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Safety of Cotinine in Humans: Physiologic, Subjective, and Cognitive Effects

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HATSUKAMI, D. K., M. GRILLO, P. R. PENTEL, C. ONCKEN AND R. BLISS. *Safety of cotinine in humans: physiologic, subjective, and cognitive effects.* PHARMACOL BIOCHEM BEHAV **57**(4) 643–650, 1997.—Preliminary data suggest that cotinine, the major metabolite of nicotine, may be behaviorally active. Studies involving the administration of cotinine at doses that produce high blood concentrations (in excess of those produced by cigarette smoking) may be of interest. This inpatient, 10-day human study examined the safety and the effects from several high doses of oral cotinine fumarate (40, 80, or 160 mg) or placebo in abstinent cigarette smokers. All subjects smoked cigarettes ad lib during the first 2 days of the study, then were required to be abstinent beginning on the third day. All subjects were given placebo on this day to wash out nicotine before the administration of cotinine. Subjects were subsequently randomly assigned in a double-blind manner to cotinine or placebo for the next 3 days to determine the safety profile of cotinine. All subjects were given placebo on the final 3 days to examine cotinine withdrawal symptoms. The results showed no significant physiologic, subjective, or performance effects across the various doses of cotinine and placebo. Furthermore, no cotinine withdrawal effects were observed. This study demonstrates that short-term administration of cotinine to humans at levels as high as 10 times that attained from cigarette smoking is safe with no observable acute or withdrawal effects from cotinine in this setting. © 1997 Elsevier Science Inc.

Cotinine Subjective effects Physiologic effects Cognitive effects Withdrawal Significance

COTININE is the major metabolite of nicotine in the tobacco user, with approximately 85% of nicotine transformed to cotinine (3). The terminal elimination half-life of cotinine is approximately 19 h compared with 2 h for nicotine. As a consequence, cotinine has become the principal biochemical marker of nicotine exposure in tobacco research paradigms. However, little attention has been given to the role of cotinine as having a pharmacologic influence in maintaining cigarette smoking behavior. The blood cotinine concentration resulting from use of tobacco products has been significantly correlated with frequency of tobacco use, severity of tobacco withdrawal symptoms experienced upon cessation, and level of nicotine dependence (30). These relationships between cotinine and smoking-related behaviors are thought to be due to the levels of nicotine intake that are reflected by cotinine levels. However, both animal and human data, albeit limited, suggest that cotinine may also have some CNS effects.

In animal studies, the effects of cotinine compared with nicotine appear to differ across species. For example, in squirrel monkeys, cotinine and nicotine produced similar effects on responses for food, and cotinine was considered to be equipotent to nicotine (25). On the other hand, in beagles (25) and rats (14), response patterns for food with cotinine differed from that of nicotine. Furthermore, drug discrimination studies showed generalization from nicotine to cotinine occurring only with very large doses of cotinine in rats (14) and squirrel monkeys (28). Of note, these investigators believed that this generalization of cotinine to nicotine may have been in part the result of nicotine impurity found in the cotinine. In a different type of study, Yamamoto and Domino (31) observed increases in electroencephalogram and behavioral arousal with cotinine administration in cats, but only with massive doses of cotinine.

Cotinine has been administered to human subjects via sev-

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eral routes, primarily for the purpose of examining its pharmacokinetics. Most of these investigations did not examine either behavioral or subjective effects from cotinine (2,7,11,23); if examined, no effects were reported (10,26). Two studies, however, have shown subjective responses to cotinine. Benowitz et al. (3) found that abstinent smokers reported experiencing significant reductions in cigarette withdrawal symptoms after administration of cotinine. However, this study was primarily a pharmacokinetic study so that there was no control (nondrug) group for comparison. Keenan et al. (19) conducted a double-blind, counterbalanced study examining the pharmacodynamic effects of 30 mg of cotinine base vs. saline administered intravenously over 15 min after 48 h of cigarette abstinence. Differences in subjective responses were observed between cotinine and placebo, with results showing increased levels of anxiety/tension and restlessness with cotinine compared with placebo. These results would indicate that cotinine has some psychoactive effect, and may possibly have a role in tobacco withdrawal, either as a potential therapeutic agent or a contributor to tobacco withdrawal symptoms.

