lines represent the 10th−90th percentile, and dots represent outliers.

were 7.9%, 9.7%, and 8.8% for NNAL-*N*-Gluc, NNAL-*O*-Gluc, and NNAL, respectively.

Urine samples from smokers, snuff-dippers, and toombak users were analyzed. Snuff-dipping is the practice of placing moist fine-cut tobacco between the cheek and gum. This practice has increased dramatically in the U.S. (*30*). Toombak is a smokeless tobacco product used as an oral snuff in Sudan (*31*). It contains exceptionally high levels of tobacco-specific nitrosamines, and consequent high levels of NNK metabolites are found in the urine of people who use this product. The results of these analyses are summarized in Table 1. Recoveries of the internal standard *iso*-NNAL ranged from 62 to 99%.

LC-ESI-MS/MS was used to confirm the presence of NNAL-*N*-Gluc in toombak users' urine. For this analysis, we used an HPLC column with a graphite stationary phase on which NNAL-*N*-Gluc was retained and gave excellent peak shape, as shown in Figure 3A. The first peak which elutes from the column is [*R*-(*E*)]NNAL-*N*-Gluc, the second is a mixture of [*R*-(*Z*)]NNAL-*N*-Gluc and [*S*-(*E*)]NNAL-*N*-Gluc, and the third is [*S*-(*Z*)]NNAL-*N*-Gluc. The resolution of these peaks is superior and the retention greater than that obtained on a C18 reverse-phase column. NNAL-*N*-Gluc is well separated from NNAL-*O*-Gluc in this system. Fraction 1 of the urine was initially purified by C18 reverse-phase HPLC prior to the LC-ESI-MS/MS analysis. Selected reaction monitoring of *m*/*z* 386 (M + 1 of NNAL-*N*-Gluc) to *m*/*z* 210 (M + 1 of NNAL) was used. The results are shown in Figure 3B. The retention time of the NNAL-*N*-Gluc peaks was confirmed by co-injection with a standard. We did not attempt to confirm NNAL-*O*-Gluc in this sample, as its presence in the urine of toombak users had already been demonstrated (*14*).

The percent contributions of NNAL-*N*-Gluc, NNAL-*O*-Gluc, and NNAL to total NNAL in the urine of smokers and snuff-dippers are summarized in Figure 4. In smokers' urine, NNAL-*N*-Gluc, NNAL-*O*-Gluc, and NNAL comprised (mean ± SD) 26.5 ± 6.2, 32.1 ± 17.6, and 41.4 ± 16.6%, respectively, of total NNAL. In snuff-dippers' urine, the corresponding figures were 13.6 ± 5.1, 46.6 ± 11.7, and 36.6 ± 9.3%. The percent contributions of NNAL-*N*-Gluc to total glucuronidated NNAL are illustrated in Figure 5. NNAL-*N*-Gluc comprised 50 ± 25% of total glucuronidated NNAL in smokers and 24 ± 12% in snuff-dippers. This difference was significant (P = 0.01).

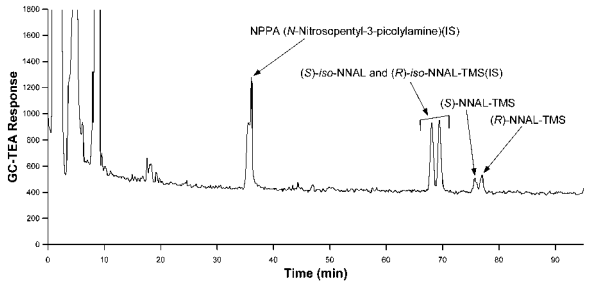


Figure 6. Chromatogram obtained upon chiral GC-TEA analysis of NNAL released by base hydrolysis of NNAL-*N*-Gluc in a smoker's urine. NPPA, *N*-nitrosopentyl-3-picolylamine, internal standard added before injection on GC-TEA; *iso*-NNAL, internal standard added as shown in Scheme 1.

Chiral stationary phase GC-TEA was used to determine the diastereomeric ratio of NNAL-*N*-Gluc in smokers. Analysis of NNAL released in Fraction 1B from base treatment of NNAL-*N*-Gluc gave the chromatogram illustrated in Figure 6. The ratio (*R*)-NNAL-*N*-Gluc:(S)-NNAL-*N*-Gluc was 1.1 ± 0.12 ($N = 3$).

Discussion

The results of this study demonstrate for the first time that NNAL-*N*-Gluc is present in human urine. Previous studies have confirmed the presence of NNAL-*O*-Gluc in urine. NNAL-*O*-Gluc was identified by NMR and MS analysis of material isolated from rodent and patas monkey urine (*10, 11*). (*R*)-NNAL-*O*-Gluc is the major diastereomer in rodent urine whereas (*S*)-NNAL-*O*-Gluc predominates in patas monkey urine (*27, 32*). NNAL-*O*-Gluc diastereomers were identified in smokers' urine by collection of the appropriate retention time fractions from HPLC, hydrolysis of the collected material with β-glucuronidase, and analysis of the released NNAL by GC-TEA (*13*). Similar experiments with toombak users' urine demonstrated that (*S*)-NNAL-*O*-Gluc was the predominant diastereomer in these samples (*14*). (*S*)-NNAL-*O*-Gluc also predominates in smokers' urine (*19*). Collectively, these results provide compelling evidence for the presence of NNAL-*O*-Gluc in human urine, and the present study is consistent with these data. However, none of our previous studies of NNAL glucuronides in human urine excluded the presence of NNAL-*N*-Gluc.

