Original Investigation

Effect of Menthol on Nicotine Pharmacokinetics in Rats After Cigarette Smoke Inhalation

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Abstract

Introduction: The effect of menthol on nicotine disposition is important in understanding smoking behaviors among different racial groups. The present study was to evaluate whether menthol affects the pharmacokinetics of nicotine after cigarette smoke inhalation.

Methods: Rats were exposed to mainstream smoke from either a nonmentholated or mentholated cigarette (1 puff/min for 10 min) using a smoke inhalation apparatus. For the multiple-cigarette smoke inhalation, rats received the smoke from either nonmentholated or mentholated cigarette (10 puffs) every 12 hr for a total of 17 cigarettes. Serial blood samples were collected during the 10-min inhalation phase for the single-cigarette smoke or the 17th cigarette inhalation and for 30 hr thereafter. Nicotine and its major metabolite cotinine were assayed by radioimmunoassay methods.

Results: Following single-cigarette smoke inhalation, mentholated cigarettes significantly decreased the mean peak concentrations of nicotine in plasma (C_{max}) from 27.1 to 9.61 ng/ml and the total area under the plasma concentration–time curves (AUC) from 977 to 391 ng min/ml as compared with those after nonmentholated cigarette smoke inhalation. C_{max} and AUC values for cotinine were also significantly reduced by menthol. Similarly after multiple smoke inhalation, C_{max} , AUC, and the mean average steady-state plasma concentration of nicotine as well as cotinine were significantly lower in mentholated cigarette inhalation. Interestingly, there was a significant increase in the cotinine to nicotine AUC ratio from 13.8 for the nonmentholated to 21.1 for the mentholated cigarette.

Conclusions: These results suggest that menthol in mentholated cigarettes can substantially decrease the absorption and/or increase the clearance of nicotine.

Introduction

Black smokers are known to have a 50% higher probability of developing lung cancer and a more than threefold higher rate of

cancers of the upper respiratory and digestive tracts than Whites, even though they smoke fewer cigarettes. This high cancer rate can be attributed to their addiction to tobacco products. One striking difference in the smoking habits of Blacks and Caucasian is a strong preference for mentholated cigarettes by Blacks (Sidney, Tekawa, & Friedman, 1989; US Department of Health and Human Services, 1986). About 75%–90% of Black smokers report a preference for menthol compared with 23%–25% of Caucasian smokers (Castro, 2004; Cumming et al., 1987; Pickworth, Moolchan, Berlin, & Murty, 2002; Sutton & Robinson, 2004). The potential contribution of menthol to the cigarette addiction and toxicity is gaining special attention for researchers to redefine the role of menthol on the biochemical and physiological effect of cigarette smoking (Ahijevych & Garrett, 2004; Henninfield et al., 2003). A recent large epidemiologic study found that menthol cigarettes are no more harmful than nonmenthol cigarettes in terms of lung cancer risk and mortality (Blot et al., 2011).

Earlier studies on mentholated cigarettes have no significant effects of menthol on number of puffs, puff volume of smoked cigarettes and has little or no effect on heart rate, blood pressure, uptake of carbon monoxide, tar intake or retention, or blood cotinine concentration (Werley, Coggins, & Lee, 2007). A nationwide study of 7,182 subjects reported, for the first time, that serum cotinine (a major nicotine metabolite) levels are higher among Black smokers than among White or Mexican American smokers (Caraballo et al., 1998). This finding could be explained in part by the results of a controlled pharmacokinetic study in which Perez-Stable, Herrera, Jacob, & Benowitz (1998) reported that Blacks had both a slower clearance of cotinine and a higher intake of nicotine per cigarette than those of Whites. Benowitz et al. (1999) further demonstrated that Blacks had a slower oxidative metabolism of nicotine to cotinine and slower N-glucuronidation than Whites. On another pharmacokinetic study, Benowitz, Herrera, & Jacob (2004) showed from a group of seven Black and seven White healthy smokers that there is no difference in systemic nicotine and carbon monoxide intake from smoking mentholated compared with nonmentholated cigarettes. The study did indicate that menthol cigarette smoking could contribute to