In summary, previous studies suggest that cotinine may be psychoactive. The status of these results indicate that more studies with cotinine are warranted, particularly at higher doses. However, few data exist at this time regarding the safety of administering high doses of this compound in humans. The effects of high doses of cotinine on safety are necessary so that future studies can examine a broader range of dose effects. In addition, any potential adverse effects resulting from higher cotinine levels attained from a combination of nicotine containing products with cotinine need to be ruled out. Furthermore, no studies have examined whether cotinine in and of itself produces withdrawal symptoms. The purpose of the current study was to examine the effects of oral cotinine fumarate during nicotine abstinence. Cotinine was administered at doses intended to produce serum concentrations in excess of those usually observed with cigarette smoking to determine: (a) the serum cotinine concentrations attained, (b) its safety, and (c) the presence of withdrawal signs or symptoms.

METHODS

Subjects

Subjects (37 male and female smokers) were recruited from the Minneapolis–St. Paul metropolitan area via newspaper advertisements. Subjects were initially screened over the telephone. If they met the telephone screening criteria, they were seen by the research coordinator and physician. At this screening session, informed written consent was obtained. Subjects were required to complete a smoking history and Fagerstrom Nicotine Tolerance Questionnaire (13). Alveolar carbon monoxide (CO) and serum cotinine and nicotine levels were obtained. Subjects were included if they: (a) smoked at least one pack of cigarettes per day for at least 1 year; (b) submitted a CO level of > 10 ppm; and (c) were in good health as verified by medical history, screening examination, electrocardiogram (ECG), and laboratory tests. Subjects were excluded if they: (a) required any form of regular psychotropic medication; (b) chronically used systemic steroids or antihistamines; (c) abused alcohol or any other recreational or prescription drug; (d) used any tobacco products other than cigarettes.

Procedure

This study used a between-subject design with one of three doses of cotinine fumarate or placebo as the between-subject variable. The study was run at the University of Minnesota General Clinical Research Center, a federally funded inpatient research ward. Total duration of participation in this study was 10 days. The study was undertaken during tobacco abstinence, since the examination of the use and effects of cotinine is likely to be conducted within this context. However, the effects of cotinine on tobacco withdrawal symptoms were not a focus of this study, since the extent of withdrawal symptoms experienced in the inpatient setting tended to be minimal (16).

Subjects were admitted to the research ward at noon. During the first 2 days of the study, baseline measures were obtained while the subjects were smoking cigarettes on an ad lib basis. Subjects were required to be abstinent from cigarettes beginning on the morning of the third day. All subjects were given placebo at this time to allow a 24-h washout of nicotine. On the morning of the fourth day, subjects were administered one of the following oral doses of cotinine fumarate: placebo, 40 mg, 80 mg, and 160 mg. Nine subjects were to be run per each condition. For purposes of safety, doses of cotinine were tested in ascending order. The subjects assigned to placebo were interspersed across the active dose conditions so that the blinded condition would be maintained. If no adverse effects were detected for a particular dose, then the next higher dose was tested with the next group of subjects. Subjects were given one of the oral doses of cotinine fumarate or placebo for the next 3 days. Since this study was a preliminary investigation of safety, only 3 days of cotinine administration was considered sufficient. Beginning on the seventh day, all subjects were required to take placebo again for 3 more days. This placebo condition allowed observation of withdrawal signs and symptoms from cotinine fumarate. Three days of placebo dosing was chosen, since the maximum tobacco withdrawal effects are observed during 24–72 h of abstinence (30). To minimize experimenter bias, the investigators and nurses involved with assessment were led to believe that during this placebo phase, subjects were randomly assigned to continue to take the medication given to them the prior 3 days or assigned to placebo. Subjects were discharged in the morning of the 10th day if medical and psychological status was considered normal.

