Elimination Kinetics of the Tobacco-Specific Biomarker and Lung Carcinogen 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanol

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Abstract

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) is tobacco specific and has a longer half-life than other tobacco biomarkers studied thus far. An accurate measurement of the NNAL half-life is important for optimal use to assess exposure to tobacco smoke. We determined the half-life of NNAL in urine in eight daily smokers on a clinical research ward and in five occasional smokers in a real-life environment. Total NNAL in urine was monitored for 14 days in daily smokers after stopping smoking and for up to 60 days in occasional smokers. The average half-life for the terminal phase in the daily smoker group using a two-compartmental body model was 10.3 days (beta phase), and using a noncompartmental model, it was 9.1 days. In the occasional group, these values were 17.6 and 16.0 days, respectively. The alpha-phase half-lives were 14.3 and 27.8 hours for the two groups, respectively. The inter-subject coefficient of variation of the NNAL terminal half-life ranged from 14% to 30%, and the intra-subject coefficient of variation ranged from 3% to 18%. There was very good agreement between the plasma and urinary half-lives in two subjects with plasma analyses: 7.4 versus 7.9 days and 9.2 versus 10.7 days. Mean renal clearance of NNAL was 13 ± 2.3 mL/min. The terminal half-life of NNAL of 10 to 18 days indicates that this biomarker can be used to detect tobacco smoke exposure for 6 to 12 weeks after cessation of exposure and requires a similar time to assess the steady levels of NNAL after switching from one tobacco product to another. (Cancer Epidemiol Biomarkers Prev 2009;18(12):3421–5)

Introduction

A variety of biomarkers have been used to access tobacco exposure in smokers and nonsmokers (1-3). 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) is highly tobacco specific and seems to have the longest half-life of all the biomarkers studied thus far (4, 5). This long half-life has an advantage over the currently most widely used biomarker, cotinine, because it allows for a time-averaged assessment of exposure, which is less dependent on sampling time with respect to when the last cigarette was smoked. In addition, NNAL is itself a carcinogen and is derived from another carcinogenic tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Therefore, studies on NNAL concentrations in smokers and nonsmokers are expected to be better correlated with the carcinogenic effects of tobacco, as has been shown in recent case control studies of lung cancer in smokers (3, 6, 7).

Published data on the half-life of NNAL are quite variable, ranging from 4 to 45 days. We recently developed a highly sensitive assay for NNAL in urine that can be used for evaluating low-level exposure (8, 9). An accurate measurement of the NNAL half-life is important to fully understand how urinary NNAL measurements relate to exposure to NNK over time, such as after smoking cessation or when studying tobacco smoke constituent exposure when people switch from one tobacco product to another. For these reasons, we studied the half-life of NNAL under highly controlled research conditions. We also compared this half-life to that in occasional smokers in a real-life environment.

Materials and Methods

Study Design. The study aimed to determine the half-life of NNAL in two groups of smokers: (*a*) research subjects on a clinical research ward, and (*b*) occasional smokers in a real-life environment. The study was approved by the Institutional Review Board at the University of California, San Francisco and the Commission on Bioethics at the Medical University of Silesia, Poland.

The clinical research ward subjects participated in a 3-wk inpatient study. Participants were not allowed to leave the hospital ward unless accompanied by staff. For the first week, subjects smoked 20 cigarettes per day. For the second and third weeks, subjects were not allowed to

Received 8/27/09; revised 10/21/09; accepted 10/22/09; published online 12/3/09.

Grant support: Center for Tobacco Control Research and Education (NIH/R25CA113710) and Flight Attendants Medical Research Institute, National Institute on Drug Abuse (DA012393).

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doi:10.1158/1055-9965.EPI-09-0874

smoke. (During that time, nicotine or placebo patches were provided to all participants as part of a different study.) Nonsmoking status was verified three times per day with an exhaled air CO monitor. Twenty-four-hour urine samples were collected from each subject on each day of the study. Plasma samples were collected on days 0, 2, 3, 6, 7, 10, 13, and 14 after smoking from two of the subjects.