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slower nicotine metabolism but provides evidence against the idea that mentholated cigarette smoking is responsible for slower cotinine metabolism in Blacks. More recently, Benowitz, Dains, Dempsey, Wilson, & Jacob (2011) reported that Blacks on average smoke cigarettes differently than White smokers such that nicotine and carcinogen exposure per individual cigarette was inversely related to cigarettes per day. This inverse correlation was stronger in Black compared with White smokers and stronger in menthol compared with regular cigarette smokers. The data suggest that Blacks who smoke less cigarettes per day and smoke mentholated cigarettes would have higher nicotine and carcinogen exposure. That study along with others (Heck, 2009; Muscat et al., 2009) comparing smokers of regular versus menthol cigarettes found no significant difference in exposure to tobacco smoke constituents, assessed by plasma and urine nicotine metabolites. However, like many other studies involving test subjects smoking menthol and nonmenthol styles, a major limitation is the possibility of multifactors such as smoking habits of test subjects, lung permeability, and physiological differences including age, gender, and ethnicity, all of which could affect absorption and disposition characteristics of nicotine and its metabolites. Thus, one may not attribute these results to menthol smoking per se. At the present time, no data are available as to the effects of menthol on the absorption, distribution, and metabolism of nicotine. A closer examination of the pharmacokinetics of nicotine and its metabolites should materially contribute to a better understanding of smoking addiction.

Therefore, the purpose of this study is to determine the pharmacokinetic effect of menthol on the absorption, distribution, metabolism, and excretion of nicotine after single- and multiple-cigarette smoke inhalation exposures from nonmentholated (regular) and mentholated cigarettes using rat as an animal model.

Methods

Chemicals

Nonradiolabeled nicotine and cotinine were purchased from Sigma Chemical Company (St. Louis, MO). All other reagent grade chemicals and high-performance liquid chromatography (HPLC) solvents were purchased from Fisher Scientific Co. (FairLawn, NJ).

Cigarettes

The same brand of nonmentholated and mentholated filtered cigarettes (100's) were purchased from commercial sources and had the same reported nicotine yield value of 2.2 mg/cigarette. The nicotine content of these cigarettes was determined in our laboratory by a published HPLC method (Fukumoto, Kubo, & Ogamo, 1997) to be 14.58 mg for the regular cigarette and 13.77 mg for the mentholated cigarette. These nicotine content values compare favorably with those of 14.82 mg/regular cigarette and 13.28 mg/mentholated cigarette reported for the same cigarettes by the American Cancer Society.

Smoke Exposure Apparatus

The intermittent smoke generation-inhalation system was designed by and purchased from the University of Kentucky Tobacco and Health Research Institute and has been described

in detail (Griffith & Hancock, 1985; Griffith & Standafer, 1985). The apparatus is designed specifically to simulate the smoking of a single cigarette and was used for mainstream smoke exposure only. Briefly, the rat exposure chambers are contained in a $5 \times 5 \times 30$ cm Plexiglas block. Four chambers on one side of the block are positioned opposite four chambers on the other side of the block. Opposite chambers are connected to the same smoke source at the bottom and to an open-end tube at the top of the block. Smoke to the chambers is divided into four equal fractions, with each fraction going to two opposite chambers. This distribution system provides smoke of the same age and quality to a maximum of eight rats, simultaneously. A wire mesh restrainer is used to hold the rats in place with only their noses protruding into the chamber. The restrainer consists of a Delrin head piece that fits into the exposure chamber, has a foam rubber insert to prevent the loss of smoke and to minimize injury to the rat, and a wire mesh to hold the body of the rat. For the single-cigarette exposure studies, 10 puffs/cigarette were given per day. For the multiple-cigarette exposure studies, 10 puffs/cigarette were given twice daily for 17 cigarettes. The puffs were generated with a frequency of one per minute and had duration of 2.4 s and a volume of 40 ml.

Animals

The animal study protocol was reviewed and approved by the Institutional Animal Care and Use Committee and conducted in the College of Pharmacy and Health Sciences Animal Facility at Texas Southern University. Adult male Sprague-Dawley rats (250–350 g) were housed in stainless steel cages and had free access to food and water. To facilitate the withdrawal of timed, multiple blood samples from each animal, the right jugular vein of each animal was cannulated 2 days prior to the intravenous and inhalation nicotine studies. Under ketamine:acetopromazine:xylazine (50:3.3:3.3 mg/kg intraperitoneal) anesthesia, silicone elastometer tubing (0.02×0.037 inch) was inserted into the jugular vein, secured with silk suture, and exteriorized in the dorsal infrascapular area. The surgical incision was closed with surgical staples. Patency of the cannula was maintained by flushing the line daily with sterile heparinized saline (100 units/ml) and filled with sterile normal saline.

