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Simple and reliable CYP1A2 phenotyping by the paraxanthine/caffeine ratio in plasma and in saliva

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Several procedures to monitor CYP1A2 activity *in vivo* by the use of caffeine as a probe have been proposed. They comprise caffeine clearance, based on both plasma and saliva concentrations, urinary metabolite ratios, the ^{13}C-caffeine breath test, and the paraxanthine/caffeine ratio in plasma. The latter method is fast, simple, economical and restricted to one sampling point. In this study, we retrospectively analysed four clinical trials comprising 78 subjects to validate the use of the paraxanthine/caffeine ratios in plasma and saliva for CYP1A2 activity. The validation was done by correlation of these ratios to the systemic caffeine clearance as a reference method. Additionally, urinary metabolite ratios and the caffeine breath test were included in the analysis. The paraxanthine/caffeine ratios in plasma and saliva preferably 5–7 h after administration of caffeine most closely resembled systemic caffeine clearance with correlation coefficients typically higher than $r = 0.85$. An equation to estimate systemic caffeine clearance from the paraxanthine/caffeine ratios taken at any time within 3–7 h postdose was developed. Correlations of systemic clearance with urinary metabolite ratios and the caffeine breath test were less reliable both in this investigation and in the literature. In conclusion, the paraxanthine/caffeine ratios in plasma and saliva appear a valid and inexpensive method of assessing CYP1A2 activity *in vivo*. Apparent distribution of CYP1A2 activity for all healthy subjects appeared bimodal in nonsmokers ($n = 29$) and smokers ($n = 17$).

Introduction

The human cytochrome P450 isoform CYP1A2 is involved in the metabolism of drugs, of environmental toxic compounds, and presumably of endogenous substrates such as uroporphyrinogen (Lambrecht *et al.*, 1992). The inducibility of this enzyme by polycyclic aromatic hydrocarbons is reflected by a two-fold higher activity in smokers (Campbell *et al.*, 1987; Fuhr *et al.*, 1993a).

The current list of drugs known to be metabolized at least in part by CYP1A2 includes caffeine and theophylline (Fuhr *et al.*, 1992; Tassaneeyakul *et al.*, 1992), phenacetin (Sesardic *et al.*, 1988), lidocaine (Imaoka *et al.*, 1990), imipramine (Lemoine *et al.*, 1993), verapamil (Kroemer *et al.*, 1993), and propafenone (Botsch *et al.*, 1993). Since some of these drugs have a narrow therapeutic range, the potential of any drug and ubiquitous foreign compound to induce or inhibit CYP1A2 activity should be characterized to avoid adverse reactions during concomitant administration.

Formation of ultimate carcinogens from polycyclic aromatic hydrocarbons and other compounds, such as imidazoquinoline derivatives or aflatoxin B_1 (Shimada *et al.*, 1988; Kitada *et al.*, 1990; Guengerich, 1991), are also mediated by human CYP1A2. Most of these substances are environmental pollutants. Although individual CYP1A2 activity is expected to influence the risk of exposure, the role of CYP1A2 in carcinogenesis has not yet been established. Epidemiological studies to assess this role necessitate the availability of a non-invasive and simple phenotyping procedure.

To characterize an individual for a cytochrome P450 enzyme, genetic and biochemical methods are used today. Although genetic methods are highly specific, they do not reflect the metabolic consequence of a change in enzyme activity and are currently not available for CYP1A2. For CYP1A2 phenotyping in man, to date caffeine is the only substrate that may be used *in vivo* (Kalow & Tang, 1993). Procedures to

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determine CYP1A2 activity *in vivo* by use of a caffeine test dose comprise systemic caffeine clearance, based on both plasma and saliva concentrations, ratios of urinary metabolites, the ^{13}C-[N3-methyl]-caffeine breath test, and the paraxanthine/caffeine ratio in plasma (Tanaka *et al.*, 1992; Kalow & Tang, 1993). Using urinary metabolite ratios, a polymodal distribution of CYP1A2 activity has been proposed (Butler *et al.*, 1992), that may reflect both environmental and genetic factors.