The second group of subjects (real-life settings) were "social" smokers. Each participant provided a series of 24-h or spot urine specimens. These were collected every day for 1 wk after the last smoking event, then every 2 to 3 d for up to 7 wk (thus, maximum total monitoring time was 7 + 49 = 56 d). Participants were asked not to smoke and to avoid any exposure to tobacco products or second-hand tobacco smoke during the 56-d monitoring period. Even if a subject did not stay tobacco-free for 56 d, he was asked to continue sample collection for the length of the study. However, data analysis was terminated when an increase of NNAL was observed thought to be due to tobacco reexposure.

Participants. Eight healthy adult subjects who smoked ≥15 cigarettes per day were recruited for the clinical research ward study (6 men and 2 women; 5 Whites, 2 African Americans, and one subject of unknown race). Nine healthy adult cigarette smokers living in Southern Poland were recruited for the second part of the study. These subjects were non-daily smokers who smoked <20 cigarettes per month. Four subjects were excluded from the analysis because their NNAL levels showed substantial increases in NNAL over an interval of <14 d, indicating smoking or significant second-hand smoke exposure, making it difficult to estimate a half-life with confidence.

Sample Analysis. Total NNAL (free and glucuronide) was measured in urine and plasma samples by liquid chromatography tandem mass spectrometry [Jacob et al. (8)].

Statistical Analysis. The elimination half-life of NNAL was estimated using WinNonlin 5.2 software (Pharsight Corp.) using two-compartment and noncompartmental body models. The renal clearance (CL_R) was estimated by dividing the 24-h urinary excretion rate by the serum concentration of NNAL. The urine and blood samples used for CL_R estimation were collected on the last day before smoking cessation from seven subjects participating in the clinical research ward study.

Results

The average age of subjects from the clinical research group was 45.6 ± 11.9 (SD) years, and that from the real-life group was 32.3 ± 14.4 years. The average body mass index of subjects from the clinical research group was 25.6 ± 4.4, and that from the real-life group was 24.9 ± 1.8. The average number of cigarettes smoked per day for the research ward subjects was 22 ± 8. The average creatinine-normalized NNAL concentration for the hospitalized subjects before smoking cessation was 435 ± 265 pg/mg, and that for the real-life subjects was 37.2 ± 34.7 pg/mg (the latter was measured 24 hours after smoking the last cigarette). The NNAL concentration was above the limit of quantitation (0.25 pg/mL) for all the samples collected.

Individual subject half-lives are presented in Table 1. The average half-life for terminal phase estimated for the clinical research using a two-compartmental model was 10.3 days (beta phase), and using a noncompartmental model, it was 9.1 days. For the real-life settings, these values were 17.6 and 16.0 days, respectively. The alpha-phase half-lives were 14.3 and 27.8 hours for the clinical and real-life settings, respectively. Figure 1 depicts the mean levels of total NNAL as a percentage of baseline level in the urine of eight subjects from the clinical research ward study (*upper panel*) and of one subject from the real-life settings who repeated the study three

Table 1. Individual and averaged values of urinary NNAL half-life

	Monitoring time (d)	Two-compartmental model half-life		Noncompartmental model half-life
		Alpha phase (h)	Terminal (beta) phase (d)	Terminal phase (d)
Clinical settings				
Subject A	14	12.4	11.9	10.5
Subject B	14	10.2	9.3	7.0
Subject C	14	12.0	11.3	14.8
Subject D	14	19.6	9.9	6.4
Subject E	14	10.2	9.3	8.8
Subject F	14	11.3	7.9	6.9
Subject G	14	11.7	10.7	9.6
Subject H	14	26.6	12.2	9.2
Average	14	14.3	10.3	9.1
SD (CV%)	—	5.8 (41)	1.5 (14)	2.7 (30)
Real-life settings				
Subject I	35	30.7	20.6	16.9
Subject J	42	24.0	15.6	12.0
Subject K	28	127.1*	7,011.2*	12.7
Subject L	56	45.9	22.9	21.8
Subject M1	42	5.2	11.4	13.3
Subject M2	56	11.9	11.1	19.1
Subject M3	56	14.5	11.7	16.8
Average	45	27.8	17.6	16.0
SD (CV%)	11.3 (25)	14.7 (53)	5.1 (29)	3.9 (24)

*Data excluded from statistical analysis.

