Pharmacogenetics (1996) **6**, 291–296

Original article

Role of CYP1A2 in caffeine pharmacokinetics and metabolism: studies using mice deficient in CYP1A2

Jeroen T.M. Buters[1] Bing-Kou Tang[2], Thierry Pineau[1‡], Harry V. Gelboin[1], Shioko Kimura[1] and Frank J. Gonzalez[1]

[1]*National Cancer Institute, Bethesda, MD 20892, USA*
[2]*Department of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8, Canada*

Received 2 January 1996 and accepted 13 February 1996

We investigated the involvement of CYP1A2 in the pharmacokinetics and metabolism of caffeine using mice lacking its expression (CYP1A2−/−). The half-life of caffeine elimination from blood was seven times longer in the CYP1A2 −/− than wild-type mice. The clearance was concomitantly eight times slower. No parameter that could affect the pharmacokinetics differed between CYP1A2 −/− and wild-type mice such as creatinine for kidney function; alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin for liver function; or albumin for protein binding. Other P450s CYP2A, 2B, 2C, 2E1, and 3A were also unchanged in the knockout animals. Caffeine 3-demethylated metabolites thought previously to be characteristic of CYP1A2 (especially 1-methylxanthine and 1-methylurate) were also found in the urines of the CYP1A2 −/− animals, although at 40% of the level found in wild-type mice. These data indicate that the clearance of caffeine in wild-type mice is primarily determined by CYP1A2.

Keywords: CYP1A2, caffeine, pharmacokinetics, metabolism, urine, knockout mice

Abbreviations: 1,7X: 1,7-dimethylxanthine, paraxanthine; 3,7X: theobromine; 1,3X: theophylline; 1X: 1–methylxanthine; 3X: 3-methylxanthine; 7X; 7-methylxanthine; 1,3U: 1,3 dimethylurate; 1,7U; 1,7-dimethylurate; 1U: 1-methylurate; AFMU: 5-acetylamino-6-formyl-amino-3-methyluracil.

Introduction

CYP1A2 is an enzyme implicated in the metabolic activation of environmental and food born carcinogens, including arylamines and heterocyclic amines (Boobis *et al.*, 1994; Eaton *et al.*, 1995). Up to a 40-fold variation exists in the expression of this enzyme in different human livers (Mcmanus *et al.*, 1988; Ikeya *et al.*, 1989; Schweikl *et al.*, 1993), and a test for the catalytic activity of CYP1A2 is desirable to estimate an individual's capacity to activate carcinogens. Caffeine has been used as such an *in vivo* probe for CYP1A2 (Fuhr & Rost, 1994), based on data obtained using immuno-correlation, selective inhibition and cDNA expression which indicated that CYP1A2 is the first and rate limiting step in the metabolism of caffeine (Butler *et al.*, 1992; Kalow & Tang, 1993). Because of the general acceptance and low toxicity of caffeine, it is more commonly used than other *in vivo* probes such as phenacetin and theophylline. However, direct *in vivo* evidence that CYP1A2 is involved in the metabolism of caffeine is lacking. We recently developed a mouse lacking CYP1A2 by means of gene targeting (Pineau *et al.*, 1995). This mouse model can be used to prove the involvement of CYP1A2 in the elimination of caffeine, avoiding the inherent *in vivo* problem of specificity or different pharmacokinetics of inhibitors of this enzyme. Because human and mouse CYP1A2 resemble each other in cDNA derived amino acid sequence (Kimura *et al.*, 1984; Jaiswal *et al.*, 1987), and catalytic activity (Aoyama *et al.*, 1989), mouse CYP1A2 should be a representative model for the human enzyme.

‡ Present address: INRA BP3, Toulouse CEDEX 31931, France.

0960-314X

Materials and methods

Materials

Caffeine and heparin were from Sigma Chemicals Co. (St Louis, MO, USA). A monoclonal antibody (MAb 22) that preferentially recognizes rat CYP1A2 (more than CYP1A1) and polyclonal rabbit antibodies against rat cytochromes were described previously (Chaloupka *et al.*, 1994; Gonzalez *et al.*, 1986a, b; Nagata *et al.*, 1987; Yamano *et al.*, 1989). A goat-polyclonal anti-rat CYP2E1 antibody was obtained from Gentest Corp. (Woburn, MA). All other chemicals were of a suitable grade available from commercial sources.