Any of the methods based on the use of caffeine as a probe has potential shortcomings in CYP1A2 phenotyping. As a general problem, caffeine metabolism may be influenced by the activity of other enzymes, mainly of CYP1A1 and CYP2E1, which also are supposed to contribute to primary caffeine metabolism *in vivo* (Gu *et al.*, 1992; Tassaneeyakul *et al.*, 1992). However, at concentrations observed *in vivo*, CYP1A2 is expected to mediate more than 90% of primary caffeine metabolism (Fuhr *et al.*, 1992; Tassaneeyakul *et al.*, 1992), at least in subjects without pronounced induction of the other enzymes mentioned above. Ratios of caffeine metabolites in urine are either based on secondary or tertiary metabolites, which are not exclusively formed by CYP1A2, or they might strongly vary due to variable urinary flow, interethnic differences in renal function, and by use of differing sampling protocols (Kalow & Tang, 1993). The pulmonary excretion of $^{13}CO_2$ out of a labelled caffeine dose in the caffeine breath test depends on the respiratory quotient and thus on physical activity and diet (Lambert *et al.*, 1983). Therefore, systemic caffeine clearance derived from plasma concentrations appears to reflect CYP1A2 activity better than the other parameters mentioned and is currently established as an appropriate reference for CYP1A2 phenotyping (Kalow & Tang, 1993).

We recently compared intraindividually the caffeine metabolism *in vivo* of 25 patients (see Table 1, study III) undergoing partial hepatectomy for removal of cancer metastasis with CYP1A2 activity *in vitro* (Fuhr *et al.*, 1993b). The *in vitro* activity was measured as the intrinsic clearance for caffeine 3-demethylation at the high affinity site in microsomes prepared from a small unaffected liver sample. The values were extrapolated to the whole amount of enzyme in the liver by estimating tumour-free liver volume using computed tomography. The coefficient of correlation for weighted linear regression between this value and systemic caffeine clearance following application of a 165 mg caffeine test dose was $r = 0.801$ ($n = 18$ data pairs available). Those for correlation between CYP1A2 activity *in vitro* and the paraxanthine/caffeine ratios 3 and 6 h postdose were $r = 0.702$ and $r = 0.777$ ($n = 18$) in plasma and $r = 0.700$ and $r = 0.734$ in saliva samples ($n = 17$), respectively. These results prompted us to further investigate the paraxanthine/caffeine ratios in plasma or in saliva. Paraxanthine, the major metabolite which accounts for 80% of primary caffeine metabolism (Fuhr *et al.*, 1992), and the parent compound caffeine can be measured by simple, accurate and time-saving HPLC methods. In contrast to urinary metabolic ratios or to the caffeine breath test, these methods do not require sample extraction procedures, expensive synthesis of reference substances or labelled compounds, or sophisticated technical equipment. Furthermore, long collection periods can be replaced with a one-point sample. As an additional benefit, saliva sampling is non-invasive.

Thus, if the paraxanthine/caffeine ratios in plasma or saliva are demonstrated as valid parameters of CYP1A2 activity, a simple, fast and economical method would be available. However, there is only one report on paraxanthine/caffeine ratios in plasma or saliva in man, which refers to the use of the plasma ratio 4 h postdose to monitor hepatic function in patients (Tanaka *et al.*, 1992). Therefore, in this study we would like to present further evidence for the reliability of these ratios for human CYP1A2 phenotyping.

Materials and methods

The molar paraxanthine/caffeine ratios presented in this investigation are derived from four clinical studies, which have, in part, been published previously (Fuhr *et al.*, 1993a, b; Rost & Roots, 1994). All studies were conducted in accordance to the Declaration of Helsinki and with the approval of an Ethics Committee. All volunteers gave their written informed consent. In these studies, either caffeine plasma and saliva clearance or caffeine breath test and plasma clearance were used to monitor CYP1A2 activity. We now retrospectively evaluated the paraxanthine/caffeine ratios out of the kinetics of these studies.