Experimental Procedures

Single-cigarette smoke inhalation study: Eight rats were placed individually in the restraining cylinders of the smoke exposure apparatus 30 min prior to lighting the cigarette. Either a single-nonmentholated cigarette or a -mentholated cigarette was consumed in 10 min (10 puffs), after which the rats were transferred to plastic metabolism cages for the remainder of the study. Blood samples (0.2 ml) were collected (from the jugular vein cannula with the aid of a heparinized syringe) at 3, 5, and 10 min (inhalation phase) and at 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 720, 1,440, and 1,800 min (post-inhalation phase). Aliquots of the plasma specimens were stored at -20°C pending radioimmunoassay (RIA) for nicotine and its major metabolite cotinine.

Multiple-cigarette smoke inhalation study: Eight rats were placed individually in the restraining cylinders of the smoke exposure apparatus 30 min prior to lighting the first cigarette to condition them to the apparatus. The rats received the smoke from one nonmentholated cigarette or one mentholated cigarette

(10 puffs) every 12 hr for a total of 17 cigarettes. The rats were transferred to plastic metabolism cages after the 10-min inhalation phase. Blood samples (0.2 ml) were collected (from the jugular vein cannula with the aid of a heparinized syringe) after the 17th cigarette at 3, 5, and 10 min (inhalation phase) and at 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 720, 1,440, and 1,800 min (post-inhalation phase). Aliquots of the plasma specimens were stored at -20°C pending RIA for nicotine and its major metabolite cotinine.

Plasma concentrations of nicotine and cotinine were measured by a double antibody RIA procedure developed by Langone & Van Vunakis (1982; Van Vunakis, Gjika, & Langone, 1987). The rabbit antinicotine-carbonyl-di-imidazole (CDI)-bovine serum albumin, rabbit anticotinine-CDI-thyroglobulin, $[^3\text{H}]$ nicotine, $[^3\text{H}]$ cotinine, goat antirabbit γ -globulin, and normal rabbit serum were supplied by Dr. Helen Van Vunakis from Brandeis University (Waltham, MA). The RIA was performed according to the procedure provided by Dr. Helen Van Vunakis. Briefly, plasma samples (0.04 ml for the assay of nicotine, 0.02 ml for the assay of cotinine) and 0.1-ml portions of $[^3\text{H}]$ nicotine or $[^3\text{H}]$ cotinine (8,000–20,000 cpm), approximately diluted antiserum and buffer (0.01 M Tris-HCl, 0.15 M sodium chloride, pH 7.4, containing 0.1% gelatin), were incubated in a test tube at 37°C for 1 hr. Normal rabbit serum (0.1 ml of a 1/25 dilution) was added to each tube to control nonspecific binding. Goat antirabbit immunoglobulin (0.1 ml) is then added, and the mixture was incubated at 4°C overnight. The precipitate was collected by centrifugation at $1,000 \times g$ for 30 min at 5°C , the supernatant was decanted, and the walls of the tubes were wiped dry. To count $[^3\text{H}]$ nicotine or $[^3\text{H}]$ cotinine, the precipitant was dissolved in 0.1-ml 0.1 N sodium hydroxide before adding 2.5 ml of liquid scintillation fluid (Biofluor from Packard Instrument Company, Meriden, CT). Radioactivity was measured in an LKB-Wallac liquid scintillation counter (LKB Instrument Inc., Gaithersburg, MD). Quantification of nicotine or cotinine in the experimental plasma specimens was accomplished by using their percent inhibition of immune binding to the respective antibody (i.e., antinicotine or anticotinine) versus the logarithmically transformed known concentrations of unlabeled analyte added. Cross-reactivity of the nicotine and cotinine antibodies with other nicotine metabolites was less than 5%. The detectable concentration ranges for nicotine and cotinine in rat plasma were 1.25–62.5 and 0.5–50 ng/ml, respectively. The slope and intercept values show little interday variations (coefficients of variation of less than 6%).

