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Decreased Serum Cotinine Levels in Smokers of Both Tobacco and Marijuana as Compared With Smokers of Tobacco Only

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VAN VUNAKIS, H., D. P. TASHKIN, M. SIMMONS AND V. A. CLARK. *Decreased serum cotinine levels in smokers of both tobacco and marijuana as compared with smokers of tobacco only.* PHARMACOL BIOCHEM BEHAV **30**(4) 895–898, 1988.—Serum and salivary cotinine levels were determined in tobacco smokers (n=125) who smoked only tobacco (n=47) or who smoked both marijuana and tobacco (n=78) as part of a field study of the pulmonary effects of heavy, habitual use of marijuana alone or with tobacco. After adjustment for current daily amount of tobacco use and time since the last tobacco cigarette was smoked, the smokers of both marijuana and tobacco were found to have lower levels of cotinine then those who smoked only tobacco, in serum [258±113 ng/ml (S.D.) and 332±109, respectively; $p=0.003$] and in saliva (331±170 and 395±170, respectively; $p=0.058$). Serum cotinine showed a significantly negative relationship to the daily amount of marijuana currently smoked ($p=0.026$). Possible explanations include inhibition by marijuana component(s) of the enzymes that participate in the conversion of nicotine to cotinine, differences in nicotine absorption patterns between the two groups of tobacco smokers, and acceleration of cotinine metabolism by marijuana smoking. Carefully controlled pharmacokinetic studies, not possible in a large-scale survey such as this one, are required both to confirm the differences in blood cotinine levels observed between the dual smokers and smokers of tobacco only and to define more clearly nicotine-marijuana interactions.

Salivary cotinine Serum cotinine Smoking Tobacco Marijuana

WHILE the effect of tobacco smoking on the pharmacokinetics of some drugs has been studied, considerably less is known about the changes that may occur in nicotine metabolism as a result of the concomitant use of other drugs [6, 7, 10, 11].

Recently, respiratory symptoms and lung function were studied in smokers of marijuana or tobacco alone, smokers of both marijuana and tobacco, and nonsmokers [17]. Unlike the participants in smoking cessation programs, false reporting of current tobacco use was not advantageous to these subjects. They, therefore, consituted an ideal population (n=327) in which to study (a) the sensitivity and specificity of salivary and serum cotinine in identifying tobacco-smoking status and (b) the relation between salivary and serum cotinine levels [18]. The tobacco smokers (n=125) consisted of two subgroups: tobacco smokers only (n=47); and tobacco smokers who also smoked marijuana (n=78). Information about current tobacco and marijuana use was available from their questionnaires. The data were analyzed (after adjusting for the effect of self-reported daily tobacco amount and number of hours since tobacco was last smoked) to determine if concomitant marijuana smoking had any effect on cotinine levels.

METHOD

Serum and salivary cotinine levels were determined in 327 subjects who were participating in a study of the pulmonary effects of habitual marijuana use [17]. Smoking status for both tobacco and marijuana was ascertained from a lifetime history as well as a detailed 7-day history administered at the time the specimens were obtained; current and usual number of tobacco cigarettes smoked per day and current and usual number of marijuana joints (or joint-equivalents) smoked per week were recorded. Seventy-eight subjects currently smoked both marijuana and tobacco, 47 smoked only tobacco, and the others either smoked marijuana alone or smoked neither marijuana nor tobacco. A further description of these subjects and the questionnaires used is given in Tashkin *et al.* [17]. In the present study, current marijuana smokers were defined as smokers of at least one joint of marijuana within the week prior to serum and saliva sampling who had a lifetime history of at least 3.4 joint-years (1 joint year=average of 1 joint or joint-equivalent smoked per day for 1 year). Current tobacco smokers were defined as current smokers of more than 1 cigarette a day on a regular basis for at least 1 year, including the 24-hr period prior to

TABLE 1

NUMBER, MEAN AGE, AND MEAN CURRENT AMOUNT OF TOBACCO AND MARIJUANA SMOKING OF SUBJECTS BY TOBACCO AND MARIJUANA SMOKING STATUS

Subjects	N	Age (years) Mean (±S.D.)	No. Tobacco Cigarettes/Day Mean(±S.D.)	No. Marijuana Joints/Week Mean (±S.D.)
Smokers of tobacco alone	47	36.5 (7.2)	28.5 (13.4)	0.0 (0.0)
Smokers of both tobacco and marijuana	78	32.4 (5.6)	19.2 (11.7)	21.7 (22.4)

sampling of serum and saliva. Subjects who reported chewing tobacco or using nicotine-containing chewing gum were excluded from analysis.

Cotinine in coded specimens was determined in a "blind" fashion by radioimmunoassay [12,13] on blood and saliva samples that were collected, processed, and stored as described in Van Vunakis *et al.* [18].

