

Impact of long-term ethanol consumption on CYP1A2 activity

Ethanol is a well-known inducer of CYP2E1; whether or not it is an inducer of other cytochromes has not been investigated systematically. The aim of our study was to evaluate the impact of ethanol consumption on the activity of CYP1A2, which has been shown to be influenced by drugs (inhibited or induced). We evaluated CYP1A2 activity by the ratio of the molar urinary concentrations of the three end products of paraxanthine demethylation of caffeine to the molar concentration of a paraxanthine 8-hydroxylation product. This urinary metabolite ratio has previously been shown to correlate with caffeine clearance. The caffeine metabolites were measured in urine collected during the 3 hours after oral administration of 200 mg caffeine. The caffeine test was performed in 12 smokers (>25 cigarettes/day) and 12 nonsmokers, all of whom were alcoholic inpatients (daily intake >100 gm absolute ethanol), within the first 3 days of their hospital stay and after 14 days of abstinence from ethanol. In alcoholic patients who were smokers the molar urinary concentration ratio was 3.14 ± 0.97 before withdrawal and 4.01 ± 0.92 after 14 days of abstinence from ethanol. In contrast, in alcoholic patients who were nonsmokers it was 2.62 ± 0.95 and 2.18 ± 0.96 before and after withdrawal, respectively. In volunteers who were smokers the molar urinary concentration ratio was 5.02 ± 1.51 , whereas in volunteers who were nonsmokers it was 3.22 ± 1.46 . Our results confirm the well-known induction of CYP1A2 activity by tobacco smoking and show that this induction is masked by long-term ethanol consumption. (Clin Pharmacol Ther 1997;62:505-9.)

Nathalie Rizzo, PharmD, Eric Hispard, MD, Sylvie Dolbeault, MD,
Sylvain Dally, MD, Roger Leverge, PharmD, PhD, and
Catherine Girre, PharmD, MD, PhD *Paris, France*

Environmental and physiologic factors play an important role in the regulation of activity of cytochrome P450 isozymes. Ethanol is a well-known inducer of CYP2E1,¹⁻³ whether or not it is an inducer of other cytochromes has not yet been investigated systematically. For instance, the impact of ethanol consumption on the activity of CYP1A2, which is responsible for biotransformation of several therapeutic drugs, has not been evaluated. It has been established that caffeine metabolism and, in particular, 3-demethylation are catalyzed by cytochrome P4501A2 in humans.⁴⁻⁶ The rate of caffeine metabolism can be measured in a single urine sample by the ratio of the molar urinary concentrations

of the three end products of the paraxanthine demethylation of caffeine to the molar concentration of a paraxanthine 8-hydroxylation product. This urinary metabolite ratio has previously been shown to correlate with caffeine clearance⁷ and CYP1A2 activity.^{4,8}

The purpose of this study was to evaluate the influence of long-term alcohol consumption on the activity of CYP1A2 with use of the caffeine molar urinary concentration ratio. To this end we measured CYP1A2 activity in a group of 24 patients with alcoholism; 22 volunteers (non-alcohol dependent) were studied as a control group.

MATERIAL AND METHODS

Subjects. The study was conducted in 24 alcoholic inpatients (daily intake >100 gm absolute ethanol): 12 were nonsmokers and 12 were smokers. The criterion for inclusion in this study was their decision to abstain from alcohol.

The control group consisted of 22 healthy volunteers (12 nonsmokers and 10 smokers). Their biological parameters were within the normal range

From the Pharmacie, Hôpital Lariboisière, and the Clinique Toxicologique, Hôpital Fernand Widal.

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Reprint requests: Catherine Girre, PharmD, MD, PhD, Clinique Toxicologique, Hôpital Fernand Widal, 200 Faubourg Saint-Denis 75010, Paris, France.

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and their ethanol consumption was below 100 gm per week.

Study design. The caffeine test was performed in each patient within the first 3 days of their hospital stay (after a null blood alcohol concentration had been checked) and after 14 days of abstinence from ethanol.