Data Interpretation

All pharmacokinetic analyses were made using classical techniques and the microcomputer-based program WinNonlin by Pharsight, Inc. The nicotine and cotinine plasma concentration-time data were interpreted by noncompartmental methods. The highest observed plasma concentration and its time of occurrence were defined as C_{max} and T_{max} . The rate constant governing elimination of nicotine from the body (β) or elimination of cotinine from the body (k) was determined from the least-squares slope of the terminal linear segment of a semi-logarithmic plot of plasma nicotine or cotinine concentration versus time. The biological half-life of nicotine or cotinine was calculated as $0.693/\beta$ or $0.693/k$. The total area under the plasma concentration-time curve (AUC_{0-4} or AUC_t) and the area under the first

moment of the plasma concentration-time curve (AUMC) were estimated by the trapezoidal rule with extrapolation of the terminal portion to infinity for the single-cigarette smoke inhalation studies. The mean residence time (MRT) was calculated from AUMC/AUC . Differences between any two mean pharmacokinetic parameter values were evaluated statistically by the Student's t test for unpaired (unrelated) or paired (related) samples at $p = .05$.

Results

Cigarette Mainstream Smoke Inhalation of Nicotine: Nonmentholated Versus Mentholated Cigarette

Single-Cigarette Smoke Inhalation Studies

Figure 1 shows the mean time courses of nicotine and cotinine plasma concentrations after single-nonmentholated cigarette and single-mentholated cigarette smoke inhalations, respectively. Following rapid absorption (within 10 min), the plasma levels declined with statistically identical apparent first-order terminal phase half-lives of 57.7 and 61.4 min for the regular and mentholated cigarettes, respectively (Table 1). The MRT of nicotine in the body was also independent of cigarette type (i.e., 68.9 min for the nonmentholated cigarette vs. 80.4 min for the mentholated cigarette). However, on the average, menthol significantly decreased the maximum plasma level of nicotine, which occurred at or near the cessation of smoke exposure, from 27.1 to 9.6 ng/ml, and decreased the AUC_{0-4} value for nicotine from 977 to 391 ng/min/ml for the regular and mentholated cigarettes, respectively (Table 1).

The formation of cotinine was rapid after smoke inhalation from both nonmentholated and mentholated cigarettes (Figure 1). Mean plasma cotinine concentrations peaked in 158 min (nonmentholated cigarette) or 128 min (mentholated cigarette) and then declined with statistically identical mean terminal phase half-lives of 399 min (nonmentholated cigarette) and 395 min (mentholated cigarette; Table 1). The mean maximum cotinine plasma concentration and the mean $\text{AUC}_{0-\infty}$ for cotinine after mentholated cigarette smoke inhalation were appreciably and significantly less than those observed after nonmentholated cigarette smoke inhalation. The mentholated cigarette produced a mean cotinine to nicotine AUC ratio (11.8) higher than that produced by the nonmentholated cigarette (8.35; Table 1). However, this latter difference was found not to be statistically significant.

Multiple-Cigarette Smoke Inhalation Studies

The mean time courses of nicotine and cotinine plasma concentrations after the 17th cigarette of the multiple-cigarette smoke inhalation studies are shown in Figure 2 for the nonmentholated cigarette and the mentholated cigarette. The mean elimination half-lives after multiple-nonmentholated and -mentholated cigarette smoke exposures were significantly different (49.4 vs. 37.3 min; Table 2) and were less than those observed after single-cigarette smoke exposures (57.7 and 61.4 min; Table 1). Multiple-cigarette exposures also caused decreases in the MRTs of nicotine (Tables 1 and 2). Consistent with the findings after single-cigarette exposure, multiple-mentholated cigarette exposures caused significant reductions in the mean maximum

Figure 1. Mean (+SD) time course of nicotine and cotinine plasma concentrations ($n = 8$) after single-cigarette smoke inhalation.

nicotine plasma concentration (from 27.0 to 13.9 ng/ml), in the mean AUC₀₋₄ value for nicotine (from 856.6 to 423.4 ng/min/ml), and in the mean average steady-state plasma concentration of nicotine (from 1.17 to 0.60 ng/ml; Table 2). As expected from its relative short terminal (elimination) phase half-life, nicotine did not accumulate in the plasma upon multiple-cigarette exposures.