Animals

CYP1A2 −/− mice were produced as described (Pineau *et al.*, 1995). Of the first 599 homozygous CYP1A2 −/− pups born, only nine survived to a fertile age and beyond. The other mice died shortly after birth due to respiratory distress syndrome. The offspring of these survivors produced normal litters (about eight per litter) and were used to establish our CYP1A2 −/− mouse line. All mice were kept in a barrier facility with 2 ppm chlorinated water, autoclaved food and bedding under a 12 h light-dark cycle, 40% humidity with free access to food and water. The genotypes of the animals were confirmed by southern blot analysis of tail DNA as described (Laird *et al.*, 1986).

Study design

Blank 24 h urine was collected from 7–9 week-old, age matched male animals in individual mice metabolic cages (Jencons, Leighton Buzzard, England). Urinary volume was determined by weight. The cages were rinsed with water and the diluted urine was evaporated in a vacuum centrifuge to about the original volume, and stored at −80 °C. Three days later, caffeine was dosed 2 mg kg^{-1} by intraperitoneal injection (i.p.) in phosphate buffered saline solution and the urines were collected as described above. After a washout period of 3 days, caffeine was again dosed i.p. and eight sequential blood samples per mouse of about 30 μl each were drawn suborbitally. In the CYP1A2 −/− mice, samples were drawn at 0 (before injection), 5, 15, 45, 90, 150, 250, and 400 min. In the wild-type mice, samples were drawn at 0, 5, 15, 30, 45, 60, 90, and 120 min. The blood was collected in heparinized tubes and stored at −80 °C until analysis. After a recovery period of 2 days, the mice were killed by carbon dioxide asphyxiation and the livers were perfused for 1 min with ice-cold phosphate buffered saline at 1.5 ml min^{-1} (mouse liver blood flow) using a peristaltic pump, and immediately frozen in liquid nitrogen. Microsomes were prepared by homogenizing livers in a glass-teflon homogenizer in 0.1 M KCl followed by centrifugation for 10 min at 900 × **g** and subsequently 20 min at 10 000 × **g**. The supernatant was then spun for 12 min at 400 000 × **g**. The microsomes were resuspended in 0.1 M phosphate buffer pH 7.4, P450 content was determined (Omura & Sato, 1964) and the microsomes were stored at −80 °C. SDS-PAGE was done as described (Laemmli, 1970) using 10% SDS-polyacrylamide gels. The proteins were transferred to nitrocellulose (Schleicher and Schuell, Keene, NH) and the blots were developed using specific anti-P450 antibodies and secondary antibodies coupled with horseradish peroxidase for enhanced chemiluminescence detection (ECL, Amersham, Arlington Heights, IL). Protein concentration was determined with bichinchoninic acid using bovine serum albumin as a standard (Smith *et al.*, 1985).

HPLC analysis

Caffeine in whole blood and urinary metabolites were analysed by HPLC with UV detection (Tang *et al.*, 1994). Briefly: 20 μl of whole blood was mixed with 5 μl of 10% acetic acid and 0.1 ml of acetonitrile containing 50 μl of internal standard (7-β-hydroxypropyl theophylline). The mixture was vortexed and centrifuged, and 80 μl of the supernatant was injected onto an Ultrasphere IP ODS column (5 μm, 25 × 4.6 cm, Beckman Instruments, Inc., CA). To 0.1 ml urine, 25 μl of internal standard solution (1.2 mg of *N*-acetyl-p-aminophenol in 10 ml of 50% isopropanol : water) and 3 ml of dichloromethane : isopropanol (90 : 10, v/v) were added. The mixture was vortexed for 30 s and the organic phase was separated and dried. The residue was dissolved in 0.25 ml of the HPLC mobile phase (1.3% isopropanol, 0.1% acetonitrile, 0.05% acetic acid) and 50 μl was injected onto the same column as mentioned above. Caffeine and its metabolites were eluted at 1 ml min^{-1} and detected at 280 nm. Biochemical plasma parameters were determined by Diagnostic Services and Clinical Pathology with a Kodak Ektachem 250 automated plasma analyser at the laboratory of the Uniformed Services University of the Health Sciences (Bethesda, MD).