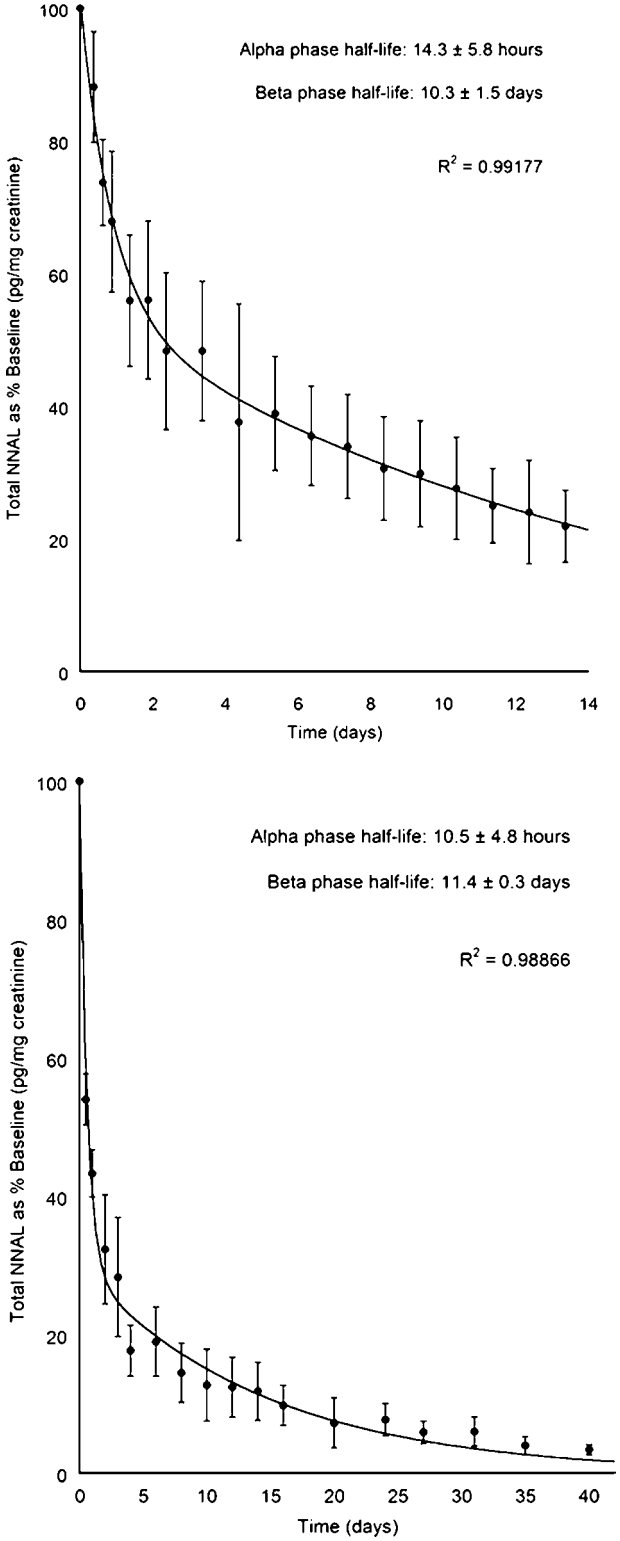

Figure 1. Mean levels of total NNAL as a percentage of baseline level in the urine of eight subjects who stopped smoking on a clinical research ward (*upper*; *bars,* SD and inter-subject variations) and one subject from the real-life setting who repeated the study three times in the course of 6 mo (*lower*; *bars,* SD and intra-subject variations).