Plasma concentrations of cotinine peaked within 120 min (Figure 2), and the terminal phase half-life was independent of cigarette type (Table 2). Similar to that observed after single-cigarette smoke inhalation, menthol appreciably reduced the

mean maximum plasma concentration of cotinine (Table 2). There was a significant increase in the cotinine to nicotine AUC₀₋₄ ratio from 13.8 for the nonmentholated cigarette to 21.1 for the mentholated cigarette.

In addition, no statistical differences were observed for nicotine C_{max} and AUC between single- and multiple-cigarette inhalation for either regular or mentholated cigarettes. Significant difference ($p < .05$, $t = -2.83$) was observed for the C_{max} of cotinine between single versus multiple inhalation of regular cigarettes but not for AUC. Interestingly, both C_{max} ($p < .001$,

Table 1. Effect of Menthol on Nicotine and Cotinine Pharmacokinetics After Single-Cigarette Smoke Inhalation by Rats of Mainstream Smoke from Nonmentholated and Mentholated Cigarettes

Parameters ^a	Nonmentholated ^b	Mentholated ^b	Unpaired t test; p and t values
For nicotine			
C_{max} (ng/ml)	27.1 ± 11	9.61 ± 2.8	<.005; $t = 4.04$
AUC ₀₋₄ (ng min/ml)	977 ± 531	391 ± 186	<.05; $t = 2.90$
$T_{1/2\beta}$ (min)	57.7 ± 31	61.4 ± 58	NS ^c
MRT (min)	68.9 ± 30	80.4 ± 70	NS
For cotinine			
C_{max} (ng/ml)	9.81 ± 1.8	5.39 ± 1.1	<.001; $t = 4.51$
AUC _{0-∞} (ng min/ml)	7142.8 ± 2067	3777.6 ± 896	<.002; $t = 4.32$
$T_{1/2k}$ (min)	399 ± 86	395 ± 84	NS
MRT (min)	617 ± 109	602 ± 120	NS
Cotinine:nicotine AUC ratio	8.35 ± 2.9	11.8 ± 5.8	NS

Notes. Data represent mean ± SD, $n = 8$.

^a C_{max} = maximum plasma concentration; AUC₀₋₄ = total area under plasma concentration–time curve from time 0- to 4-hr postsmoke inhalation; $T_{1/2\beta}$ = terminal beta (elimination) phase half-life; and MRT = mean residence time.

^bSingle dose = 1 puff/min for 10 min/cigarette × 1 cigarette.

^cNot statistically significant ($p > .05$).

Figure 2. Mean (+SD) time course of nicotine and cotinine plasma concentrations ($n = 8$) after multiple-cigarette smoke inhalation.

$t = -10.0$) and AUC ($p < .001$, $t = -4.71$) were significantly increased between mentholated single- versus multiple-cigarette inhalation groups.

Discussion

The purpose of this study was to evaluate affect of menthol on pharmacokinetics of nicotine and its metabolite cotinine

following mentholated versus nonmentholated cigarette smoke inhalation. We found that single inhalation of mentholated cigarette significantly decreased the maximum plasma level and the area under the curve of nicotine but not the terminal half-life of nicotine. The lower levels of nicotine following mentholated-cigarette smoke inhalation are most likely due to reduced intake of nicotine. This could result from the effect of menthol in the lungs to inhibit nicotine intake or mentholated smoke is just more aversive for rats such that they do not inhale

Table 2. Effect of Menthol on Nicotine and Cotinine Pharmacokinetics After Multiple-Cigarette Smoke Inhalation by Rats of Mainstream Smoke from Nonmentholated and Mentholated Cigarettes