Each subject was given 200 mg caffeine in the form of two 100 mg caffeine monohydrate capsules (Coopérative Pharmaceutique Française, Melun, France). Urine was collected during the 3 hours after administration.

Patients and volunteers were told to eat a normal diet but to avoid broccoli, white cabbage, charcoal-broiled meat, and heavy physical exercise during the entire study because these factors have previously been shown to alter CYP1A2 activity.^{9,10} Except during the 3 hours of each caffeine test when it was strictly forbidden, subjects were allowed to consume caffeine-containing food and beverages moderately.

Chemicals. 1-Methyluric acid (1U), 1-methylxanthine (1X) and 1,7-dimethyluric acid (1,7U) were obtained from Sigma Chemical Co. (St. Louis, Mo.). We used an analytical sample of 5-acetyl-amino-6-formylamino-3-methyluracil (AFMU) supplied by Dr. Galteau (Centre du Médicament, Nancy, France).

Methanol and isopropyl alcohol (Farmitalia Carlo Erba, Milano, Italy) were HPLC grade. Reagent grade chloroform was obtained from Prolabo (Paris, France). All other chemicals were of reagent grade from Sigma Chemical Co.

Methods of analysis. Urine was collected and 20 ml aliquots were acidified to pH 3 and stored at -20°C until analysis. Under these conditions AFMU is stable and quantitative determination by HPLC can be performed.¹¹ Levels of the caffeine metabolites AFMU, 1X, 1U, and 17U were measured with use of an HPLC method described previously.¹² In brief, 500 μl of 50% diluted urine was added to a tube containing 100 μl internal standard solution (240 mg/L *N*-acetyl-*p*-amino phenyl). After addition of 120 mg ammonium sulfate, metabolites were extracted in 2×8 ml chloroform/isopropyl alcohol (80:20, vol/vol) by vortexing for 2 minutes. Phases were separated by centrifugation at 3000g for 10 minutes. The organic phase was transferred and dried under a nitrogen stream at 45°C . The dry extracts were dissolved in 500 μl of 0.05% acetic acid, and 20 μl was injected into a C_{18} column (Ultrasphere ODS Beckman, 5 μm , 150×4.6 mm). The mobile phase was 0.05% acetic acid/methanol (92:8; vol/

vol) with a constant flow of 0.8 ml/min. Metabolites were detected by absorbance at 280 nm and quantitated by comparison with blank urine, spiked with pure standards. Recovery was 67%, 65%, 92%, and 95% for AFMU, 1U, 1X, and 17U, respectively. The quantitation limit was 1 mg/L for AFMU and 1U and 2 mg/L for 1X and 17U. The interassay coefficients of variation were 9.6% (2 mg/L), 5.0% (5 mg/L), and 5.0% (10 mg/L) for AFMU; 3.3% (5 mg/L), 7.7% (10 mg/L), and 7.6% (25 mg/L) for 1U; 9.9% (5 mg/L), 1.4% (20 mg/L), and 4.0% (50 mg/L) for 1X; and 5.2% (5 mg/L), 3.8% (20 mg/L), and 6.7% (50 mg/L) for 17U. Biases were less than 10% for each compound.

Determination of P4501A2 activity. CYP1A2 activity was evaluated by the caffeine urinary metabolite ratio (molar urinary concentration ratio), calculated by dividing the sum of the molar urinary concentrations of the three end products of the paraxanthine demethylation (AFMU+1U+1X) by the molar concentration of the paraxanthine 8-hydroxylation product (17U) (i.e., $\text{AFMU}+1\text{U}+1\text{X}/17\text{U}$).

Statistical analysis. The impact of long-term ethanol consumption on CYP1A2 activity was evaluated by comparison of the molar urinary concentration ratios on day 15 with those on day 1 by use of a test based on pairwise differences—the nonparametric Wilcoxon paired test. In addition, the nonparametric Mann-Whitney test was used to compare P4501A2 activity between inpatients with alcoholism and healthy volunteers. Significance was set at $p < 0.05$.