The design of the four clinical studies is given in Table 1. The pharmacokinetic properties of caffeine allow a reliable determination of the clearance and other pharmacokinetic parameters with only a few data points of the concentration time profile (Kalow & Tang, 1993). Thus, a metaanalysis of the four studies is possible despite the differences in design.

Overnight abstinence for methylxanthines was requested, but not all subjects adhered to this rule. Therefore, predose values were taken into account when calculating clearance (Fuhr *et al.*, 1993a; Rost & Roots, 1994).

Table 1. Characterization of clinical studies used for retrospective evaluation of the paraxanthine/caffeine ratios

	Study I (*unpublished*)	*Study II* (*Fuhr* et al., *1993a*)	*Study III* (*Fuhr* et al., *1993b*)	*Study IV* (*Rost & Roots, 1994*)
Subjects	24 healthy volunteers	12 healthy volunteers	25 liver tumour patients	18 healthy volunteers
sex	All male	7 f/5 m	14 f/11 m	7 f/11 m
age (mean ± SD)	29 ± 4	34 ± 13	56 ± 11	42 ± 68
weight (mean ± SD)	77 ± 9	68 ± 15	72 ± 14	68 ± 10
smoking habits	11S/13NS	6S/6NS	5S/20NS	2S/16NS
Period A	Without comedication	Without comedication	Within 2 days prior to surgery, with multiple comedications	Without comedication
Period B	—	Coadministration of grapefruit juice	Approximately 1 week after surgery, with multiple comedications	Coadministration of 40—120 mg omeprazole per day
Caffeine dose	150 mg	appr. 165 mg	appr. 165 mg	3 mg/kg
Blood sampling	Predose and 3, 6, 9, 12 h postdose	Predose and 2, 5, 9, 24 h postdose	Predose and 3, 6, 9 h postdose	Predose and 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 9, 12, 24 h postdose
Saliva sampling	—	Predose and 1, 2, 3, 5, 7, 9, 12, 15, 18, 24, 30, 36 h postdose	Predose and 2, 3, 6, 9, 12, 15 h postdose	—
Urine sampling	—	spontaneous urine 5 hours postdose	spontaneous urine 6 hours postdose	urine collected 6—8 hours postdose
Parameters determined	Cl_P	Cl_P	Cl_P	Cl_P
	—	Cl_S	Cl_S	—
	R_P 3 and 6 h postdose	R_P 5 h postdose	R_P 3 and 6 h postdose	R_P 3 and 6 h postdose
	—	R_S 3, 5 and 7 h postdose	R_S 3 and 6 h postdose	—
	—	Urinary ratios	Urinary ratios	Urinary ratios
	—	—	—	^{13}C-caffeine breath test

Abbreviations used: S, smoker; NS, nonsmoker; Cl, clearance; R, ratio paraxanthine/caffeine; P, plasma concentrations; S, saliva concentrations

The HPLC procedures used to quantitate caffeine and its metabolites have been described elsewhere (plasma and saliva: Fuhr *et al.*, 1993a; Rost & Roots, 1994; urine: Rost & Roots, 1994). Plasma sample preparation in studies I, II and III, and saliva sample preparation in study III was by protein precipitation using 20% trichloroacetic acid. Saliva samples in study II were extracted using diisopropyl ether/isopropanol. All urine samples and all samples in study IV were prepared by extraction with chloroform/isopropanol. In all studies, caffeine and its metabolites were separated by C18-reversed phase HPLC and quantitated using UV absorption at 278 or 280 nm. Relevant interference of caffeine metabolite peaks in urine with other peaks was checked by measuring samples of individuals who were methylxanthine abstinent for 72 h. Additional evaluation of peak purity during measurement of samples from subjects was not carried out.