Abstinence was confirmed by biochemical verification (e.g., alveolar carbon monoxide) obtained two times per day, randomly distributed across the day. Every morning, weight was recorded and a sleep scale (9) was completed. Subjects were required to complete subjective measures at the same times in the morning and afternoon throughout the study (Table 1). These measures included the Addiction Research Center Inventory (ARCI) (22), which measures druglike effects (benzedrine—stimulant effects, lysergic diethylamide—dysphoria, morphine benzedrine group—euphoria; and phenobarbitol chlorpromazine—sedation); the Profile of Mood States (POMS) (24), which measures various moods such as depression-dejection, tension-anxiety, confusion-bewilderment, anger-hostility, vigor, and fatigue; a Nicotine-Effects visual analogue scale (which measures nicotine-like effects on a scale of 0 to 100); Drug Effects visual analogue scale, which measures whether cotinine produced any drug effects, any bad drug effects, any good drug effects, liking for the drug, and desire for the drug on a scale of 0 to 100; a modified Nicotine Withdrawal Symptom Checklist (17) composed of symptoms of nicotine withdrawal as described in the DSM-IV (1), which subjects rated on a 0–4 scale with 0 = none, 1 = slight, 2 = mild, 3 = moderate, and 4 = severe; and the Smoking Urges Questionnaire (29), which measured two factors, one reflecting intention, desire and anticipation to smoke, and the other anticipation of relief

TABLE 1
TIMES FOR MEASUREMENT

Measures	0630	0830	0930	1000	1200	1500	1800	2100
Physiologic								
Weight	X							
Blood pressure		X	X	X	X	X	X	X
Heart rate		X	X	X	X	X	X	X
Skin temperature		X		X	X	X		
EKG		X			X			
Subjective								
Withdrawal Symptom Checklist		X		X	X	X		
Profile Of Mood States		X		X	X	X		
Nicotine Effects Visual Analog Scale		X		X	X	X		
Drug Effects Scale Visual Analog Scale		X		X	X	X		
Addiction Research Center Inventory		X		X	X	X		
Smoking Urges Questionnaire		X		X	X	X		
Adverse effects		X		X	X	X		
Sleep diary		X						
Performance								
Symbol Digit Modalities Test		X				X		

from negative affect, nicotine withdrawal, and urgent or overwhelming desire to smoke. Cognitive performance was measured by the Symbol Digit Modalities Test (27), which is a measure of complex attention functions and perceptual-motor speed. This measure was selected because of the ease of administration and evidence suggesting that it may be sensitive to the effects of nicotine deprivation (15). Sitting blood pressure and heart rate, skin temperature, a 12-lead ECG, and adverse events were also assessed at these times. Caloric intake was monitored throughout the study. All foods (meals and snacks) that were eaten by the subject were recorded by type and amount. Food content was later analyzed and calculated for daily amount of carbohydrate, protein, fat, sweets, and kilocalories. Caffeine intake was controlled and maintained at the same level throughout the study. The amount of caffeine intake allowed for each individual was based on the levels of intake before the study. Alcohol intake was prohibited. Serum nicotine and cotinine samples were obtained at noon throughout the study. At admission and on days 7 and 9 (cotinine was administered on days 4–6), routine lab tests were measured including complete blood count, serum creatinine, aminoleucine transaminase (ALT), aminospartate transaminase (AST), and alkaline phosphatase (Alk Phos). If results were abnormal, follow-up measurements were obtained after discharge from the inpatient ward. In all cases, attempts were made to obtain follow-up values until they had returned to normal.

During the study, subjects were free to engage in activities on the research ward. Their exposure to smoking related stimuli was controlled during these activities to maintain consistency in cue exposures across subjects. To maximize compliance and completion of this study, subjects were paid $700 for their participation.

Cotinine

(S)-Cotinine was synthesized and converted into its fumarate salt by the method of Bowman and McKennis (6). The crystalline material was purified and found to be $> 98\%$ pure with no nicotine contamination as measured by gas chromatography. The drug substance was formulated into capsule dosage form at the Research Pharmacy at the University of Minnesota. The doses prepared were placebo (0 mg) and 40, 80, and 160 mg of cotinine fumarate. These were tested for uniformity of content, stability, and drug release rate by standard USP dissolution testing. The doses were coded to assure double blinding. Serum cotinine concentrations were measured by gas chromatography (18).

Data Analysis

Data from each of the dosing conditions were combined and analyzed as one randomized study. Between-group comparisons for demographic and smoking history variables were made using one-way analysis of variance or chi-square tests. When the analysis of variance indicated a significant difference, multiple comparisons between treatment groups were performed using Tukey's HSD.

