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CYP1A2 activity, gender and smoking, as variables influencing the toxicity of caffeine

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We have investigated several factors that might be related to the occurrence of toxic effects during the performance of a urinary test with caffeine (300 mg p.o), in 120 healthy volunteers. A total of 218 toxic effects were self-reported by eighty-two (68%) subjects. Females and nonsmokers were at the highest risk (chi-square test, $P=0.01$). Furthermore, two nonsmoking females experienced a symptomatology with delirium, restlessness, muscle tremor, vomiting and wakefulness. Among females and nonsmokers, those subjects who experienced toxic effects had lower caffeine N3-demethylation index (CYP1A2 activity) compared with unaffected females (1.87 ± 0.51 *vs* 1.47 ± 0.27, $P<0.0005$) and nonsmokers (1.69 ± 0.23 *vs* 1.49 ± 0.31, $P<0.02$). Caffeine N1- and N7-demethylations indices were also lower among females ($P<0.0005$) and nonsmokers ($P<0.02$) who reported toxic symptoms. We conclude that CYP1A2 activity, gender and smoking are variables to be considered as influencing the toxicity of caffeine.

Keywords CYP1A2 activity caffeine smoking gender toxicity

Introduction

Caffeine is probably the most frequently ingested drug in the world and is present in a variety of common beverages (coffee, tea, soft-drinks), products containing cocoa or chocolate, and in medications, including headache or pain remedies and over-the-counter stimulants [1]. On the other hand, in the last decade caffeine has become a drug of choice for assessing CYP1A2, xanthine oxidase and *N*-acetyltransferase activities in humans [2–4]. The term *caffeinism* refers to a state of acute or chronic toxicity resulting from the ingestion of high doses of caffeine (500–600 mg day^{-1}) and includes a wide variety of symptoms such as restlessness, agitation, anxiety, irritability, muscle tremor, palpitations, arrhythmia, tachycardia, diuresis and gastrointestinal disorders among others [5, 6]. This symptomatology might also occur whenever the rate of caffeine elimination is reduced, leading to a greater systemic accumulation of this xanthine and consequent toxicity [7, 8]. Widespread caffeine use prompted us to deal with those variables that might be influencing the occurrence of toxic effects during the performance of a urinary test with caffeine in a healthy population [4].

Methods

One hundred and twenty healthy volunteers (59 males and 61 females), took part in a urinary caffeine test in a population study [4]. They were aware of the purpose and gave written informed consent. The protocol was approved by the Ethics Committee at the University Infanta Cristina Hospital (Badajoz, Spain). Average age was 22.5 ± 3.6 (mean $\pm$ s.d., range 20 to 37 years), mean ($\pm$ s.d.) body weight was 65.1 ± 12.4 kg (males: 74.5 ± 10.2, females: 56.0 ± 5.6) and mean height ($\pm$ s.d.) was 169 ± 9.5 cm (males: 176 ± 7.1, females: 162 ± 6.1). Ninety subjects were only occasional alcohol drinkers with a low intake of alcohol averaging 69.5 ± 7.5 (s.e.mean) g per week. Fifty-seven (19 males, 38 females; chi square test, $P<0.001$) were habitual caffeine drinkers, averaging 162.5 ± 14.8 (s.e. mean) mg day^{-1}. Forty-four (20 males, 24 females) stated that they were smokers of 12.5 ± 1.4 (s.e. mean) cigarettes day^{-1}. Urinary concentrations of caffeine and its metabolites were measured according to Carrillo & Benítez [4]. Subjects were requested to avoid methylxanthine-containing foods and beverages for at least 48 h before and during the study. Before the administration of caffeine, each subject emptied their bladder and collected a blank urine sample to detect compounds that might interfere with the analysis and,

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evaluate the compliance in the caffeine-free period. All subjects received a 300 mg oral dose of caffeine. The concentrations of caffeine and metabolites were assessed by h.p.l.c. in the urine collected for 24 h after drug intake [4]. When the subjects were recruited, they were provided with a protocol which explained the purpose of the study and contained a wide list of common symptoms that might occur after intake of caffeine. All self-reported toxic effects occurring in the volunteers during the performance of the caffeine test were considered in the present study.