Pharmacokinetic data were calculated using the equation:

$$Cl = D \cdot F/AUC$$

in which Cl is whole blood clearance, D is doses of caffeine, and AUC is area under the curve (model independent pharmacokinetics). F was assumed unity because the oral availability of caffeine is complete (Vozeh *et al.*, 1990). The AUC was calculated with the linear trapezoidal rule using $C = 0$ and $t = 0$

Table 1. Characteristics of male CYP1A2 −/− and wild-type mice. Mean ± SD are given

	Unit	n[b]	*CYP1A2 −/−*	*Wild type*
Physiological[a]				
Body weight	g	8/6	35.2 ± 3.4**	27.1 ± 2.4
Urine volume	ml per 24 h	8/6	1.7 ± 0.8	2.7 ± 1.5
Urinary pH[a]		6/6	6.2 ± 0.2	6.1 ± 0.2
Feces volume	g per 24 h	8/6	0.14 ± 0.12	0.20 ± 0.18
Liver weight	g	8/6	1.0 ± 0.2	1.1 ± 0.2
Total liver protein	mg g^{-1} liver	8/6	162 ± 23	169 ± 22
P450	nmol mg^{-1}	8/6	0.63 ± 0.11	0.55 ± 0.12
Biochemical[a]				
Sodium	meq l^{-1}	10/7	167 ± 18*	195 ± 30
Potassium	meq l^{-1}	10/8	9.5 ± 1.1	11.0 ± 2.3
Glucose	mg dl^{-1}	10/8	218 ± 43	230 ± 62
Creatinine	mg dl^{-1}	10/6	0.18 ± 0.04	0.18 ± 0.08
Albumin	g dl^{-1}	4/4	2.7 ± 0.4	2.7 ± 0.4
Total blood protein	g dl^{-1}	10/8	5.5 ± 0.5	6.2 ± 1.0
Total bilirubin	mg dl^{-1}	10/6	0.56 ± 0.08	0.63 ± 0.05
Alkaline Phosphatase	U l^{-1}	3/4	205 ± 60	172 ± 70
AST	U l^{-1}	3/4	208 ± 86	244 ± 22
ALT	U l^{-1}	3/4	99 ± 36	93 ± 12
LDH	U l^{-1}	4/4	2935 ± 1100	3815 ± 88
Cholesterol	mg dl^{-1}	10/8	108 ± 19	107 ± 19

[a] Values of age matched animals not used in the caffeine experiments. For some animals, not enough plasma was obtained to measure all parameters.
[b] Number of knockout versus wild-type animals.
* $p < 0.05$; ** $p < 0.01$. AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactic dehydrogenase.