RESULTS

Subjects. Twenty-four alcoholic inpatients participated in this study: 12 nonsmokers (six men and six women) and 12 smokers (10 men and two women; mean smoking status, 36 ± 21 cigarettes/day). Demographic and biological parameters in both smoking and nonsmoking alcoholic inpatients were determined on the day of admission in hospital (Table I). Characteristics of the 22 healthy volunteers (eight men and 14 women) were as follows: mean age \pm SD, 32 ± 7 years and 27 ± 4 years; body weight, 61.5 ± 10 kg and 60.6 ± 8.5 kg; height, 167 ± 6 cm and 163 ± 8 cm for smokers (mean smoking status, 25 ± 9.4 cigarettes/day) and nonsmokers, respectively. All subjects completed the study, and none reported adverse events or subjective discomfort after the caffeine intake.

Table I. Demographic and biological parameters in 12 alcoholic smokers and 12 alcoholic nonsmokers inpatients at admission

	Smokers (mean \pm SD)	Nonsmokers (mean \pm SD)
Age (yr)	42 \pm 12	48 \pm 9
Height (cm)	170 \pm 9	164 \pm 10
Weight (kg)	64 \pm 13	70 \pm 11
Alcohol consumption (gm absolute ethanol/day)	222 \pm 94	160 \pm 71
Blood alcohol (gm/L)	1.15 \pm 1.24	1.00 \pm 1.30
γ -Glutamyl transferase (IU/L)	183 \pm 205	564 \pm 618
ALT (IU/L)	96 \pm 64	84 \pm 57
AST (IU/L)	80 \pm 66	71 \pm 58
MCV (fl)	94.6 \pm 5.5	93.7 \pm 3.5
Thrombotest (%)	99 \pm 2	90 \pm 13
CL _{CR} (ml/min)	122 \pm 42	131 \pm 36

ALT, Alanine aminotransferase; AST, aspartate aminotransferase; MCV, mean cellular volume; CL_{CR}, creatinine clearance.

P4501A2 activity: molar urinary concentration ratio.

On day 0, before admission of patients to a rehabilitation program, their mean alcohol consumption was 197 ± 87 gm absolute ethanol per day (range, 100 to 350 gm). On day 1, their P4501A2 activity was considered to be influenced by alcohol. At day 15, after 2 weeks of abstinence, the P4501A2 activity was considered to be the reference activity (i.e., not influenced by alcohol).

Individual molar urinary concentration ratios on day 1 and on the fourteenth day of abstinence (day 15) are shown in Figs. 1 and 2 for smoking and nonsmoking patients with alcoholism, respectively. Mean \pm SD values of the molar urinary concentration ratio in alcoholic inpatients and healthy volunteers are given in Table II.

In patients with alcoholism who were smokers, the molar urinary concentration ratio was significantly higher ($p = 0.015$) after 14 days of abstinence (4.01 ± 0.92) than before withdrawal of alcohol (3.14 ± 0.97), whereas the difference was not significant in patients with alcoholism who did not smoke (2.62 ± 0.95 and 2.18 ± 0.96 , respectively). Therefore ethanol consumption significantly decreased the molar urinary concentration ratio values in patients with alcoholism who smoked. In contrast, there was no significant difference between molar urinary concentration ratios before and after withdrawal of alcohol in patients with alcoholism who did not smoke.

