Effects of cigarette smoking and carbon monoxide on chlorzoxazone and caffeine metabolism

Objectives: **Our objectives were to examine the effects of cigarette smoking on the disposition kinetics of chlorzoxazone and caffeine as probes of cytochrome P450 (CYP) 2E1, CYP1A2, xanthine oxidase, and *N*-acetyltransferase-2 activity and to test the hypothesis that carbon monoxide inhibits drug metabolism via these pathways.**

Methods: **Twelve cigarette smokers were studied in 3 treatment conditions, each lasting 7 days, during which they smoked cigarettes, breathed carbon monoxide to achieve carboxyhemoglobin levels similar to those associated with cigarette smoking, or breathed air. In each treatment condition, subjects received oral chlorzoxazone (250 mg) and caffeine (250 mg) with measurement of disposition kinetics and urine metabolite profiles.**

Results: **Compared with the air condition, cigarette smoking significantly induced the metabolism of chlorzoxazone (oral clearance, 5.9 ± 1.5 mL · min^{-1} · kg^{-1} versus 4.8 ± 1.0 mL · min^{-1} · kg^{-1}, $P < .005$) and caffeine (2.0 ± 0.8 mL · min^{-1} · kg^{-1} versus 1.5 ± 0.7 mL · min^{-1} · kg^{-1}, $P < .001$) but had no effect on caffeine urine metabolite ratios that reflect xanthine oxidase and *N*-acetyltransferase-2 activity. Considerable individual variability was noted in the extent of induction of metabolism by cigarette smoking, particularly as it affects chlorzoxazone (change in oral clearance ranged from −10% to +71%). Carbon monoxide had no effect on chlorzoxazone or caffeine metabolism or caffeine metabolic profile.**

Conclusions: **This study provides novel evidence that cigarette smoking accelerates chlorzoxazone metabolism, most likely reflecting induction of CYP2E1 activity, in humans. Induction of CYP2E1 activity by cigarette smoking could contribute to tobacco-induced cancer, alcohol-induced liver disease, and the risk of acetaminophen hepatotoxicity. (Clin Pharmacol Ther 2003;74:468-74.)**

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Cigarette smoking accelerates the metabolism of a number of drugs.[1] Metabolic pathways known to be induced by cigarette smoking include cytochrome P450 (CYP) 1A1 and CYP1A2 and some, but not all, glucuronidation pathways. Cigarette smoking also appears to inhibit the metabolism of nicotine, which is metabolized primarily by CYP2A6.[2]

CYP2E1 is involved in the metabolism of a number of drugs, including acetaminophen and ethanol, and is involved in the activation of many carcinogens, including nitrosamines.[3,4] Induction of CYP2E1 may be associated with a greater risk of acetaminophen- or alcohol-related hepatotoxicity and may enhance carcinogenesis. Cigarette smoke induces CYP2E1 activity in mouse liver, lung, and kidney.[5-7] Nicotine has recently been shown to induce CYP2E1 protein and to accelerate metabolism of CYP2E1 substrates in rat liver microsomes.[8,9] Chlorzoxazone metabolism has been widely used as a probe of CYP2E1 enzymatic activity in humans.[10,11] Previously published studies comparing chlorzoxazone metabolism in smokers and nonsmokers

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have reported no differences, and it has been concluded that smoking does not induce CYP2E1 activity in humans.[12,13]

Caffeine is primarily metabolized by CYP1A2, and its metabolism is well known to be accelerated by cigarette smoking.[14,15] Caffeine is also metabolized to a lesser extent by *N*-acetyltransferase-2 (NAT-2) and xanthine oxidase. The effects of cigarette smoking on these latter pathways of metabolism have not been described.

Carbon monoxide is a widespread environmental contaminant, with high levels in cigarette smokers and workers with occupational exposures to combustion products. It is known to inhibit CYP in vitro and has been shown to inhibit the metabolism of some drugs in animals.[16,17] We have previously reported that carbon monoxide, at levels commonly found in cigarette smokers, had no effect on nicotine metabolism,[2] but the effects of carbon monoxide on other metabolic pathways in humans have not been described.

The aims of our study were to examine the effects of cigarette smoking and carbon monoxide on the disposition kinetics and metabolism of chlorzoxazone, a probe for CYP2E1 activity, and caffeine, a probe for CYP1A2, NAT-2, and xanthine oxidase activity.