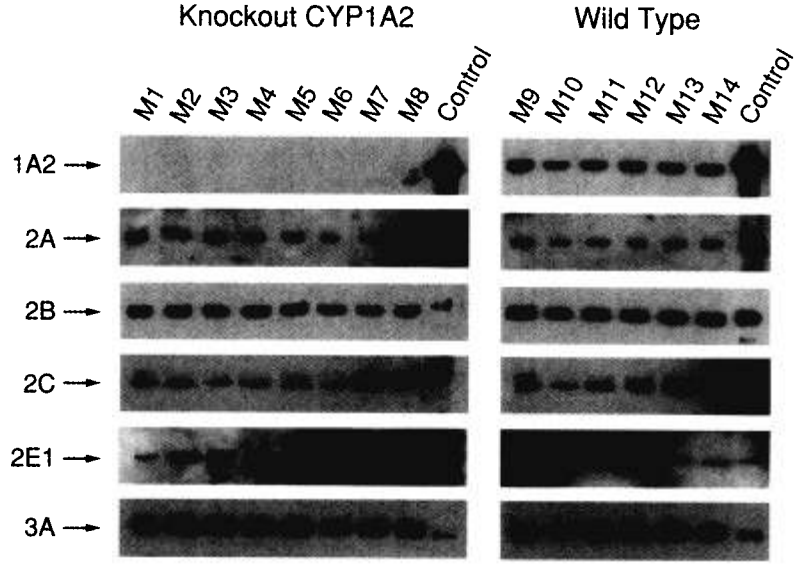

Fig. 1. Analysis of P450 expression levels in male CYP1A2 −/− and wild-type mice. Ten μg of liver microsomal protein were separated by electrophoresis on 10% SDS-PAGE and blotted onto nitrocellulose. The antibodies were incubated with the blots and probed with a secondary antibody coupled to horseradish peroxidase and detected using enhanced chemiluminescence. Microsomes (0.5–3 μg) from rat liver were used as positive control (induced with methylcholanthrene for CYP1A2, phenobarbital for CYP2B, acetone for CYP2E1, dexamethasone for CYP3A, and uninduced microsomes for CYP2A and CYP2C).

Table 2. Pharmacokinetic parameters of caffeine elimination from whole blood after 2 mg kg^{-1} i.p. dosage in male CYP1A2 −/− and wild-type mice. Mean ± SD are given

		CYP1A2 −/− n = 8	*Wild type* n = 6
Half-life	(min)	184 ± 60***	25 ± 15
Clearance/F[a]	(ml min^{-1} kg^{-1})	4.6 ± 1.0***	37.8 ± 11.3
$AUC_{0\rightarrow\infty}$[b]	(μmol min l^{-1})	2372 ± 649***	292 ± 78

[a] Caffeine resorption from i.p. dosing is considered complete, thus F is unity.
[b] Caffeine concentration at t = 0 was treated as zero.
*** $p < 0.001$.

and was extrapolated to infinity using the computer program TOPFIT (Gödecke AG, Freiburg, Germany). The half-life of caffeine in blood was calculated by linear regression of the log transformed blood concentrations.

Statistical analysis

Comparisons between groups were done with Student's *t*-test (Lorenz, 1989).

Results and discussion

CYP1A2 −/− mice weighed 30% more than wild-type control animals (Table 1, $p < 0.001$). Liver homogenate protein content per g liver and liver weights were the same for CYP1A2 −/− and wild-type mice (Table 1). Total microsomal P450 per mg protein was not different between CYP1A2 −/− 0.63± 0.11 nmol mg^{-1} protein) and wild-type mice (0.55 ± 0.12 nmol mg^{-1} protein) and was similar to values reported by others (0.69 ± 0.18 nmol mg^{-1} protein) (Aubrecht *et al.*, 1995). From the biochemical parameters measured in plasma (of age matched groups not used in the caffeine experiments), only the sodium content was significantly lower in the CYP1A2 −/− animals ($p < 0.05$). All parameters that might have had an impact on the pharmacokinetics of caffeine such as albumin (for protein binding), creatinine (for kidney function), or bilirubin, alanine transaminase (ALT), aspartate transaminase (AST) (for liver function (Sherlock, 1989)), were not different between CYP1A2 −/− and wild-type mice. No difference was also noticed for the expression of other P450s CYP2A, 2B, 2C, 2E1 and 3A as determined by Western blotting (Fig. 1).