In volunteers who smoked, the molar urinary concentration ratio was 5.02 ± 1.51 , whereas it was 3.22 ± 1.46 in volunteers who did not smoke (Table II). These

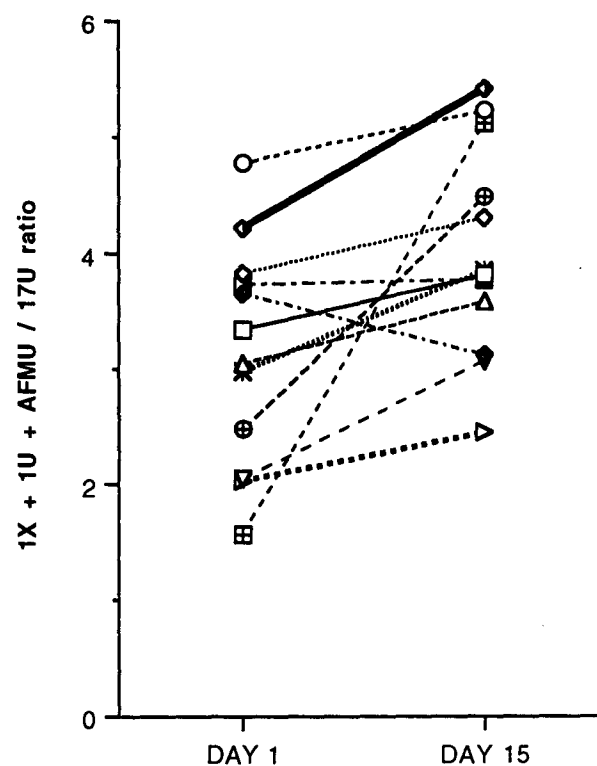


Fig. 1. Individual values of the urinary caffeine metabolite ratio before withdrawal of alcohol (day 1) and on the fourteenth day of abstinence from ethanol (day 15) in 12 inpatients with alcoholism who were smokers.

results show an increased activity of CYP1A2 in smokers, confirming the well-known induction of cytochrome P4501A2 activity by tobacco smoking.

Interestingly, the mean molar urinary concentration ratio in volunteers who did not smoke (3.22 ± 1.46) was close to that in patients with alcoholism who did smoke, before withdrawal of alcohol (3.14 ± 0.97).

DISCUSSION

In this study we evaluated the influence of ethanol consumption on the activity of CYP1A2. The induction or inhibition potency was measured as the molar urinary concentration ratio; that is, the molar ratio of the three urinary end products of caffeine demethylation (AFMU+1U+1X) to the 8-hydroxylation product (17U). Our results showed that in alcoholic inpatients who were smokers the metabolism of caffeine was significantly modified by abstinence from alcohol. Furthermore, the expected induction of CYP1A2 by tobacco smoking was not found in alcoholic patients who were smokers be-

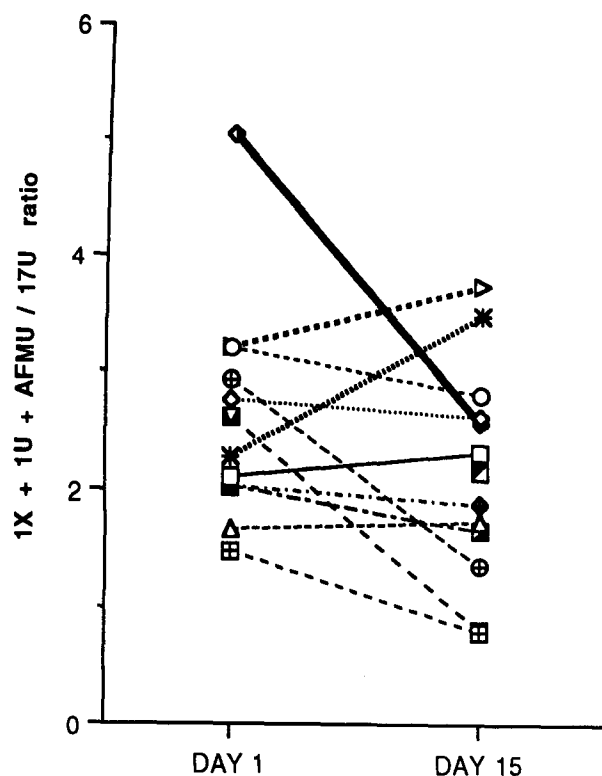


Fig. 2. Individual values of the urinary caffeine metabolite ratio before withdrawal of alcohol (day 1) and on the fourteenth day of abstinence from ethanol (day 15) in 12 inpatients with alcoholism who were nonsmokers.