The primary statistical analysis for the present study was performed using an unbalanced repeated-measures analysis comparing differences on each measure by dosage level, day, and interaction of Dose and Day. For measurements collected at multiple time points within a day, the average measurement of all time points for that day was used in the analysis. A baseline measure consisting of the average of all measurements during the 2-day ad lib smoking period (for effects of cotinine) or for the final day of cotinine administration (for withdrawal from cotinine) was used as a covariate. When *F*-tests indicated that a term was significant, posthoc tests were conducted on the adjusted means to determine which of the levels of the term were significantly different from each other. Owing to the large number of analyses conducted, a significance level of 0.005 was adopted for each *F*-test. Likewise, a significance level of 0.01 was adopted for each posthoc test.

Physiologic, Subjective, and Cognitive Effects of Cotinine

The primary dependent measures included all assessments except the Nicotine Withdrawal Symptom Checklist and Smoking Urges Questionnaire. The following dependent measures were analyzed using methods other than those outlined above. Because very few adverse events were reported, data for each subject were collapsed over all time points in the

TABLE 2
DEMOGRAPHIC AND SMOKING HISTORY

Variables	Dose Mean (SD)			
	Placebo	40 mg	80 mg	160 mg
Age	27.3 (6.1)	26.6 (3.0)	30.9 (4.2)	33.8 (8.2)
Number of cigarettes	23.0 (5.0)	26.6 (6.1)	28.3 (5.6)	33.8 (9.2)
Years of smoking	11.4 (6.6)	11.0 (3.9)	15.4 (7.1)	20.3 (7.1)
Fagerstrom score	5.3 (1.1)	6.6 (1.1)	6.2 (1.2)	6.9 (1.0)
Serum cotinine	228.4 (100.1)	288.1 (119.1)	263.9 (99.6)	350.2 (94.9)

3-day period during which subjects were administered cotinine. The maximum severity of response to each adverse event was then used to obtain the frequencies of response by dosage level. For the 160-mg cotinine group, changes in the electrocardiographic PR, QRS, and QT intervals and QRS axis between baseline and 2 h postdose on day 6 (after 3 days of cotinine administration) were compared using paired Wilcoxon tests. Descriptive statistics for the Addiction Research Center Inventory items revealed no need to perform additional analysis on these variables.

Withdrawal Effects From Cotinine

The primary dependent measures used to assess for withdrawal effects from cotinine included all measures except the Drug Effects visual analogue scales, Nicotine Effects visual analogue scales, and adverse events checklist. The following dependent measure was analyzed using methods other than those outlined above. Because most withdrawal symptoms were reported to be of minimal severity, the data for each subject on the Withdrawal Symptom Checklist were collapsed over all time points in the 3-day period. The maximum severity of response to each withdrawal symptom over the 3-day period was then used to obtain the frequencies of response by dosage level.

RESULTS

Demographics, Smoking History, and Cotinine Levels

Thirty-seven subjects entered the study and 35 subjects completed it. Two of the subjects were discharged before assignment to the medication. One subject experienced family problems while on the unit, and the other experienced a reoccurrence of peptic ulcer disease. Nine subjects completed the protocol in each group except the 160-mg one, in which eight subjects completed the study. The demographic and smoking history variables are shown in Table 2. Significant differences were observed in age [$F(3, 31) = 3.0$, $p = 0.05$], number of cigarettes per day [$F(3, 31) = 3.9$, $p = 0.02$], years of smoking [$F(3, 31) = 3.9$, $p = 0.02$], Fagerstrom Tolerance Questionnaire Score [$F(3, 31) = 3.1$, $p = 0.04$], and baseline serum cotinine [$F(3, 31) = 6.1$, $p < .001$]. Posthoc analyses showed that most of the significant differences were between the 160-mg and placebo groups. Figure 1 shows the mean cotinine level attained for each of the 3 days with the medication. Repeated-measures analysis of variance of the serum cotinine levels for the nine days indicated significant differences across the doses of cotinine ($F(3, 31) = 59.38$, $p < 0.001$). Univariate analysis of variance for each day indicated significant differences between all groups on days 4–7. In addition, each dose was significantly different from the 160-mg dose on days 8 and 9.