Pharmacokinetic parameters

Mice can be dosed with at least 100 mg kg^{-1} caffeine (Bonati *et al.*, 1985). However, we observed non-linear pharmacokinetics at a dose of 10 mg kg^{-1} i.p. in CYP1A2 −/− females (data not shown). Since a 170 ml cup of coffee contains about 120 mg caffeine (Anonymous, 1995), equaling a dose of about 2 mg kg^{-1} in an average individual, we used 2 mg kg^{-1} (about 10 μM peak plasma concentration) to mimic 'physiological' concentrations. The half-life of caffeine elimination from blood was 74 times longer and the clearance was 8.2 times slower in the CYP1A2 −/− mice as compared to wild-type mice (Table 2). The AUC was also increased 8.1 times in the CYP1A2 −/− mice (the extrapolated area under the curve consisted of 12 ± 6% in the wild-type mice and was 28 ± 9% in the CYP1A2 −/− mice, $p < 0.01$). The concentration at t = 5 min was 7.6 ± 1.5 μM in the CYP1A2 −/− and 7.42 ± 4.5 μM in the wild-type mice, suggesting that there is no difference in volume of distribution per kg (Fig. 2). Because no parameter determining the pharmacokinetics of caffeine other than the lack of expression of CYP1A2 was found to differ between wild-type mice and CYP1A2 −/− mice, we conclude that 87% of the caffeine clearance in wild-type mice is mediated by this enzyme (clearance wild-type minus clearance CYP1A2 −/− divided by clearance wild-type).

Urinary metabolite profile

Of the administrated dose of caffeine, about 30% was recovered in urine (Table 3). The average sum of 3-demethylated xanthines in the CYP1A2 −/− mice (metabolites believed to be predominantly generated by CYP1A2) was only 40% of the amount found with wild-type mice. concomitantly, the sum of all other xanthines (not 3-demethylated) was increased by 207%. The commonly used caffeine metabolic ratio (AFMU + 1X + 1U)/1,7U to assess the involvement of CYP1A2 in caffeine metabolism (Kalow & Tang, 1993) was 5.6 ± 2.4 in CYP1A2 −/− and 2.6 ± 1.4 in the wild-type mice (AFMU is a metabolite only found in primates and was not detected in our mice urines). Of interest is the finding of the 7-demethylated product from 1,7 dimethylxanthine

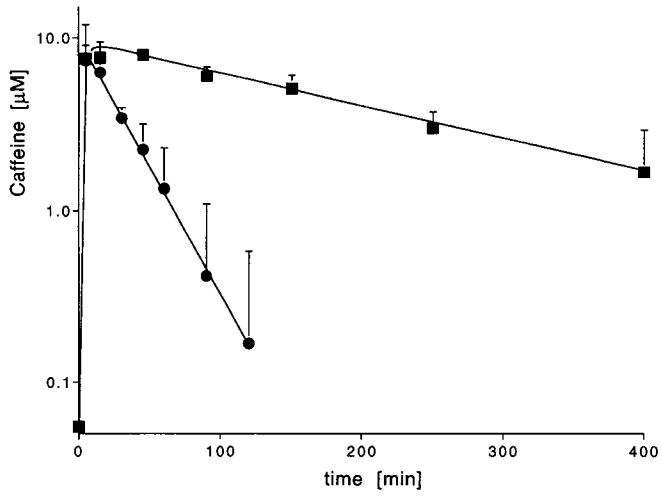

Fig. 2. Whole blood caffeine elimination in male CYP1A2 −/− (■) and wild-type (●) mice. Caffeine was dosed at 2 mg kg^{-1} i.p. and eight sequential blood samples of each 30 µl for each mouse were drawn suborbitally and analysed by HPLC. Mean ± SD are given. Samples with no detectable amounts of caffeine were treated as zero.

Table 3. Urinary recovery of caffeine metabolites after a 2 mg kg^{-1} i.p. dose to male CYP1A2 −/− and wild type mice. Mean [molar % of dose] ± SD are given

	CYP1A2 −/− n = 8	*Wild type* n = 6
3-Demethylated		
1,7 X	1.4 ± 1.3	5.6 ± 4.7
1,7 U	1.1 ± 0.4*	5.1 ± 3.7
1 X	3.0 ± 1.3	5.4 ± 4.0
1 U	2.2 ± 1.5***	5.1 ± 0.6
7 X	1.0 ± 0.6	0.4 ± 0.5
Sum	8.7 ± 2.3**	21.7 ± 4.6
Not 3-demethylated		
1,3 X	Below	Below[a]
1,3 U	6.0 ± 2.2**	1.8 ± 0.5
3,7 X	9.0 ± 3.1**	4.3 ± 1.2
3 X	0.6 ± 0.6	Below[b]
Sum	15.5 ± 4.6**	7.5 ± 3.5[c]
Total	24.2 ± 5.8	29.1 ± 11.4

[a] Only detectable in one wild type animal at 4.9%.
[b] Only detectable in another wild type animal at 3.7%.
[c] Without these two animals the sum was 6.0 ± 1.6%.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

(paraxanthine to 1-methylxanthine) in the CYP1A2 −/− mice as this product was previously thought to be formed almost exclusively by CYP1A2 (Kalow & Tang, 1993) showing that other enzymes are also capable of forming these metabolites.