METHODS

The study methods have been described in detail in a previous publication that reported on the effects of cigarette smoking and carbon monoxide inhalation on the metabolism of nicotine.[2] In brief, the methods were as follows.

Subjects. The subjects were 12 healthy men who were regular cigarette smokers. They ranged in age from 27 to 47 years, smoked an average of 28 cigarettes per day, and had smoked for an average of 22 years. All smoked American cigarette brands containing blond tobacco. These subjects were recruited by advertisement in local newspapers and were financially compensated for participation. Five of the subjects consumed alcohol at least weekly (range, 147-400 g/wk). All subjects denied illicit drug use, including smoking marijuana.

Written informed consent was obtained for each subject. The study was approved by the Committee on Human Research at the University of California, San Francisco.

The number of subjects was based on a power analysis for repeated-measures ANOVA to detect an effect size of 25%, assuming a coefficient of variation of 25% with $\alpha = .05$ and $\beta = .2$.

Experimental protocol. The subjects were studied as inpatients at the General Clinical Research Center at San Francisco General Hospital Medical Center, San Francisco, Calif, where they were confined for 21 days. Subjects consumed a regular diet except that no alcohol or caffeinated beverages were permitted for the duration of the study. The study was conducted as a within-subject crossover design with the following 3 treatments: cigarette smoking, inhalation of carbon monoxide, and inhalation of air. Each treatment was administered for 7 days, and the sequence of treatments was balanced across subjects by use of 3 × 3 Latin squares. In the smoking treatment, subjects smoked 20 cigarettes per day, 1 every 45 minutes, from 8 AM to 10:15 PM. In the air and carbon monoxide conditions, subjects inhaled air or carbon monoxide from 1-L bags once every minute for 10 minutes to simulate the intake of carbon monoxide from cigarette smoking. The inhalation sequence was repeated 20 times per day, on the same schedule as cigarette smoking. The concentration of carbon monoxide in the tank ranged from 1200 to 1500 ppm, concentrations calculated to deliver similar doses of carbon monoxide to the smoker as that delivered by a cigarette. Three subjects inhaled 1200 ppm carbon monoxide, and nine subjects inhaled 1500 ppm carbon monoxide. The inhaled carbon monoxide concentration was increased after the first 3 subjects to produce carboxyhemoglobin levels in the mid range (as opposed to the lower range) of those observed in the average cigarette smoker. The subjects were blinded to whether they were receiving the air or carbon monoxide treatment. Details of the carbon monoxide administration system have been published previously.[2]

On the fifth day of each treatment block, subjects received 250 mg caffeine orally at 4 PM. Earlier in the day, at noon, subjects had received an infusion of nicotine and its metabolite cotinine, followed by lunch at about 1 PM. Blood samples for measurement of caffeine concentrations were obtained at 30 minutes and at 1, 2, 4, 6, 8, and 12 hours after caffeine dosing. Urine was collected for 6 hours after caffeine dosing. On the sixth day of each treatment block, subjects were administered 250 mg chlorzoxazone at 8 AM, after an overnight fast. Blood samples were collected for chlorzoxazone analysis at 30 minutes and at 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 hours after ingestion. Urine was collected for 24 hours for measurement of 6-hydroxychlorzoxazone concentrations.

Analytic chemistry. Nicotine and metabolite concentrations were determined by gas chromatography–mass spectrometry.[18] Caffeine concentrations in plasma were measured by gas chromatography by use

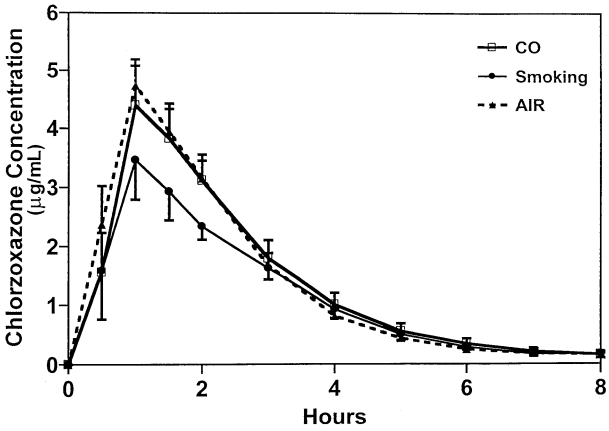

Fig 1. Plasma chlorzoxazone concentrations during cigarette smoking (CS) and air and carbon monoxide (CO) treatments (N = 10). Data are presented as mean ± SEM.