Data analysis

The following molar metabolic ratios were calculated and used as indices of five caffeine enzymatic pathways:

Caffeine N1-demethylation
(CYP1A2/CYP2E1) = 7X + 37U + 37X/137X
Caffeine N3-demethylation
(CYP1A2) = AAMU + 1U + 1X + 17U + 17X/137X
Caffeine N7-demethylation
(CYP1A2/CYP2E1) = 3X + 13U + 13X/137X
Xanthine oxidase = 1U/1X + 1U
N-acetyltransferase (NAT2) = AAMU/1X

AAMU: 5-acetylamino-6-amino-3-methyluracil; 7X: 7-methylxanthine; 1U: 1-methyluric acid; 3X: 3-methylxanthine; 37U: 3,7-dimethyluric acid; 1X: 1-methylxanthine; 13U: 1,3-dimethyluric acid; 37U: 3,7-dimethyluric acid; 37X: 3,7-dimethylxanthine (theobromine); 17U: 1,7-dimethyluric acid; 17X: 1,7-dimethylxanthine (paraxanthine); 13X: 1,3-dimethylxanthine (theophylline); 137X: 1,3,7-trimethylxanthine (caffeine).

Results

The anthropometric characteristics such as height and weight were significantly lower in females (Student's *t*-test, $P < 0.0001$). A total of 218 toxic effects were self-reported by eighty-two subjects (68%) after the intake of caffeine, 300 mg orally. The list of symptoms and frequency included: restlessness or muscle tremor, 69; palpitation, 30; dizziness, 27; headache, 22; diarrhoea, 20; wakefulness, 14; polyuria, 11; increased sweating, 8; abdominal pain, 7; tinnitus or photopsia, 5; vomiting or nausea, 3; and delirium, 2.

Table 1 shows that females and nonsmokers had a higher rate of toxic symptoms in the population ($n = 120$) (chi-square test, $P = 0.01$). Thus, nonsmoker females were also at the highest risk (Fisher's exact test; $P = 0.003$) and two of them, experienced a short-lasting but highly unpleasant symptomatology that included delirium, restlessness, muscle tremor, vomiting and wakefulness. The performance of the caffeine test in both females denoted a low enzymatic activity in the three caffeine *N*-demethylation indices compared with the average in the global population of 120 volunteers (0.14 *vs* 0.58 for N1-; 1.2 *vs* 1.55 for N3- and 0.2 *vs* 0.57 for N7-demethylation). The three caffeine

Table 1 Influence of sex, smoking as well as habitual caffeine intake from dietary sources on the incidence of toxic effects in the population with the tested dose of caffeine (300 mg p.o)

	n	*No toxic effects*	*Toxic effects*
Males	59	25	34
Females	61	13	48(†)
Smokers	44	20	24
Nonsmokers	76	18	58(*)
Smoking males	20	10	10
Nonsmoking males	39	15	24
Smoking females	24	10	14
Nonsmoking females	37	3	34(**)
Caffeine drinkers	57	18	39
Noncaffeine drinkers	63	20	43

The following intergroup comparison values were statistically significant (chi square and *P*, respectively): (†) 6.14, 0.01; (*) 6.10, 0.01; (**) 9.77, 0.003.

N-demethylation indices (mean ± s.d.) were significantly lower among females (Student's *t*-test, $P < 0.0005$) and nonsmokers ($P < 0.02$) who experienced toxic effects compared with unaffected females and nonsmokers (Table 2). The metabolic ratios of xanthine oxidase and *N*-acetyltransferase did not differ between groups (Table 2). The amount of unchanged caffeine excreted in urine, expressed as the percentage of the total metabolite excretion in each subject, was significantly higher (mean ± s.d., $P < 0.02$) in the group of volunteers who reported toxic effects ($n = 82$) compared with unaffected subjects ($n = 38$) (3.0 ± 2.14 *vs* 2.01 ± 1.72; mean difference -1.0, 95% confidence interval -1.7, -0.18). There was no difference in the urinary excretion of the remaining metabolites.