Physiologic, Subjective, and Cognitive Effects of Cotinine

No adverse effects were noted by the subjects that would warrant termination from the study. Ten of 35 subjects had asymptomatic increases in the serum ALT to above the normal range during the study (Table 3). Increases occurred in all treatment groups, including those receiving placebo. Most elevations were modest, with seven of the nine elevations being less than two times the upper limit of normal (50 IU/liter). One subject receiving 160 mg of cotinine had an ALT elevation to four times normal, with a lesser elevation in AST and no elevation of Alk Phos. This elevation was still present on day 17 but had returned to nearly normal (with a normal AST) on day 28. One subject receiving placebo had an ALT elevation to 2.7 times normal, which returned to nearly normal by day 18. None of the subjects with liver function test abnormalities had symptoms or abnormalities on the physical exam. One subject with a minor elevation of the Alk Phos (127 IU/liter, normal up to 120 IU/liter) but normal ALT and AST at baseline was allowed to participate in the study and had no further elevation of the Alk Phos during or after the study. No other laboratory results showed abnormal effects.

Table 4 shows the side effects reported by the subjects. No

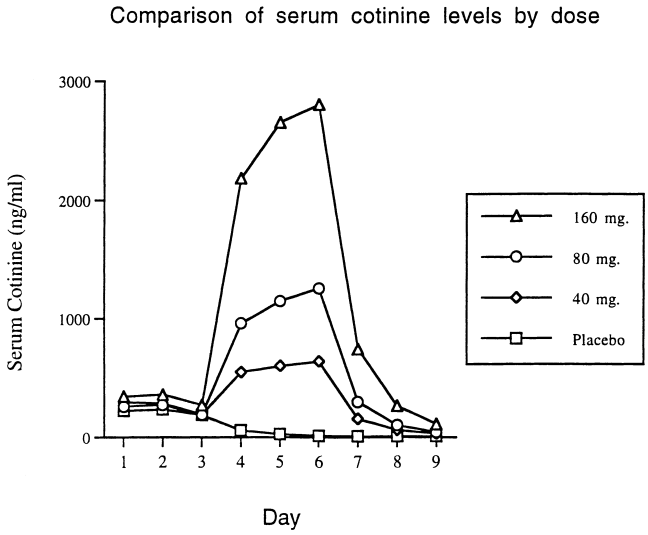

FIG. 1. Mean cotinine levels across the various doses of medication.

TABLE 3

LIVER FUNCTION TESTS DURING AND AFTER COTININE ADMINISTRATION

Subject	Cotinine Dose (mg)	Study Day*	ALT IU/liter†	AST IU/liter	Alk Phos IU/liter
102	0	7	57	N	N
		9	57	N	N
		46	N	N	N
106	0	7	136	60	N
		9	133	N	N
		18	54	N	N
118	0	7	61	N	N
		9	69	N	N
		13	N	N	N
132	0	7	59	N	N
		9	87	N	N
		16	113	N	N
108‡	40	7	n/a	n/a	n/a
		9	57	N	N
109	80	7	64	N	N
		9	87	N	N
		16	55	N	N
110‡	80	7	n/a	n/a	n/a
		9	57	N	N
116	80	7	84	N	N
		9	78	N	N
		15	N	N	N
129‡	160	7	69	N	N
		9	89	N	N
130	160	7	149	93	N
		9	199	94	N
		17	173	59	N
		28	60	N	N

*Cotinine was administered on days 4–6.

†N, normal; n/a, not available; ALT, aminoleucine transferase; AST, aminospartate transferase; Alk Phos, alkaline phosphatase. All subjects had normal values for each of these tests at baseline. Normal values; ALT $<$ 50 IU/liter, AST $<$ 50 IU/liter, Alk Phos 50–120 IU/liter.

‡Follow-up values not available.

dose-related pattern was observed. Palpitations were reported by two of the subjects (one each in the 40- and 160-mg groups). Those symptoms were not accompanied by dizziness, numbness, chest pain, or other symptoms. Physical exams and ECGs remained normal after these events.