of the same assay. Concentrations of chlorzoxazone in plasma and 6-hydroxychlorzoxazone in urine were measured by reversed phase HPLC, with the use of pentoxifylline as internal standard, as described by Kharasch et al,[10] with minor modifications. Caffeine metabolites in urine were measured by HPLC as described previously.[19]

Data analysis. Pharmacokinetic parameters were estimated from blood concentration data with model-independent methods. Oral clearance was computed as follows: CL = Dose/AUC, in which CL is clearance and AUC is area under the plasma concentration–time curve extrapolated to infinity. The clearance of chlorzoxazone via the 6-hydroxychlorzoxazone pathway was estimated as oral clearance multiplied by the percentage of chlorzoxazone dose recovered as 6-hydroxychlorzoxazone in the urine.

Urine caffeine metabolite ratios were used to estimate acetylation and xanthine oxidase activity as follows: Acetylation ratio = AAMU/(AAMU + 1U + 1X), in which AAMU is 5-acetylamino-6-amino-3-methyluracil, 1U is 1-methyluric acid, and 1X is 1-methylxanthine.[19] The xanthine oxidase activity was estimated as 1U/1X.

Statistical analysis was performed by repeated-measures ANOVA with a Tukey post hoc test.

RESULTS

During cigarette smoking, the 24-hour average plasma nicotine concentration was 14 ng/mL, the average plasma cotinine concentration was 270 ng/mL, and the average carboxyhemoglobin level was 5.3%. During carbon monoxide inhalation, the average carboxyhemoglobin level was 4.6%. Concentration-time curves for these analytes have been published previously.[2]

Plasma concentration–time curves for chlorzoxazone in the 3 treatment conditions are shown in Fig 1 and pharmacokinetic data in Table I. Blood level data are presented for 10 subjects because in 2 cases the clearance could not be estimated. In these 2 subjects, in one or more treatment conditions, chlorzoxazone blood levels were sustained for most of the 12-hour sampling period such that a terminal half-life could not be satisfactorily estimated. In one case blood levels were sustained in all treatment conditions, whereas in the other case levels were sustained in the smoking condition only. Urine 6-hydroxychlorzoxazone data were available for only 9 of the remaining 10 subjects, because in 1 subject the 24-hour urine collection was incomplete. The oral clearance of chlorzoxazone was significantly higher during cigarette smoking (488 ± 144 mL/min) compared with air (396 ± 151 mL/min) and carbon monoxide (414 ± 131 mL/min) conditions ($P < .005$). As shown in Fig 2, the majority of subjects showed a greater clearance when smoking cigarettes compared with the air condition, but 3 subjects showed little or no difference (<5% increase). With the air condition used as a reference, the average increase in chlorzoxazone clearance during cigarette smoking was 24.4% (95% confidence interval, 7.4%-41.5%), with a range of −10% to 71%.

The maximum plasma drug concentration (C_{max}) of chlorzoxazone tended to be lower during cigarette smoking, whereas the terminal half-life was similar in the 3 conditions. The fractional clearance of chlorzoxazone to 6-hydroxychlorzoxazone tended to be higher during cigarette smoking, but this difference was not significant.

Plasma concentration–time curves for caffeine in the 3 treatment conditions are shown in Fig 3, and pharmacokinetic data are given in Table II. Data are presented for 11 subjects. The data from 1 subject were excluded because blood levels were sustained without much decline over the entire 12-hour period of blood sampling so that a terminal half-life could not be estimated. This was observed for this subject in all 3 treatment conditions. The clearance of caffeine was significantly higher during cigarette smoking (164 ± 67 mL/min) compared with air (125 ± 52 mL/min) and carbon monoxide (115 ± 41 mL/min) conditions ($P < .001$). As shown in Fig 4, caffeine clearance was higher (28% or more) in all but one of the subjects during cigarette smoking compared with air and carbon monoxide conditions. With use of the air condition as a reference, the average increase in clearance was 35.0%

Table I. Effect of cigarette smoking and carbon monoxide on disposition kinetics of chlorzoxazone