Four different urinary metabolite ratios were determined in studies II, III and IV:

(a) $\frac{17X}{137X}$ (Kadlubar *et al.*, 1990

(b) $\frac{17X + 17U}{137X}$ (Butler *et al.*, 1992)

(c) $\frac{1X + 1U + AFMU}{17U}$ (Campbell *et al.*, 1987)

(d) $\frac{1X + 1U + AFMU + AAMU}{17U}$

(modified from Kalow & Tang, 1991)

Abbreviations used: 17X, 1,7-dimethylxanthine (paraxanthine); 137X, 1,3,7-trimethylxanthine (caffeine); 17U, 1,7-dimethyluric acid; 1X, 1-methylxanthine; 1U, 1-methyluric acid; AFMU, 5-acetylamino-6-formylamino-3-methyluracil; AAMU, 5-acetylamino-6-amino-3-methyluracil.

The correlations between systemic caffeine clearance (ml min^{-1} kg^{-1} body weight), derived from plasma concentrations, and other CYP1A2 parameters (denoted as y) were estimated by linear regression using equation (1).

$$y = \text{intercept} + \text{slope} \cdot \text{clearance} \quad (1)$$

Dependent variables were weighted by their inverse values to avoid a strong influence of extremes on correlation coefficients.

The values for the paraxanthine/caffeine ratios increased with the time (h) after caffeine administration. Therefore, we included postdose time into the calculation of correlation and pooled all data from different time points and from all studies. The explorative correlation was calculated separately for plasma and for saliva ratios using equation (2)

$$\text{ratio} = a + b1 \cdot \text{clearance} + b2 \cdot \text{time} + b3 \cdot \text{clearance} \cdot \text{time} \quad (2)$$

Multiple stepwise linear regression (Armitage & Berry, 1987) was carried out to eliminate those variables that did not make a significant ($p < 0.05$) contribution to the correlation.

Finally, the two equations obtained, one for the plasma ratio and one for saliva, were combined. To this end, the intercept a was replaced by $(a + f \cdot \delta a)$. The value of f, which was used to discriminate between plasma and saliva, was 0 for plasma ratios and 1 for saliva ratios. Thus, a was the intercept for plasma ratios, and $(a + \delta a)$ was the intercept for saliva ratios. The same procedure was applied to slopes. After this transformation, all ratios could be included into one equation. Multiple stepwise linear regression analysis was again used to eliminate those differences between saliva and plasma ratios that did not significantly influence the correlation.

Reproducibility of the paraxanthine/caffeine ratios between different caffeine tests could not be determined retrospectively since all subjects took part only once in a test under the same conditions, e.g. without comedication. As an explorative estimation of reproducibility of the ratios within one caffeine test, those obtained for each individual at different postdose times and in different matrices in studies II and III (altogether 4 ratios per subject and per period) were transformed, using equation (5) (see Results), to clearance values for which the coefficient of variation (%) was calculated as $100 \cdot SD/mean$.

To compare the distribution of CYP1A2 activity in our subjects as determined by systemic caffeine clearance and as derived from the metabolic ratio, both values were displayed as histograms. Only healthy subjects (studies I, II, IV) without comedication (period A) were evaluated. Histograms were plotted separately for nonsmokers ($n = 29$) and smokers (defined as cigarette consumption of at least one per day; actual consumption was 14 ± 7 cigarettes per day [mean $\pm$ SD], $n = 17$). We used the paraxanthine/caffeine plasma ratios 5 or 6 h after the caffeine test dose, which were transformed to clearance values using equation (5) (see Results) to enable a better comparability.

Results

In all studies systemic caffeine plasma clearance, which ranged from 0.3 to 3.3 ml min^{-1} kg^{-1} was used as reference method to validate the paraxanthine/caffeine ratios. The correlation with other parameters as dependent variables are shown in Table 2. Values obtained for caffeine clearance derived from saliva concentrations most closely correlated to the reference method ($r \geqslant 0.947$).