Data Analysis

The equality of cotinine levels between the sexes was tested using two-way analysis of covariance with sex and marijuana smoking (i.e., marijuana smoker vs. nonsmoker) as grouping factors, and with the current daily amount of tobacco smoked and the time since tobacco was last smoked as covariates [8]. This analysis was performed to determine if gender needed to be considered in subsequent analyses. The effects on cotinine levels of height, weight, and body surface area were determined by using these parameters as the independent variables in a stepwise multiple regression with the cotinine level as the dependent variable; only variables that were entered into the equation were considered to be related to the cotinine level [8]. Since gender and anthropometric measurements were not found to be related to cotinine level, these variables were not included in further analyses.

To compare the cotinine levels of the smokers of both marijuana and tobacco with those of the smokers of tobacco alone, analysis of covariance was performed with the current daily amount of tobacco smoked and the time since tobacco was last smoked as covariates. To investigate how cotinine levels might be affected by (1) the time since tobacco was last smoked, (2) the average daily amount of tobacco smoked, and (3) the average daily amount of marijuana smoked, these parameters were used as independent variables in a multiple regression with the cotinine level as the dependent variable.

RESULTS

The number and mean age of subjects by tobacco and marijuana smoking status is shown in Table 1. Significant differences in cotinine levels (when adjusted for the number of cigarettes smoked per day) were not found between the sexes nor were cotinine levels affected by weight, height, or body surface area. Therefore, further classification of subjects based on these parameters is not shown. The mean current amount of tobacco and marijuana smoking of subjects by tobacco and marijuana smoking status indicates that the tobacco-only smokers smoked about 10 cigarettes more than the dual smokers (28.5 vs. 19.2 cigarettes/day, respectively). The marijuana smokers smoked an average of 21.7 joints/week.

The effect of amount of concomitant marijuana smoking on serum and salivary cotinine levels was determined using multiple regression (Table 2). Serum (but not salivary) cotinine was negatively related to the current weekly amount of marijuana smoked ($p<0.03$) after adjusting for the effect of daily tobacco amount and the number of hours since tobacco was last smoked. Both serum and salivary cotinine were positively related to the current amount of tobacco smoked ($p<0.001$) and both showed a significant negative relationship to the number of hours since the last cigarette was smoked ($p<0.01$). The slope of the linear relationship between serum cotinine level and the number of tobacco cigarettes smoked per day for marijuana-plus-tobacco smokers (5.1) was nearly identical to that for tobacco-only smokers (5.3) but showed a parallel downward shift from that for the latter group; as a result, the y intercept (serum cotinine level) for the dual smokers was lower (141 ng/ml) than that for the tobacco-only smokers (206 ng/ml).

Covariance analysis which adjusted for the amount of tobacco smoked indicated that those who smoked marijuana in addition to tobacco had significantly lower levels of serum cotinine than smokers of tobacco alone ($p=0.003$) (Table 3). The smokers of marijuana and tobacco also had lower salivary cotinine levels than tobacco-only smokers, although this difference was of only borderline significance ($p<0.06$).

DISCUSSION

The evaluation of nicotine-drug interaction from data obtained in large-scale surveys is subject to distinct limitations. In our study, the smoke of tobacco and marijuana was inhaled as was customary for each subject. The time when the last cigarette was smoked, the number of cigarettes or joints smoked, and inhalation patterns were obtained from oral testimony. Since dosage was not accurately known and only single specimens were obtained from each subject (at convenient rather than exact times), the data on nicotine serum levels yielded relatively little information. Although nicotine is the parent and the pharmacologically active compound, its short half-life and great individual variation [2] limit its usefulness in this type of study. Also, salivary nicotine levels do not reflect blood nicotine levels. Nicotine is introduced directly into the mouth during smoking and levels as high as several μg/ml can be present in the saliva of active smokers compared to peak serum levels of 60–70 ng/ml (data not shown).

TABLE 2

MULTIPLE REGRESSION OF SERUM AND SALIVARY COTININE ON DAILY TOBACCO AMOUNT (CIGARETTES/DAY), WEEKLY MARIJUANA AMOUNT (JOINTS/WEEK) AND HOURS SINCE LAST SMOKED TOBACCO*

	Independent Variable	Simple Correlation Coefficient (r)	Slope Coefficient	p Value (for slope coefficient)
Serum	Tobacco (T) cigarettes/day	.53	4.5	0.000
	Marijuana joints/week	−.18	− 8.2	0.026
	Hr since last smoked T	−.42	−11.7	0.000
	Intercept	—	241	—
Saliva	Tobacco (T) cigarettes/day	.43	4.9	0.000
	Marijuana joints/week	−.11	− 5.0	0.342
	Hr since last smoked T	−.37	−12.0	0.003
	Intercept	—	303	—

*Multiple r values from the multiple regressions of serum and salivary continine on all three independent variables were .62 and .51, respectively.