	No.	*Cigarette smoking*	*Carbon monoxide*	*Air*	*P value*
Clearance (mL/min)	10	488 ± 144	414 ± 131	396 ± 115	<.005*
Clearance (mL · min^{-1} · kg^{-1})	10	5.9 ± 1.5	5.0 ± 1.4	4.8 ± 1.0	<.005*
$t_{1/2}$ (min)	10	66.0 ± 9.9	62.6 ± 9.9	62.2 ± 9.0	NS
C_{max} (μg/mL)	10	4.0 ± 2.2	4.8 ± 2.0	4.9 ± 1.2	NS
t_{max} (min)	10	84 ± 42	84 ± 52	69 ± 20	NS
Percent recovery of 6-hydroxychlorzoxazone	9	47.2 ± 8.6	47.6 ± 16.2	53.6 ± 12.8	NS
Formation clearance of 6-hydroxychlorzoxazone (mL/min)	9	236 ± 96	194 ± 84	210 ± 78	NS

$t_{1/2}$, Terminal half-life; NS, not significant; C_{max}, maximum plasma drug concentration; t_{max}, time to maximum plasma drug concentration.
*Higher during cigarette smoking compared with carbon monoxide and air.

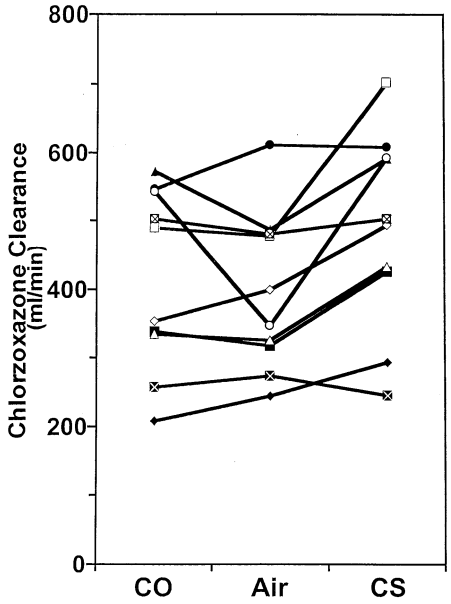

Fig 2. Plasma clearance of chlorzoxazone for individual subjects across 3 treatment conditions. Data (mean ± SD) for clearance in each treatment are provided in Table I.

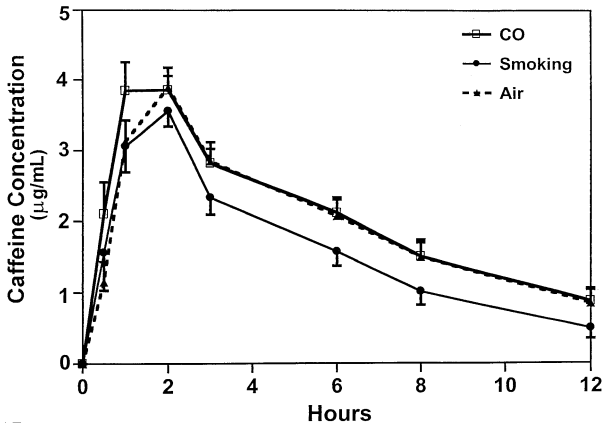

Fig 3. Plasma caffeine concentrations during CS and air and CO treatments (N = 11). Data are presented as mean ± SEM.

(95% confidence interval, 20.7-49.3), with a range of 23% to 60%. The half-life of caffeine was significantly shorter during cigarette smoking, whereas there was no difference in C_{max}. The urine metabolite ratios, AAMU/(AAMU + 1U + 1X), representing NAT-2 activity, and 1U/1X, representing xanthine oxidase activity, were similar in the 3 treatment conditions.

DISCUSSION

Our study provides novel information on the effects of cigarette smoking on drug metabolism. We have determined that cigarette smoking accelerates the metabolism of chlorzoxazone, presumably by inducing CYP2E1. However, the induction effect was not seen in all subjects. The finding that cigarette smoking increased oral clearance but did not affect elimination half-life suggests that the main consequence of the cigarette smoking effect on CYP2E1 was to increase first-pass rather than systemic metabolism of chlorzoxazone.