These results clearly demonstrate that 87% of the elimination of caffeine at physiological concentrations is due to CYP1A2. Further, the pharmacokinetic parameters varied at least seven-fold between CYP1A2 −/− and wild-type mice whereas the urinary caffeine metabolic ratio varied only two-fold. Therefore, we conclude that whole blood elimination of caffeine is more indicative of CYP1A2 catalytic activity than the urinary profile. The possibility of salivary sampling of caffeine and the simple one compartment pharmacokinetics of caffeine (Jost *et al.*, 1987) makes non-invasive, one sample analysis of pharmacokinetic parameters of caffeine feasible and indeed, more predictive than the urinary metabolite pattern (Fuhr & Rost, 1994). This is also exemplified by the presence of caffeine metabolites that previously were assumed to be characteristic for CYP1A2 in the urines of CYP1A2 −/− mice.

Acknowledgement

The help of Joyce Mordenti in evaluating the pharmacokinetic parameters is greatly appreciated.

References

Anonymous. What you should know about caffeine. Washington DC: International Food Information Council Foundation, 1995.

Aoyama T, Gonzalez FJ, Gelboin HV. Human cDNA-expressed cytochrome P450 1A2: mutagen activation and substrate specificity. *Molec Carcinogen* 1989: **2**, 192–198.

Aubrecht J, Hirsch-Ernst KI, Becker-Rabbenstein V, Kahl GF, Taniguchi H, Hoehne MW. Induction of cytochrome P-4502B1-related mouse cytochrome P-450 and regulation of its expression by epidermal growth factor/transforming growth factor α in primary hepatocyte culture. *Biochem Pharmacol* 1995: **50**, 781–785.

Bonati M, Latini R, Tognoni G. Interspecies comparison of *in vivo* caffeine pharmacokinetics in man, monkey, rabbit, rat, and mouse. *Drug Metabol Rev* 1985: **15**, 1355–1383.

Boobis AR, Lynch AM, Murray S, de la Torre R, Solans A, Farre M, Segura J, Gooderham NJ, Davies DS. CYP1A2-catalyzed conversion of dietary heterocyclic amines to their proximate carcinogen is their major route of metabolism in humans. *Cancer Res* 1994: **54**, 89–94.

Butler MA, Lang NP, Young JF, Caporaso NE, Vineis P, Hayes RB, Teitel CH, Massengill JP, Lawsen MF, Kadlubar FF. Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. *Pharmacogenetics* 1992: **2**, 116–127.

Chaloupka K, Santostefano M, Goldfarb IS, Liu G, Myers MJ, Tsyrlov IB, Gelboin HV, Krishnan V, Safe S. Aryl hydrocarbon (Ah) receptor-independent induction of *Cyp1a2* gene expression by acenaphtylene and related compounds in B6C3F1 mice. *Carcinogenesis* 1994: **15**, 2835–2840.

Eaton DL, Gallagher EP, Bammler TK, Kunze KL. Role of

cytochrome P4501A2 in chemical carcinogenesis: implications for human variability in expression and enzyme activity. *Pharmacogenetics* 1995: **5,** 259–274.

Fuhr U, Rost KL. Simple and reliable CYP1A2 phenotyping by the paraxanthine/caffeine ratio in plasma and in saliva. *Pharmacogenetics* 1994: **4,** 109–116.

Gonzalez FJ, Kimura S, Song BJ, Pastewka J, Gelboin HV, Hardwick JP. Sequence of two related P-450 mRNAs transcriptionally increased during rat development. *J Biol Chem* 1986a: **261,** 10667–10672.