Parameters ^a	Nonmentholated ^b	Mentholated ^b	Unpaired t test; p and t values
For nicotine			
$C_{\max}$ (ng/ml)	27.1 ± 12	13.9 ± 3.8	$<.05$; $t = 2.48$
AUC _t (ng min/ml)	856.6 ± 189	423.4 ± 82.7	$<.0002$; $t = 6.50$
$T_{1/2\beta}$ (min)	49.4 ± 11	37.3 ± 8.6	$<.03$; $t = 2.67$
MRT (min)	45.1 ± 12	55.3 ± 16	NS ^c
C_{average} (ng/ml)	1.17 ± 0.2	0.60 ± 0.11	$<.001$; $t = 6.49$
For cotinine			
$C_{\max}$ (ng/ml)	18.3 ± 3.2	17.2 ± 3.0	NS
AUC _t (ng min/ml)	12443.7 ± 3189.7	8952.7 ± 2066	$<.03$; $t = 2.46$
$T_{1/2\alpha}$ (min)	312 ± 33	333 ± 46	NS
MRT (min)	509 ± 36	467 ± 34	NS
C_{average} (ng/ml)	16.0 ± 3.4	11.9 ± 2.9	$<.03$; $t = 2.46$
Cotinine:nicotine AUC _t ratio	13.8 ± 3.0	21.1 ± 4.5	$<.004$; $t = -3.75$

Notes. Data represent mean $\pm$ SD, $n = 8$.

^a $C_{\max}$ = maximum plasma concentration; AUC_t = total area under plasma concentration–time curve for the dosing interval; $T_{1/2\beta}$ = terminal beta (elimination) phase half-life; MRT = mean residence time; and C_{average} = average nicotine plasma concentration at steady state (calculated as AUC_t/ t , where t = dosing interval).

^bMultiple dose = 1 puff/min for 10 min/cigarette repeated every 12 hr for a total of 17 cigarettes.

^cNot statistically significant ($p > .05$).

as much of it. It is worth to note that the inhalation apparatus is designed to provide smoke of the same age and quality to each smoking chamber and thus minimizing potential bias among the animal groups. It is also possible that the animals may prefer a particular type of cigarette smoking and hence inhale more smoke and to a deeper degree of inhalation. Similar reductions in the mean maximum nicotine plasma concentration observed in multiple-mentholated cigarette exposures further confirmed such an inhibition effect of menthol on nicotine absorption. However, the exact mechanisms underlying the significant reductions in the maximum plasma concentrations and in the AUC_{0-4} and AUC_t for nicotine observed in the single- and multiple-mentholated cigarette smoke inhalation studies must await additional pharmacokinetic studies for confirmation. These plasma nicotine level reductions cannot be attributed to nicotine content or nicotine yield value differences between the nonmentholated cigarettes (14.58 and 2.2 mg, respectively) and mentholated cigarettes (13.77 and 2.2 mg, respectively). Based on currently available information, it appears that these reductions can best be attributed to an inhibitory effect of menthol on nicotine absorption. It has been shown (Benowitz et al., 2004) that in the mentholated cigarette smoking condition, total clearance of nicotine was statistically significantly slower compared with the nonmentholated cigarette condition. If reduced plasma nicotine levels also occur in Black-mentholated cigarette smokers, then this would lead to Black smokers to smoke more intensely to maintain a desirable nicotine effect when smoking mentholated cigarettes. This assumption was supported by a previous study where the nicotine intake per cigarette was found to be 30% greater in Blacks compared with Whites (1.41 vs. 1.09 mg per cigarette, respectively; $p = .02$; Perez-Stable et al., 1998).

Plasma cotinine levels were also lower in mentholated group as compared with nonmentholated group. Interestingly, the mean elimination half-lives of nicotine after multiple-nonmentholated cigarette smoke inhalation were significantly longer than that after mentholated cigarette smoke inhalation, which indicates an increased nicotine metabolic conversion to cotinine by menthol. This observation is contrary with what observed by Benowitz et al. (2004) where there is no significant difference in cotinine disposition kinetics by mentholated cigarette smoking. It is worth to note that our multiple-cigarette smoke inhalation study showed a significant increase in the cotinine to nicotine AUC_t ratio from 13.8 for the nonmentholated cigarette to 21.1 for the mentholated cigarette, which further suggest increased nicotine to cotinine metabolism by menthol. Further study is warranted to confirm this changed nicotine metabolism rate process by menthol.