times in the course of 6 months (*lower panel*). The inter-subject coefficient of variation (CV) of the NNAL terminal half-life was 14% and 30% for the beta phase 2 compartment body model and noncompartmental model half-lives, respectively. The intra-subject CVs were 3% and 18% for the two half-lives, respectively (estimated based on one subject who had half-life measurements three times). Figure 2 compares the plasma and urine half-lives for two subjects who provided both urine and plasma samples. There was excellent agreement between the plasma and urinary half-lives. The average AUC estimated for

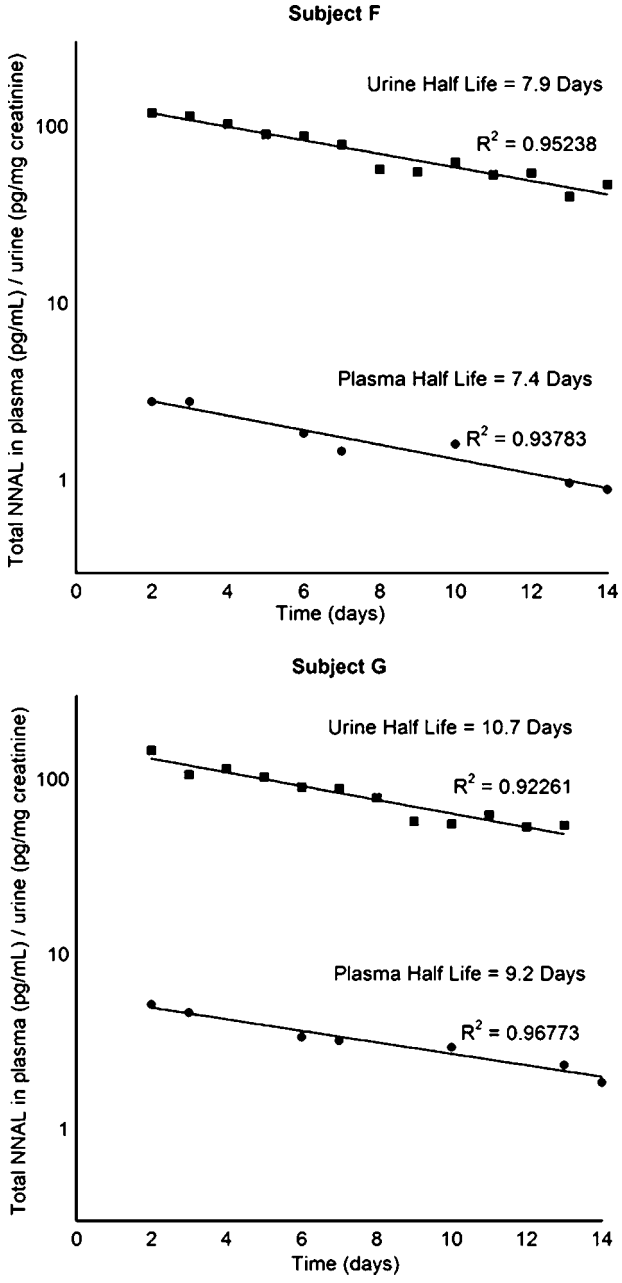

Figure 2. Plasma and urine concentrations and half-lives of NNAL in two subjects who stopped smoking on a clinical research ward.

the times covered in the study were 65% and 78% of the AUC projected to infinity for the clinical and real-life settings, respectively. Mean renal clearance of NNAL was 13 ± 2.3 mL/min (CV, 18%).

Discussion

Novel findings of our study include (*a*) a detailed analysis of the disposition kinetics of NNAL after cessation of regular smoking in a controlled setting in which tobacco exposure was prohibited, (*b*) determination of the half-life of NNAL after smoking cessation in occasional smokers, and (*c*) demonstration of concordance of urine and plasma half-lives of NNAL. Our data generated in a controlled setting provide the best available estimate of the half-life of NNAL and refine the previously published range of 4 to 45 hours (4, 5, 10, 11).