Discussion

The initial population study [4] was not a standardized and controlled phase I clinical trial with caffeine examining toxicity profiles. However, the self-reporting of symptoms by the volunteers during the performance of the caffeine test for a metabolic investigation, prompted us to undertake the current analysis. Eighty-two (68%) subjects experienced a total of 218 toxic effects with caffeine (300 mg, p.o.) which was higher than that observed in another study using an identical dose [9]. The reported consumption of caffeine by the 57 habitual drinkers (19 males, 38 females; $P < 0.001$) was relatively low in our population (162.5 ± 14.8 mg day^{-1}) compared with other similarly characterized populations with roughly 350 mg of caffeine per day [10, 11] and since a certain degree of insensitivity, habituation and tolerance to caffeine might be expected [12], it seems contradictory that females registered a higher incidence of toxic effects in our population ($P = 0.01$) (Table 1). However, it has been described that changes in the physiological pattern of regular caffeine

Table 2 Urinary caffeine metabolite ratios after intake of caffeine (300 mg) in the two groups of subjects (with and without toxic effects) stratified by sex and smoking status

		No toxic effects	*Toxic effects*	*Mean difference (95% confidence interval)*
Caffeine N1-demethylation	Males	0.63 ± 0.33	0.59 ± 0.38	0.036 (−0.16, 0.23)
	Females	0.95 ± 0.46	0.43 ± 0.31	0.51 (0.29, 0.73)†
	Smokers	0.78 ± 0.46	0.62 ± 0.30	0.15 (−0.08, 0.39)
	Nonsmokers	0.69 ± 0.35	0.45 ± 0.36	0.24 (0.05, 0.43)*
Caffeine N3-demethylation	Males	1.65 ± 0.23	1.61 ± 0.33	0.043 (−0.11, 0.20)
	Females	1.87 ± 0.51	1.47 ± 0.27	0.39 (0.18, 0.61)†
	Smokers	1.76 ± 0.45	1.63 ± 0.27	0.13 (−0.09, 0.35)
	Nonsmokers	1.69 ± 0.23	1.49 ± 0.31	0.20 (0.03, 0.35)*
Caffeine N7-demethylation	Males	0.54 ± 0.27	0.53 ± 0.34	−0.03 (−0.20, 0.14)
	Females	0.80 ± 0.42	0.34 ± 0.25	0.45 (0.27, 0.64)†
	Smokers	0.63 ± 0.42	0.49 ± 0.26	0.14 (−0.069, 0.35)
	Nonsmokers	0.59 ± 0.27	0.40 ± 0.32	0.19 (0.023, 0.36)*
Xanthine oxidase	Males	−0.22 ± 0.07	−0.23 ± 0.12	0.01 (−0.04, 0.07)
	Females	−0.29 ± 0.16	−0.22 ± 0.10	−0.07 (−0.14, 0.002)
	Smokers	−0.27 ± 0.13	−0.21 ± 0.08	−0.06 (−0.12, 0.007)
	Nonsmokers	−0.21 ± 0.08	−0.23 ± 0.12	0.023 (−0.03, .08)
NAT2	Males	1.89 ± 1.82	1.62 ± 1.63	0.27 (−0.63, 1.18)
	Females	2.10 ± 2.89	1.39 ± 1.46	0.71 (−0.44, 1.86)
	Smokers	2.53 ± 2.57	1.89 ± 1.75	0.64 (−0.68, 1.96)
	Nonsmokers	1.34 ± 1.56	1.32 ± 1.40	0.02 (−0.76, 0.79)

The results (mean ± s.d.) are expressed in logarithmic form, except NAT2 index. The 95% confidence intervals corresponding to the differences in the urinary metabolic ratios between groups are given in parenthesis. †$P < 0.0005$, *$P < 0.02$.