NNAL-*N*-Gluc makes a substantial contribution—24% in snuff-dippers and 50% in smokers—to total NNAL glucuronides in human urine. The smokers' urine data are influenced by one outlier (Table 1 and Figure 5) in which no NNAL-*O*-Gluc was detected. However, even without this data point, the contribution of NNAL-*N*-Gluc to total NNAL glucuronides is 44.3 ± 16.5%, still significantly higher ($P < 0.01$) than in snuff-dippers. These results suggest that smoking may induce *N*-glucuronidation of NNAL. Smoking is known to induce glucuronidation of the nicotine metabolite 3′-hydroxycotinine as well as glucuronidation of a number of drugs (*33*−*35*). However, smoking had no effect on the *N*-glucuronidation of nicotine or cotinine (*33*). Further studies are required to determine whether smoking affects *N*-glucuronidation of NNAL.

Total NNAL in toombak users' urine was about 50% as great as previously reported in analyses carried out on the same urine samples (*14*). The lower levels measured here probably reflect some decomposition over time. It has been more than 7 years since these samples were previously analyzed, and our unpublished data indicate that NNAL and NNAL-Gluc slowly decompose in urine samples stored for long periods of time.

Free NNAL in smokers' urine comprised 41.4% of total NNAL compared to 30.8% in a previous study (*18*), while the corresponding figures for snuff-dippers were 36.6% vs 26.3% (*36*). This comparison is limited because different smokers and snuff-dippers participated in this study vs the earlier ones, and the number of subjects in this study was limited. Nevertheless, the results suggest that the present method, in which *iso*-NNAL is added to the urine, gives a higher and presumably more accurate measure of free NNAL than the published method, in which *iso*-NNAL is added to the ethyl acetate extract of urine (*15*).

Total NNAL amounted to 1.22 pmol/mL in smokers in this study compared to 1.95 pmol/mL in a previous one while the corresponding figures for snuff-dippers were 4.30 and 4.22 pmol/mL (*18, 36*). Thus, the results presented here are consistent with those published previously.

(*R*)-NNAL-*O*-Gluc was inactive as a lung tumorigen in A/J mice (*26*). This contrasts to the potent lung tumorigenicity of (*S*)-NNAL and NNK in this model (*26*). Therefore, (*R*)-NNAL-*O*-Gluc is a detoxification product of NNAL and NNK. No tumorigenicity data are available for NNAL-*N*-Gluc, but it would be expected to be inactive. Thus, it is likely that NNAL glucuronides in total are a good measure of NNAL and NNK detoxification.

NNAL-*N*-Gluc in urine is comprised of about 50% of each diastereomer, based on analysis of urine from three smokers. In an earlier study, we showed that total NNAL-Gluc in smokers' urine was comprised of 68 ± 8.1% of the (*S*)-diastereomer ($N = 30$)(*19*). In that study, the chirality of NNAL released by β-glucuronidase treatment was determined. Part of the released NNAL would have been from NNAL-*N*-Gluc. Since the diastereomeric ratio for NNAL-*N*-Gluc is apparently about 1, the results indicate that (*S*)-NNAL-*O*-Gluc predominates over the (*R*)-diastereomer in smokers' urine. However, further analyses of the diastereomeric ratio of NNAL-*N*-Gluc are necessary to confirm this conclusion.

Ren et al. investigated the glucuronidation of NNAL by human UDP-glucuronosyltransferases (*37*). They reported that human liver microsomes preferentially catalyze the formation of (*S*)-NNAL-*O*-Gluc from racemic NNAL. They identified UGT2B7, which preferentially produces (*S*)-NNAL-*O*-Gluc and is expressed in human liver, as one of the enzymes involved in this reaction. Thus, UGT2B7 is believed to play a major role in human hepatic NNAL glucuronidation. Polymorphisms in UGT2B7 have been characterized, but their significance with respect to enzyme activity is unknown (*38*). Ren et al. also suggest that UGT1A9 is involved in human hepatic formation of (*R*)-NNAL-*O*-Gluc. Preliminary data indicate that NNAL-*N*-Gluc is a major product of NNAL glucuronidation in human liver microsomes and that UGT1A9 may be involved as a catalyst (*25*).

In summary, the data presented here demonstrate that NNAL-*N*-Gluc contributes substantially to NNAL glucuronides in human urine. These results are important for further understanding the enzymology of NNAL glucuronidation, which may in turn be critical in determining cancer risk upon exposure to NNK. It is likely that NNAL-*N*-Gluc is a detoxification product of NNAL and NNK, as already demonstrated for NNAL-*O*-Gluc.

Therefore, total glucuronidation of NNAL should be a good measure of NNAL and NNK detoxification.

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