The terminal half-life of NNAL averaged about 10 days in regular smokers after stopping smoking for 14 days and about 18 days for occasional smokers who reported not smoking for periods of 28 to 56 days. After cessation of exposure, levels of the substance decline to <5% of initial levels in five half-lives. Using the range of half-lives from 8 to 23 days observed in our subjects and using a high-sensitivity assay, one should be able to measure NNAL for 6 to 12 weeks or longer after cessation of exposure. NNAL is thus a superior biomarker for investigating tobacco exposure over prolonged periods of time, when exposure is sporadic, which is the case when studying exposure to second-hand smoke and in occasional smokers. Our data show that even after a single exposure from smoking a few cigarettes, resulting in a urine NNAL concentration of <40 pg/mg of creatinine, one can measure NNAL for up to 8 weeks.

We observed a longer half-life in the occasional smokers studied in an uncontrolled environment compared with the regular smokers on a research ward. The longer half-life in social smokers could be due to unreported reexposure to NNK due to cigarette smoking or second-hand smoke exposure. Another explanation could be the presence of a deep tissue compartment for NNAL with a long terminal half-life that is not seen in the 14-day hospital study, but is detected during longer periods of follow-up. The hospital study spanned 1 to 1.5 half-lives; the occasional smokers were followed for up to 3.5 half-lives. Even if there were a deep tissue compartment, we estimate that it would account for a relatively small percentage of the total AUC of NNAL. Therefore, the 10-day terminal half-life describes the elimination kinetics of most of the NNAL in the body and is expected to be predictive of the time course of accumulation or decrease when rates of exposure to NNK change. We also document for the first time the excellent agreement between the plasma and urinary half-lives for two subjects studied in a clinical setting.

We report half-life data for total NNAL in urine, combining both free and conjugated NNAL. Previous observations by Hecht et al. (4) indicate no difference in the half-life of free versus conjugated NNAL, and thus our use of total NNAL is warranted.

The average terminal half-life of NNAL was previously reported by Hecht et al. to be 26 and 45 days in smokers (4) and users of smokeless tobacco (5), respectively. The reason why the Hecht estimates of half-life were 2- to 3-fold higher than what we have found might be that their subjects were reexposed to NNK, such as from surreptitious smoking or from second-hand smoke exposure. In an uncontrolled setting, the probability of being reexposed to NNK increases with the length of the study. Other observations reported by Hecht et al. include the high inter-subject variation of the estimated half-lives (average CV of 62%) and the difference in half-life for smokeless tobacco users versus cigarette smokers (26 versus 42 days, respectively). Our data show much less inter-subject variation in the NNAL half-life compared with Hecht. We have not studied smokeless tobacco users, but would not expect the half-life of NNAL to differ compared with that of smokers. Recent data from Carmella et al. (11) measuring eight urinary toxicant biomarkers for 56 days after smoking cessation found a terminal half-life for NNAL of 15.5 days (raw data provided in the cited article were reanalyzed by us using two-compartment modeling), which is in agreement with the findings of our study.

We also present data on the renal clearance of NNAL in 7 smokers, averaging 13 mL/min. Our data are similar to those measured by Hecht et al, 9.8 mL/min, in three subjects (4).

In conclusion, we provide novel data on the elimination half-life of NNAL in urine of subjects who were studied under continuous observation after stopping smoking on a research ward, as well as in occasional smokers. We show the concordance of urine and plasma half-lives in two subjects. The 10- to 18-day half-life of NNAL makes this the best available biomarker for assessing intermittent exposure to tobacco, such as with exposure to second-hand smoke or in occasional smokers. Our data indicate that NNAL can be detected in urine for 6 to 12 weeks after smoking cessation, and that when subjects are switched from one tobacco product to another, such as in testing of novel products, it will take 6 to 12 weeks for NNAL levels to reach a new steady state. Measuring NNAL is also useful in assessing exposure to tobacco in people using nicotine medications, such as in smoking cessation clinical trials.

Disclosure of Potential Conflicts of Interest

N.L. Benowitz has been a paid expert witness in litigation against tobacco companies. No other potential conflicts of interest were disclosed.

Acknowledgments

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We thank Marc Olmsted for editorial assistance.

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