consumption, produced strongly disturbed sleep in people who are accustomed to drink large amounts of coffee over long periods [13]. Likewise, women usually have a lower body weight and height (in our population, $P < 0.0001$), as well as blood volume and, for equivalent levels of caffeine consumption, this might more easily result in toxic concentrations of circulating caffeine [1, 6, 8, 11]. The abstinence period of at least 48 h required before the administration of caffeine, could influence the incidence of toxic effects regardless of the daily caffeine intake in our volunteers [4, 14]. On the other hand, a number of drugs used commonly in psychiatric patients are putative CYP1A2 substrates namely, clozapine [15], imipramine [16, 17] and clomipramine [18], and the selective serotonin reuptake inhibitor (SSRI) fluvoxamine, has recently been confirmed as a potent and selective inhibitor of CYP1A2, the main metabolic pathway of caffeine [19]. Thus, clinically defined pharmacokinetic interactions at the CYP1A2 site have caused a higher risk of toxic effects with the mentioned drugs [20–22] and some authors have pointed out that restriction of caffeine should be considered as part of the treatment plan [1, 6, 23–25]. Nonsmokers were at the highest risk of toxicity with caffeine and the differences were particularly remarkable in nonsmoking females ($P = 0.01$ and $P = 0.003$ respectively) (Table 1). Although we can not rule out pharmacodynamic differences in sensitivity to caffeine effects, one explanation could be that polycyclic aromatic hydrocarbon (PAH) as contained in cigarette smoke, would accelerate the metabolism of caffeine dependent on CYP1A2 [4, 26] and hence, result in diminished plasma caffeine levels and toxicity. Moreover, caffeine toxicity has been described to increase after tobacco withdrawal [27]. There was significantly lower CYP1A2 activity among females and nonsmokers who experienced toxic effects with caffeine compared with unaffected females and nonsmokers ($P < 0.0005$ and $P < 0.02$ respectively) (Table 2) and the excretion of unchanged caffeine was higher in the urine of those volunteers reporting toxic effects ($P < 0.02$). There was no difference in the remaining urinary caffeine metabolite indices and excreted metabolites between groups. These findings support the idea that in our study, caffeine was more toxic than the other metabolites. Indeed, it has been reported that individuals with a history of coffee-induced wakefulness, eliminate caffeine from the body more slowly than those not affected [7]. A marked difference in the plasma levels of clozapine was also related to factors such as sex, smoking and age [28].

In summary, a very unpleasant symptomatology could easily be produced following the ingestion of sufficient amounts of caffeine-containing beverages or over-the-counter medications. The additional finding of two nonsmoker females, with low caffeine *N*-demethylation indices who experienced a very unpleasant symptomatology with delirium, restlessness, muscle tremor, vomiting and wakefulness, strengthened the evidence about the importance of taking sex, smoking, as well as CYP1A2 activity into consideration when caffeine and/or cosegregated compounds are given to the population. Since caffeine is a widely used drug for certain metabolic assessments (CYP1A2, xanthine oxidase and *N*-acetyltransferase) either low doses or a body weight adjustment is recommended. In our laboratory we use lower doses successfully (100 or 150 mg).