The paraxanthine/caffeine ratios in plasma and in saliva also highly correlated with systemic caffeine clearance. In 14 of 20 correlations calculated for one period of a study, r values exceeded 0.85. In eight of them, they even exceeded 0.9 (see Table 2). Slope and intercept of regression lines were similar between corresponding ratios for the two limbs of each study (not shown). The correlation coefficients of the paraxanthine/caffeine ratios increased with time. They were 0.628–0.949, 3 h postdose and improved to 0.719–0.955, 6–7 h after caffeine intake.

Only in study IV could the r values be calculated with respect to the caffeine breath test with 0.623 and 0.679.

Correlation coefficients for urine ratios ranged from 0.236 to 0.744 for paraxanthine/caffeine, from 0.015 to 0.810 for (17X + 17U)/137X, from 0.271 to 0.703 for (1X + 1U + AFMU)/17U, and from 0.232 to 0.662 for (1X + 1U + AFMU + AAMU)/17U. These coefficients were the lowest of all the tests evaluated and often failed to reach significance (Table 2). Even if the correlation to the reference was significant, slope and intercept of the regression line showed pronounced differences between the different studies and between the two limbs of each study.

Multiple stepwise linear regression calculated explorative between the pooled paraxanthine/caffeine

Table 2. Coefficients for linear correlations between caffeine parameters used for CYP1A2 phenotyping and systemic clearance based on plasma concentrations as reference

Correlation with Cl_P (*ml min*$^{-1}$ *kg*$^{-1}$) *of:*	*Study I* (n = 24)	*Study IIA* (n = 12)	*Study IIB* (n = 12)	*Study IIIA* (n = 16–19)	*Study IIIB* (n = 16–19)	*Study IVA* (n = 10)	*Study IVB* (n = 17)
Cl_S (ml min^{-1}) kg^{-1}	Not done	0.947***	0.975***	0.989***	0.985***	Not done	
R_{P3h}	0.653***	Not done		0.843***	0.949***	0.628 NS	0.629**
R_{P5h}	Not done	0.911***	0.854***	Not done		Not done	
R_{P6h}	0.903***	Not done		0.886***	0.955***	0.869***	0.719***
R_{S3h}	Not done	0.846***	0.747**	0.852***	0.915***	Not done	
R_{S5h}	Not done	0.875***	0.901***	Not done		Not done	
R_{S6h}	Not done	Not done		0.874***	0.950***	Not done	
R_{S7h}	Not done	0.943***	0.910***	Not done		Not done	
Breath test (^{13}C-CO_2 exhaled as % of dose)	Not done	Not done		Not done		0.623 NS	0.679***
Urinary ratios	*Sampling procedure*	*Spontaneous urine 5 h postdose*		*Spontaneous urine 6 h postdose*		*Urine collected 6–8 h postdose*	
$\frac{17X}{137X}$	Not done	0.236 NS	0.204 NS	0.744***	0.671***	0.736*	0.736***
$\frac{17X+17U}{137X}$	Not done	0.471 NS	0.015 NS	0.683***	0.810***	0.698*	0.615**
$\frac{(1X+1U+AFMU)}{17U}$	Not done	0.372 NS	0.271 NS	0.456 NS	0.703***	0.371 NS	0.328 NS
$\frac{(1X+1U+AFMU+AAMU)}{17U}$	Not done	0.332 NS	0.232 NS	0.465 NS	0.662***	0.339 NS	0.408 NS

Cl, clearance; R, ratio paraxanthine/caffeine; P, plasma concentrations; S, saliva concentrations; for urinary ratios, see 'materials and methods'; *n*, number of data pairs available; ***, $p < 0.005$; **, $p < 0.01$; *, $p < 0.05$; NS, not significant.