During the 3-day period when the subjects were administered a dose of cotinine, no significant differences were observed among the doses for heart rate, diastolic and systolic blood pressure, mean arterial blood pressure, pressure-rate product, weight, skin temperature, or electrocardiographic in-

TABLE 4

NUMBER OF SUBJECTS WITH SELECTED SIDE EFFECTS FROM VARIOUS DOSES OF COTININE

Symptoms	Placebo (n = 9)	40 mg (n = 9)	80 mg (n = 9)	160 mg (n = 9)
Constipation				1 (mild)
Diarrhea	1 (severe)	1 (mild)		
Dizzy	2 (mild)	2 (mild)	1 (mild)	2 (mild and moderate)
Headache	1 (mild)	1 (mild)	4 (mild)	1 (moderate)
Palpitations		1 (mild)		1 (mild)
Salivation		2 (mild)	1 (mild)	
Stomach aches	1 (mild)		1 (mild)	
Sweaty			1 (mild)	
Tremors			1 (moderate)	

tervals and axis. No differences were observed for sleep, caloric and macronutrient intake, the various scales on the Addiction Research Inventory, the Profile of Mood States, the Drug Effects and Nicotine Effects visual analogue scales, or performance on the Symbol Digit Modalities Test.

Withdrawal Effects From Cotinine

No significant differences on withdrawal measures as assessed by the sleep scale, Nicotine Withdrawal Symptom Checklist, Profile of Mood States, the Smoking Urges Questionnaire, or performance on the Symbol Digit Modalities Test were observed across the doses of cotinine upon withdrawal from the medication. In addition, no significant differences were observed for skin temperature, systolic blood pressure, pressure-rate product, and weight. Significant Dose × Day interaction effects were observed for diastolic blood pressure, heart rate, and mean arterial pressure. On the second day off medication, the diastolic blood pressure and mean arterial pressure were higher for the 80-mg group than for the 40-mg one, and heart rate was higher for the 40-mg group than the 160-mg one. The results from the total caloric intake and macronutrient intake data showed no significant differences across the various doses with the exception of carbohydrates. A significant group effect was observed with the placebo and 160-mg cotinine group experiencing greater carbohydrate intake than the 40- and 80-mg cotinine groups.

DISCUSSION

This study shows that the short-term administration of cotinine at doses that result in mean serum concentration over 2500 ng/ml (almost 10 times higher than the concentration observed during smoking cigarettes) is well tolerated by normal subjects during nicotine abstinence. Ten subjects had elevations in their ALT during the study, but their clinical importance is unclear. Elevations occurred in all treatment groups, including placebo. Most elevations were small, and in all cases where follow-up was possible, these values returned to normal or nearly so within days to weeks. None of the subjects had associated signs or symptoms suggestive of liver disease. The one observation of potential concern is that the highest elevation of ALT occurred in the highest-dose cotinine group. However, the second highest elevation of ALT occurred in the placebo group. Some of these minor elevations in ALT likely represent random fluctuations that were evident because of frequent sampling. Nevertheless, these data suggest that caution and careful monitoring of liver function tests is warranted in future studies of cotinine, particularly in studies in which high doses are administered.

In general, previous studies in animals and humans have found no adverse effects of cotinine on physiological measures. Borzelleca et al. (5) found very high doses of cotinine transiently lowered blood pressure in a dose-dependent manner in anesthetized dogs. Similar depressor findings have been observed in sleeping cats given large doses of cotinine (31) and in anesthetized rats (12). The mechanism that underlies this decrease in blood pressure has been postulated to be vascular smooth muscle relaxation (5,20). Heart rate has also been observed to decrease in a dose-dependent manner in anesthetized rats (12), whereas another study with dogs observed no changes in heart rate (5).

In humans, Benowitz and Sharp (4) observed an inverse correlation between serum cotinine concentration and blood pressure in cigarette smokers. However, most human laboratory studies of cotinine administration to abstinent smokers (3,26) or nonsmokers (10) have found no decreases in either blood pressure or heart rate at doses of cotinine that typically did not exceed levels of cotinine attained by moderate to heavy cigarette smokers. Keenan et al. (19), on the other hand, found a small but significant decrease in mean arterial blood pressure with 30 mg of intravenously administered cotinine base compared with placebo in smokers. The results from the present study are consistent with most of the previous human studies in which no significant cardiovascular effects were observed with the use of cotinine, even at doses as high as 160 mg. In addition, as in other studies (19,26), no changes in the ECG were observed when cotinine was administered.