We confirmed the well-established observation that cigarette smoking accelerates caffeine metabolism, which is mediated primarily via CYP1A2.[1] By using caffeine metabolite ratios in the urine, we have also shown that cigarette smoking does not affect the activity of NAT-2 or xanthine oxidase, which has not been demonstrated previously to the best of our knowledge.

Table II. Effect of cigarette smoking and carbon monoxide on disposition kinetics of caffeine (N = 11)

	Cigarette smoking	*Carbon monoxide*	*Air*	*P value*
Clearance (mL/min)	164 ± 62	115 ± 41	125 ± 52	<.001*
Clearance (mL · min^{-1} · kg^{-1})	2.0 ± 0.8	1.4 ± 0.6	1.5 ± 0.7	<.001*
$t_{1/2}$ (min)	205 ± 94	289 ± 130	276 ± 132	<.0001†
C_{max} (μg/mL)	4.0 ± 0.8	4.3 ± 0.9	4.1 ± 0.8	NS
t_{max} (min)	90 ± 35	79 ± 34	98 ± 30	NS
Urine [AAMU/(AAMU + 1U + 1X)]‡	0.33 ± 0.13	0.33 ± 0.15	0.31 ± 0.12	NS
Urine (1U/1X)§	2.56 ± 0.94	2.38 ± 0.78	2.38 ± 0.84	NS

Data are presented as mean ± SD.
AAMU, 5-Acetylamino-6-amino-3-methyluracil; 1U, 1-methyluric acid; 1X, 1-methylxanthine.
*Higher during cigarette smoking compared with carbon monoxide and air.
†Higher during carbon monoxide and air compared with cigarette smoking.
‡Index of *N*-acetyltransferase-2.
§Index of xanthine oxidase.

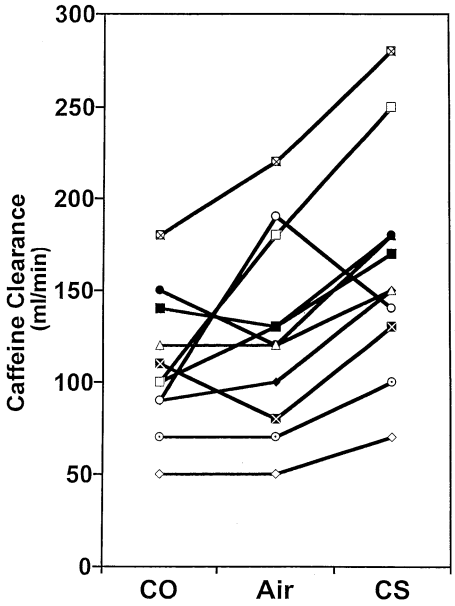

Fig 4. Plasma clearance of caffeine for individual subjects across 3 treatment conditions. Data (mean ± SD) for clearance in each treatment condition are provided in Table II.

Finally, our study indicates that inhalation of carbon monoxide at levels similar to those achieved during cigarette smoking does not significantly affect drug metabolism via CYP2E1, CYP1A2, NAT-2, or xanthine oxidase pathways.

A strength of our study compared with most other studies of the effects of cigarette smoking on drug metabolism is the within-subject crossover design. A possible limitation of our study design is that a no-smoking condition of 7 to 14 days might not be adequate to demonstrate complete dissipation of the enzyme-inducing effects of cigarette smoking. Thus, although we did find significant effects of cigarette smoking on CYP2E1 and CYP1A2 activity, these differences between the smoking and nonsmoking conditions might have been larger had the period of nonsmoking been longer.

Animal studies support the idea that cigarette smoking induces CYP2E1 activity in humans. Tobacco smoke significantly enhances CYP2E1 expression in mouse lung[6] and metabolic activity in mouse liver.[5] CYP2E1 expression and activity in mouse kidneys are highly inducible by cigarette smoke exposure.[7] Nicotine per se has recently been shown to increase levels and activity of CYP2E1 in liver microsomes of rats that have been treated with nicotine for 7 days.[8] Two human studies of cigarette smoking and CYP2E1 comparing chlorzoxazone metabolism in smokers and nonsmokers have been published. Girre et al[12] found no difference in the clearance of chlorzoxazone in 10 nonalcoholic smokers compared with 10 nonsmokers. Lucas et al[13] studied larger groups of both abstinent alcoholic subjects and nonalcoholic subjects and reported no difference in the ratio of 6-hydroxychlorzoxazone/chlorzoxazone measured in the blood 2 hours after chlorzoxazone dosing. Because CYP2E1 activity is widely variable in different persons and is affected by a number of environmental factors, it is likely that a between-subject study design such as that used by Girre et al and Lucas et al will be relatively insensitive to detect moderate effects of smoking. Our within-subject study demonstrates that cigarette smoking does have a moderate inducing effect on CYP2E1, although this effect is not seen in all smokers.