TABLE 3

ADJUSTED GROUP MEANS (±S.D.) FROM COVARIANCE ANALYSIS*

Group	N	Serum Cotinine (ng/ml)	Salivary Cotinine (ng/ml)	
Tobacco alone	47	332 (109)	43	395 (170)
Tobacco + marijuana	76	258 (113)	72	331 (170)
p		0.003		0.058

*From covariance analysis with daily tobacco amount and hours since last smoked as covariates.

An analysis of the cotinine data yielded some information of interest. Cotinine has a relatively long half-life and, in the habitual smoker, shows only minor fluctuations in blood concentrations over long periods of time [2,13]. The metabolic patterns in our subjects are established; they are habitual tobacco smokers and, in some cases, heavy, habitual marijuana smokers as well. After adjustment for the number of cigarettes smoked and the time of the last cigarette, tobacco smokers who also smoked marijuana had levels of serum cotinine (ng/ml) that were significantly lower than those of smokers of tobacco only. Serum levels of cotinine also showed a significantly negative relationship to the current amount of marijuana subjects reported smoking; failure to find a similar relationship with salivary cotinine could reflect the greater variability of salivary cotinine levels, as well as gross inaccuracies in our estimates from self-reported marijuana use of actual exposures of the lung to the combustion products of cannabis. Both marijuana and tobacco smoke contain several thousand individual compounds; a large number of these are found in both tobacco and marijuana smoke although not in the same concentrations [15]. The nicotine-related alkaloids in tobacco and the cannabinoids in marijuana are, of course, unique to each plant product.

The microsomal cytochrome P_{450} enzyme system participates in the oxidation of nicotine to cotinine [9] and is also implicated in the oxidation of cannabinoid derivatives [1]. These detoxifying enzymes are limited in number, have wide and overlapping specificities, and metabolize xenobiotics of vastly diverse structures [5]. Many cases of tobacco smoke-drug interaction are thought to be caused by inhibition or induction of these enzyme systems [7,10]. The findings that serum cotinine showed a significantly negative relationship to the daily amount of marijuana smoked and that serum cotinine levels were significantly lower in the smokers of both marijuana and tobacco compared to the tobacco-only smokers would be consistent with the suggestion that marijuana component(s) are inhibiting enzyme systems that participate in the nicotine-to-cotinine conversion. If induction had taken place as a result of marijuana smoking, cotinine levels might be expected to be higher (rather than lower) in the dual smokers as a result of increased nicotine metabolism.

If differences in inhalation patterns for tobacco existed between smokers of tobacco only and smokers of marijuana plus tobacco, these differences could influence the inhaled dose of nicotine and its absorption in the lung. However, unpublished data on tobacco smoking topography recently collected in our laboratory failed to show significant differences between tobacco-only smokers and dual smokers of tobacco and marijuana in puff number, puff volume, puff duration, interpuff interval, inhaled volume or breathholding time.

The route by which nicotine is taken into the body can also affect cotinine metabolism [3]. Nicotine ingested by the oral route (e.g., in chewing gum) leads to higher-than-

expected blood cotinine levels since a large part of the alkaloid is absorbed from the gastrointestinal tract, enters the portal circulation, and undergoes extensive single-pass metabolism to cotinine in the liver. In contrast, smokers absorb nicotine via the lung and buccal cavity into the systemic circulation, and only a portion undergoes single-pass metabolism in the liver while the remainder is distributed to the tissues and recirculated.

Dryness of the mouth has been known to be a side-effect of smoking marijuana [16]. In dogs and cats, Δ^9-tetrahydrocannabinol was found to decrease salivary flow [14]. While statistically significant differences were noted in cotinine levels between our two groups of smokers, the difference was not large. It is conceivable that a side effect of marijuana (i.e., decreased salivary flow) could decrease swallowing of nicotine deposited in the mouth, thus reducing gastrointestinal absorption and contributing to the modestly but significantly lower blood cotinine levels found in the dual smokers.

Cotinine is a major (but not the sole) product of nicotine metabolism and is itself subject to additional catabolism [9]. In addition, other complex processes may control the ultimate fates of nicotine and cotinine. For example, marijuana inhibits the metabolism of drugs such as pentobarbital and antipyrine by mechanisms that may involve changes in the volume of drug distribution and diminished metabolic clearance [4]. Alterations in other processes that affect absorption, distribution, metabolism and excretion of nicotine and cotinine may influence their plasma levels but could not be adequately assessed in a large-scale survey such as this one. Classic pharmacokinetic studies on individual subjects are required to more clearly define nicotine-marijuana interactions.

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