Adverse clinical symptoms from cotinine have generally not been noted in human subjects. Most of the studies with humans, however, have focused on characterizing the pharmacokinetic and pharmacodynamic effects of cotinine, and have not directly examined its safety. Nonetheless, in human studies, doses as high as 1800 mg/day had been administered for 4–6 days in nonsmoking male volunteers without any mention of adverse effects (7,23). All other studies undertaken with humans using doses no higher than 30 mg of intravenous cotinine also made no mention of any adverse side-effects from cotinine (3,10,11,19,26). Only transient dryness of mouth and coldness of hands and feet were mentioned in two of nine subjects administered 20 mg infusion of cotinine in one of the studies using nonsmokers (10).

Cotinine had no effect on body weight or food intake in this short-term study. However, a previous study in rats showed that cotinine may have weight-suppressing effects (21). These investigators found that rats given water containing cotinine weighed significantly less than those given tapwater. No differences in water intake were observed between the two groups, so the weight loss cannot be attributed to difference in fluid intake. Cotinine substitution in rats was also observed to have an acute suppression of caloric intake following nicotine deprivation, whereas substitution with saline created an immediate increase in caloric intake (8). In humans, no other study examined the effects of cotinine on food intake or weight as directly as the present study.

With regards to behavioral effects of cotinine in animals, the data are mixed. Results vary across species, with some studies showing minimal effects from cotinine (14,28,31) and others showing effects similar to nicotine (25). In human studies, the results show minimal subjective or behavioral effects in both nicotine-experienced and nicotine-naive subjects at cotinine levels consistent with that obtained from moderate cigarette smoking (10,26). Again, however, these studies were focused primarily on understanding the pharmacokinetics of cotinine and did not systematically address the subjective and behavioral effects from cotinine. Benowitz et al. (3) examined the effects of cotinine in abstinent smokers. Doses of 4 μg base/kg per min and 50 μg/kg per min were infused over 60 or 5 min, respectively, to attain concentrations of approximately 387 ng/ml. Although no spontaneous reports of subjective changes were noted, when subjects were asked to complete a subjective measurement, significant reductions were observed for the desire to smoke, irritability, low energy, and the Tension/Anxiety subscale of the Profile of Mood States. However, these changes were considered by the researchers to be similar in magnitude to those observed from intravenous infusion of saline found in their other studies of abstinent smokers. On the other hand, Keenan and associates (19) observed some differences when intravenous saline administration was compared with 30 mg of cotinine base administration

in abstinent male smokers. These investigators observed smaller decreases relative to baseline in ratings of anxiety/tension, restlessness, insomnia, and smaller increases for sedation and pleasantness when subjects were administered cotinine compared with placebo. The results from the present study are consistent with the studies showing a lack of subjective effects from cotinine even at high doses, but whether cotinine suppresses tobacco withdrawal symptoms could not be examined because of the minimal degree of withdrawal experienced by participants in the inpatient setting. Withdrawal symptoms may have been minimized owing to the hospital environment and the lack of cues that are typically associated with smoking (16). In addition, subjects were given a placebo or active medication throughout the withdrawal period. Perhaps an interaction of both these factors led to greatly reduced withdrawal symptomotology.

Finally, this study examined withdrawal symptoms from cotinine. As of yet, no studies have examined withdrawal symptoms from cotinine. Our results would indicate no withdrawal effect. The few effects that were observed in this study were inconsistent in direction of effect and dose relationship; therefore, these results were considered to have been significant by chance. The lack of withdrawal symptoms from cotinine is consistent with the lack of physiologic and subjective responses to cotinine.

Several limitations exist in this study that may have precluded finding significant results. These limitations include limited sample size and the differences that were observed across the groups on level of nicotine dependence and other smoking history variables, which may have confounded the results. Further, another weakness is the short duration of time with as well as without cotinine. Three days of cotinine dosing is not sufficient to reach steady-state serum levels. Reaching steady state and maintaining that level for several days may be necessary to see any significant effects from cotinine. Similarly, although subjects in this study had chronic exposure to cotinine from smoking, this study does not eliminate the possibility that with long-term administration of cotinine alone, withdrawal symptoms would be observed. Nonetheless, this preliminary study shows that the short-term administration of high doses of cotinine does not result in clinically important adverse effects. The only potential negative effect was elevated liver function values that were noted in some of the subjects. Although these elevated tests were found to be randomly distributed across doses of cotinine and placebo, careful monitoring of liver functions is warranted until additional data are available. In general, future research exploring the effects of cotinine at the levels examined in the present study appears to be feasible and safe.

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