Chlorzoxazone clearance has been widely used as an in vivo probe for CYP2E1 activity in humans.[11] In concluding that cigarette smoking induces CYP2E1, the possibility that other enzymes induced by cigarette smoking might be mediating chlorzoxazone metabolism needs to be considered. There is evidence that chlorzoxazone may be metabolized to some extent by CYP1A1 and CYP1A2 (which are known to be induced by cigarette smoking), but the contributions of CYP1A1 and CYP1A2 compared with CYP2E1 appear to be quite small.[11,20-22] CYP1A1 is not expressed in human liver and appears to be expressed to only a small extent in the small intestine.[23] Thus it seems most likely that an effect of cigarette smoking on chlorzoxazone clearance does reflect a change in CYP2E1 activity.

Our data indicating that cigarette smoking accelerates caffeine metabolism confirm numerous prior observations that cigarette smoking induces CYP1A2, which is the major enzyme involved in caffeine demethylation.[14,15] Caffeine is metabolized to minor metabolites by the enzymes xanthine oxidase and NAT-2. Caffeine urine metabolite ratios have been used to assess metabolism via these pathways.[19] We found no effect of cigarette smoking on caffeine urine metabolite ratios reflecting either xanthine oxidase or NAT-2 activity. We are not aware of other studies that have previously examined smoking effects on these metabolic pathways. Although cigarette smoking did not affect acetylation in this study, we have previously shown that cigarette smoking can affect other phase 2 reactions, namely, glucuronidation.[2]

Carbon monoxide directly inhibits drug-metabolizing enzyme activity in vitro.[16,24] In living rats carbon monoxide exposure inhibits hexobarbital metabolism.[17] The level of carbon monoxide exposure in the in vivo rat study was 1000 to 1500 ppm, similar to levels to which cigarette smokers are exposed. In our study carbon monoxide levels typical of smokers had no effects on drug metabolism via CYP2E1, CYP1A2, xanthine oxidase, or NAT-2. In a previous study we found no effects of carbon monoxide on nicotine metabolism, mediated by CYP2A6 or by glucuronidation.[2] Thus, to date, we are aware of no data indicating that carbon monoxide at levels seen in smokers or associated with typical environmental exposures has effects on drug metabolism in humans.

Of note was our observation that there is considerable individual variability in the extent of enzyme induction related to cigarette smoking. In the case of caffeine, all subjects did have increased clearance, but the extent of induction ranged from 23% to 60%. For chlorzoxazone, most subjects showed induction but one third did not. The extent of induction ranged from −10% to 71%. The mechanisms underlying individual differences in inducibility by cigarette smoking are not well understood but may be related to the presence of CYP2E1 gene alleles that are associated with higher or lower susceptibility to induction, as described by Hu et al.[25] The extent of induction of chlorzoxazone metabolism is small compared with the inducing effects of ethanol, which can increase clearance several-fold.[26]

Our findings that cigarette smoking induces CYP2E1 metabolism may have implications for understanding susceptibility to smoking-related cancer, as well as other diseases. CYP2E1 bioactivates tobacco smoke carcinogens such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).[27] CYP2E1 metabolizes ethanol, and there is evidence that CYP2E1 activity contributes to the pathogenesis of alcoholic liver disease putatively by generating free radicals and promoting lipid peroxidation.[28] CYP2E1 also metabolizes acetaminophen, and induction of CYP2E1 activity, such as with long-term alcohol use, appears to increase the risk of acetaminophen-induced hepatitis.[28] Thus cigarette smoking, acting through induction of CYP2E1, could contribute to tobacco-induced cancer, alcohol-induced liver disease, and acetaminophen hepatotoxicity. Furthermore, individual differences in CYP2E1 inducibility by cigarette smoking might explain some individual variability in smoking-related risks of such diseases.

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