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References

1 Benowitz NL. Clinical pharmacology of caffeine. *Ann Rev Med* 1990; **41**: 277–288.
2 Tang BK, Kadar D, Qian L, Iriah J, Yip J, Kalow W. Caffeine as a metabolic probe: validation of its use for acetylator phenotyping. *Clin Pharmacol Ther* 1991; **49**: 648–657.
3 Kalow W, Tang BK. The use of caffeine for enzyme assays: a critical appraisal. *Clin Pharmacol Ther* 1993; **53**: 503–514.
4 Carrillo JA, Benítez J. Caffeine metabolism in a healthy Spanish population: *N*-acetylator phenotype and oxidation pathways. *Clin Pharmacol Ther* 1994; **55**: 293–304.
5 Ellinwood EH, Rockwell WJK. Central nervous system stimulants and anorectic agents. In *Meyler's Side Effects of Drugs*, ed Dukes MNG, Amsterdam: Elsevier Science 1992: 1–29.
6 Stavric B. Methylxanthines: toxicity to humans. 2. Caffeine. *Food Chem Toxic* 1988; **26**: 645–662.
7 Levy M, Zylber-Katz E. Caffeine metabolism and coffee-attributed sleep disturbances. *Clin Pharmacol Ther* 1983; **33**: 770–775.
8 Lane JD, Steege JF, Rupp SL, Kuhn CM. Menstrual cycle effects on caffeine elimination in the human female. *Eur J Clin Pharmacol* 1992; **43**: 543–546.
9 Hardy BG, Lemieux C, Walker SE, Bartle WR. Interindividual and intraindividual variability in acetylation: Characterization with caffeine. *Clin Pharmacol Ther* 1988; **44**: 152–157.
10 James JE, Bruce MS, Lader MH, Scott NR. Self-report reliability and symptomatology of habitual caffeine consumption. *Br J Clin Pharmacol* 1989; **27**: 507–514.
11 Barone JJ, Roberts H. Human consumption of caffeine. In *Caffeine: Perspectives from recent research,* ed Dews PB, Berlin: Springer-Verlag, 1984: 59–85.
12 Snel J. Coffee and caffeine: Sleep and wakefulness. In *Caffeine, Coffee, and Health,* ed Garattini S, New York: Raven Press, 1993: 255–290.
13 Ogunremi OO, Mamora AO. *Cola acuminata* (Kolanut) and coffee: acute effects on sleep of Nigerians. *Psychopathologie Africaine* 1980; **16**: 69–75.
14 Shi J, Benowitz NL, Denaro CP, Sheiner LB. Pharmacokinetic-pharmacodynamic modelling of caffeine: tolerance to pressor effects. *Clin Pharmacol Ther* 1993; **53**: 6–14.
15 Bertilsson L, Carrillo JA, Dahl ML, *et al.* Clozapine disposition covaries with the CYP1A2 activity determined by a caffeine test. *Br J Clin Pharmacol* 1994; **38**: 471–473.
16 Lemoine A, Gautier JC, Azoulay D, *et al.* Major pathway of imipramine metabolism is catalyzed by cytochromes P-450 1A2 and P-450 3A4 in human liver. *Mol Pharmacol* 1993; **43**: 827–832.
17 Skjelbo E, Brøsen K. Inhibitors of imipramine metabolism by human liver microsomes. *Br J Clin Pharmacol* 1992; **34**: 256–261.
18 Nielsen KK, Brøsen K, Hansen J, Gram LF. Single-dose kinetics of clomipramine: Relationship to the sparteine and S-mephenytoin oxidation polymorphisms. *Clin Pharmacol Ther* 1994; **55**: 518–527.
19 Brøsen K, Skjelbo E, Rasmussen BB, Poulsen HE, Loft S. Fluvoxamine is a potent inhibitor of cytochrome P450 1A2. *Biochem Pharmacol* 1993; **45**: 1211–1214.
20 Jerling M, Lindström L, Bondesson U, Bertilsson L. Fluvoxamine inhibition and carbamazepine induction of the metabolism of clozapine: Evidence from a Therapeutic Drug Monitoring Service. *Ther Drug Monit* 1994; **16**: 368–374.
21 Hiemke C, Weigmann H, Härtter S, Dahmen N, Wetzel H, Muller H. Elevated levels of clozapine in serum after addition of Fluvoxamine. *J Clin Psychopharmacol* 1994; **14**: 279–281.
22 Vainer JL, Chouinard G. Interaction between caffeine and clozapine. *J Clin Psychopharmacol* 1994; **14**: 284.
23 Carrillo JA, Jerling M, Bertilsson L. Comments to 'Interaction between caffeine and clozapine' *J Clin Psychopharmacol* 1995; **15**: 376–377.
24 Baldwin HA, File SE. Caffeine-induced anxiogenesis: The role of adenosine, benzodiazepine and noradrenergic receptors. *Pharmacol Biochem Behav* 1989; **32**: 181–186.
25 Curry ML, Curry SH, Marroum PJ. Interaction of phenothiazine and related drugs and caffeinated beverages. *DICP, Annals Pharmacother* 1991; **25**: 437.
26 Kalow W, Tang BK. Caffeine as a metabolic probe: exploration of the enzyme-inducing effect of cigarette smoking. *Clin Pharmacol Ther* 1991; **49**: 44–48.
27 Oliveto AH, Hughes JR, Terry SY, *et al.* Effects of caffeine on tobacco withdrawal. *Clin Pharmacol Ther* 1991; **50**: 157–164.
28 Haring C, Fleischhacker WW, Schett P, Humpel C, Barnas C, Saria A. Influence of patient-related variables on clozapine plasma levels. *Am J Psychiatry* 1990; **147**: 1471–1475.

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