Gonzalez FJ, Song BJ, Hardwick JP. Pregnenolone 16α-carbonitrile-inducible P-450 gene family: gene conversion and differential regulation. *Mol Cell Biol* 1986b: **6,** 2969–2976.

Ikeya K, Jaiswal AK, Owens RA, Jones JE, Nebert DW, Kimura S. Human *CYP1A2*: sequence, gene structure comparison with the mouse and rat orthologous gene, and difference in liver 1A2 mRNA expression. *Molec Endocrinol* 1989: **3,** 1399–1408.

Jaiswal AK, Nebert DW, McBride OW, Gonzalez FJ. Human P(3)450: cDNA and complete protein sequence, repetitive Alu sequences in the 3′ nontranslated region, and localization of gene to chromosome 15. *J Exp Pathol* 1987: **3,** 1–17.

Jost G, Wahllaender A, von Mandach U, Preisig R. Overnight salivary caffeine clearance: A liver function test suitable for routine use. *Hepatology* 1987: **7,** 338–344.

Kalow W, Tang BK. The use of caffeine for enzyme assays: A critical appraisal. *Clin Pharmacol Ther* 1993: **53,** 503–514.

Kimura S, Gonzalez FJ, Nebert DW. Mouse cytochrome P3-450: complete cDNA and aminoacid sequence. *Nucleic Acids Res* 1984: **12,** 2917–2928.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970: **227,** 680–685.

Laird PW, Zijderveld A, Linders K, Rudnicki MA, Jaenisch R, Berns A. Simplified mammalian DNA isolation procedure. *Nucleic Acids Res* 1986: **19,** 4293–4295.

Lorenz RJ, Biometrie, Grundbegriffe der Biometrie, 2 edition. Stuttgart: Gustav Fischer Verlag, 1989.

Mcmanus ME, Stupans I, Ioannoni B, Burgess W, Robson RA, Birkett DJ. Identification and quantitation in human liver of cytochromes P-450 analogous to rabbit cytochromes P-450 forms 4 and 6. *Xenobiotica* 1988: **18,** 207–216.

Nagata K, Matsunaga T, Gillette J, Gelboin HV, Gonzalez FJ. Rat testosterone 7α-hydrolase: isolation, sequence, and expression of cDNA and its developmental regulation and induction by 3-methylcholanthrene. *J Biol Chem* 1987: **262,** 2787–2793.

Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J Biol Chem* 1964: **239,** 2370–2385.

Pineau T, Fernandez-Salguero P, Lee SST, Mcphail T, Ward JM, Gonzalez FJ. Neonatal lethality associated with respiratory distress in mice lacking cytochrome P450 1A2. *Biochemistry* 1995: **92,** 5134–5138.

Schweikl H, Taylor JA, Kitareewan S, Linko P, Nagorney D, Goldstein JA, Expression of *CYP1A1* and *CYP1A2* genes in human liver. *Pharmacogenetics* 1993: **3,** 239–249.

Sherlock S. Diseases of the liver and biliary system, 8 edition London: Blackwell Scientific Publications, 1989.

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. Measurement of protein using bichinchoninic acid. *Anal Biochem* 1985: **150,** 76–85.

Tang BK, Zhu Y, Kadar D, Kalow W. Caffeine as a probe for CYP1A2 activity: potential influence of renal factors on urinary phenotypic trait measurements. *Pharmacogenetics* 1994: **4,** 117–124.

Vozeh S, Schmidlin O, Taeschner W. Pharmacokinetic drug data. In: Mammen G ed. Clinical Pharmacokinetics. Drug data handbook 1990, 2nd edition. Hong Kong: ADIS Press, 1990: 6–30.

Yamano S, Nhamburo PT, Aoyama T, Meyer UA, Inaba T, Kalow W, Gelboin HV, McBride OW, Gonzalez FJ. cDNA cloning and sequence, and cDNA-directed expression of human P450 2B1: identification of a normal and two variant cDNAs derived from the *CYP2B* locus on chromosome 19 and differential expression of 2B mRNAs in human liver. *Biochemistry* 1989: **28,** 7340–7348.