The fact that menthol increases metabolic conversion of nicotine to cotinine is consistent with the study of Clark, Gautam, & Gerson (1996) where menthol was found to be associated with higher cotinine levels among 161 smokers after adjusting for race, cigarettes per day, and mean amount of each cigarette smoked. In other words, more nicotine was metabolized to cotinine for those who smoke mentholated cigarettes versus nonmentholated cigarettes. It was also evident in the present study where C_{max} and AUC of cotinine were significantly higher after multiple-mentholated cigarette inhalations as compared with single-mentholated cigarette inhalation. However, in a study of menthol and nonmentholated cigarettes, Benowitz et al. (2004) showed that menthol cigarette smoking did not

significantly affect blood nicotine concentrations measured throughout the day as compared with nonmentholated cigarette smoking. The differences in nicotine and cotinine disposition between that study and our current investigation may be due to (a) the fact that the human study had active smoke inhalation whereas our animal study was passive smoke inhalation and (b) these human subjects were smokers with more than 20 cigarettes/day and having a prior experience of smoking both mentholated and nonmentholated cigarettes. Also, consist with previously observed dose dependency pharmacokinetics of nicotine and cotinine by Miller, Rotenberg, & Adir (1977), our intravenous studies of nicotine at 0.05 versus 0.5 mg/kg dose showed significant dose-dependency disposition of nicotine and cotinine in rats, that is, nicotine clearance was significantly decreased from 65.9 to 45.8 ml/min/kg when dose was increased from 0.05 to 0.5 mg/kg (our unpublished data). It is therefore that the human study is more reflective of heavy smokers and does not necessary represent light smokers (i.e., <20 cigarettes/day), where it is still unclear whether there is an effect of menthol on disposition kinetics of nicotine and cotinine. Mainstream smoking inhalation studies in rodents were well-established and widely used to study nicotine addiction and cigarettes toxicities. This laboratory apparatus has low cost and maybe easily controlled for experimental purpose. Nevertheless, one should always be cautious in translating these laboratory findings to actual human smoking.

Earlier studies in nose-only smoke generation-inhalation system reported a 68% of nicotine delivered to the inhalation chamber was absorbed in rats exposed to a single-cigarette smoke (Rotenberg, Miller, & Adir, 1980). The amount of nicotine absorbed from multiple-cigarette smoke was reportedly 10-fold greater than that absorbed from a single cigarette (Rotenberg & Adir, 1983). However, our results showed no significant difference in the rate and extent of nicotine absorption as well as the formation of cotinine between single-cigarette versus multiple-cigarette smoke inhalation groups. This difference may be due to the greater specificity of our RIA for nicotine compared with their assay method that involved thin layer chromatography separation of nicotine and its metabolites followed by liquid scintillation spectrometry.

The effect of menthol on smoke topography (e.g., puff volume and puff frequency) is still unclear because of inconsistent reports. Cigarette smoking topography parameters provide information regarding smoke constitute exposure through puff volume, puff duration, and lung exposure measures. Jarvik, Tashkin, McCarthy, & Rosenblatt (1994) reported no change in number of puffs taken from menthol cigarettes compared with regular and decreased puff volume but increased carbon monoxide absorption with menthol cigarettes. Ahijevych & Parsley (1999) found that menthol smokers had significantly larger puff volumes and higher cotinine levels compared with nonmenthol smokers. McCarthy et al. (1995) reported a significant high in puff volume (13%) and puff frequency (22%) with a nonmentholated brand. In a recent study of modified, controlled-dose rapid smoking procedure comparing menthol versus nonmenthol cigarettes, Caskey et al. (1993) reported no difference for the number of puffs taken from regular as opposed to menthol cigarettes.

In conclusion, we found that mentholated cigarette smoke inhalation resulted in significantly lower plasma nicotine and

cotinine levels as compared with nonmentholated cigarettes. However, menthol may significantly increase metabolic process of nicotine to cotinine in vivo as indicated in the present study that the plasma cotinine to nicotine ratio was significantly increased by mentholated-cigarette smoke inhalation. If this animal data translate to humans, it might result in increased inhalation of tobacco smoke in an attempt to compensate when smoking mentholated cigarettes. This might explain the fact that smoking mentholated cigarettes has higher incidents of cancer. The observed results provide an important information on the effect of menthol on pharmacokinetics of nicotine and its metabolism. Further studies are warranted to investigate mechanisms of such metabolic activation and eventually define the role of menthol on nicotine addiction and cancer risk.

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Declaration of Interests

None declared.

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