cause their molar urinary concentration ratios on day 1 were much lower than those in volunteers who smoked (3.14 ± 0.97 versus 5.02 ± 1.51 ; $p = 0.021$). These results confirm the findings of George et al.,¹³ who showed a strong inhibitory effect of alcohol consumption on caffeine kinetics. George et al.¹³ reported that alcohol consumption significantly prolonged caffeine half-life and reduced caffeine clearance. In the present study no significant difference was found between P4501A2 activity before and after alcohol withdrawal in alcoholic inpatients who were nonsmokers. This contrast between our smoking and nonsmoking alcoholic groups might be explained by the fact that chronic ethanol consumption could mask the inducing potency of tobacco smoking.

It has been established that smoking induces caffeine elimination and thus reduces its half-life and increases its clearance in smokers.¹⁴⁻¹⁷ Our data in the volunteers group showed an increased CYP1A2 activity in smokers, thus confirming the inducing potency of tobacco smoking and the validity of the

Table II. Mean values \pm SD of the urinary caffeine metabolite ratio representing cytochrome P4501A2 activity in alcoholic patients and volunteers

	P4501A2 activity*	
	Smokers	Nonsmokers
Alcoholic patients		
Day 1	$3.14 \pm 0.97^{\dagger\dagger}$	$2.62 \pm 0.95^{\dagger\$}$
Day 15	$4.01 \pm 0.92^{\dagger\dagger}$	$2.18 \pm 0.96^{\dagger\$}$
Volunteers	$5.02 \pm 1.51 $	$3.22 \pm 1.46^{\dagger}$

*AFMU+1U+1X/17U molar ratio.

$n = 12$.

$^{\dagger\dagger}p = 0.015$.

$^{\$}$ Not significant.

$||n = 10$.

molar urinary concentration ratio as a useful probe for the in vivo evaluation of CYP1A2 activity. Several procedures for monitoring CYP1A2 activity in vivo by means of caffeine have been proposed and extensively discussed^{7,8,18,19} and the urinary caffeine metabolic ratio is referred to as the CYP1A2 index.⁷ Campbell et al.⁷ reported a correlation between caffeine clearance and the molar urinary concentration ratio based on the molar recovery of paraxanthine 7-demethylation products relative to a paraxanthine 8-hydroxylation product in 15 volunteers ($r = 0.91$; $p < 0.001$). In addition, Rost et al.²⁰ recently found almost the same close correlation between caffeine clearance and the molar urinary concentration ratio ($r = 0.872$; $p < 0.001$; $n = 131$). Furthermore, this molar urinary concentration ratio correctly indicates increased CYP1A2 activity in children²¹ and in subjects who eat broccoli.¹⁰ In addition, the molar urinary concentration ratio is 66% to 70% higher in male and female subjects who smoke 10 cigarettes per day or more than in the corresponding nonsmoking groups, showing the expected induction of CYP1A2 by tobacco,¹⁰ which correlates with the number of cigarettes smoked per day and with the urinary cotinine levels.²² These observations are in agreement with the recent study of Catteau¹¹ in 245 healthy subjects: in smokers ($n = 164$) the molar urinary concentration ratio was 6.96 ± 3.41 , whereas in nonsmoking volunteers it was 4.07 ± 1.79 . Similarly, in our study comparison of smoking and nonsmoking groups in both volunteers and patients with alcoholism showed the inducing effect of tobacco on CYP1A2 activity.

In conclusion, our results show that the inducing potency of smoking on CYP1A2 activity is masked

by long-term ethanol consumption and confirm that the caffeine molar urinary concentration ratio correctly indicates the increased activity of CYP1A2 in subjects who smoke. These results should prompt investigators who use caffeine as a probe for the *in vivo* evaluation of CYP1A2 status to take into account consumption of both alcohol and tobacco.

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