ratios and systemic caffeine plasma clearance (equation (2)) showed that only intercept (a) and slope of the product of clearance and sampling time (b3) made significant contributions to the correlation between plasma ratios ($n = 196$ data pairs) or saliva ratios ($n = 141$) and clearance (equations (3) and (4)).

$$\text{plasma ratio} = 0.0936 + 0.0761 \cdot \text{clearance}$$
$$(\text{ml min}^{-1}\,\text{kg}^{-1}) \cdot \text{time (h)}\ [r = 0.977] \quad (3)$$

$$\text{saliva ratio} = 0.0581 + 0.0771 \cdot \text{clearance}$$
$$(\text{ml min}^{-1}\,\text{kg}^{-1}) \cdot \text{time (h)}\ [r = 0.976] \quad (4)$$

Likewise, parameters describing the difference between saliva and plasma ratios (δa and δb3) in data pooled from both matrices ($n = 337$) did not significantly contribute to the correlation and were therefore omitted (equation (5)).

$$\text{overall ratio} = 0.0751 + 0.0769 \cdot \text{clearance}$$
$$(\text{ml min}^{-1}\,\text{kg}^{-1}) \cdot \text{time (h)}\ [r = 0.976] \quad (5)$$

The plot of paraxanthine/caffeine ratios versus clearance and postdose time together with the function defined by equation (5) (Fig. 1) shows that residuals are distributed equally, independent of sample matrix and sampling time. The values estimated using this equation reached (mean ± SD) 103.6% ± 30.4% of the measured values for saliva ratios and 97.4% ± 25.3% for plasma ratios.

Reproducibility of the paraxanthine/caffeine based estimation of clearance within one caffeine test is reflected by coefficients of variation (CV), given as mean ± SD for individual CVs of $n = 4$ ratios per test, of 10.9% ± 4.5% and 14.5% ± 5.5% in study II, periods A and B, and 13.0% ± 4.4% and 22.2% ± 22.3% in studies IIIA and IIIB, respectively.

Caffeine clearance appeared not to be either normally or log-normally distributed (Fig. 2), irrespective of the measured values or the values derived from paraxanthine/caffeine ratios were used. A polymodal, presumably bimodal distribution with different cut-off points for smokers and for nonsmokers to distinguish

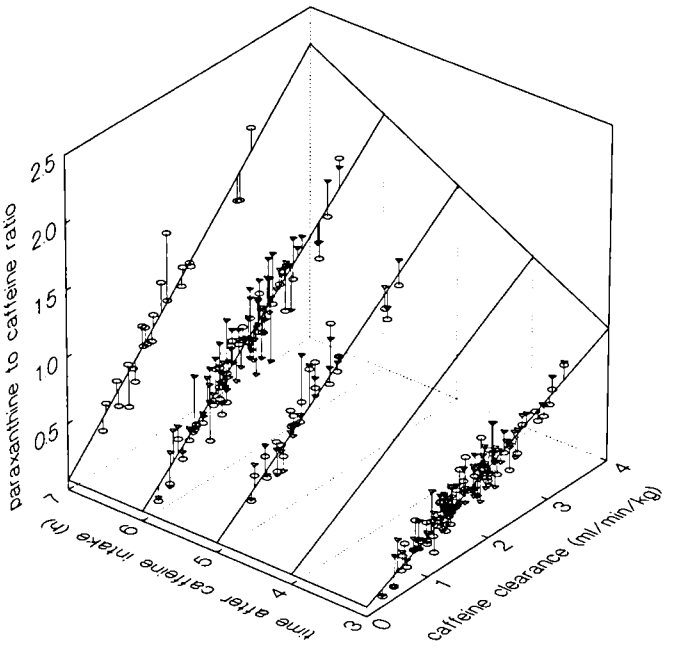

Fig. 1. 3D-plot of relationship between the paraxanthine/caffeine ratio as dependent variable and systemic caffeine clearance ($ml\,min^{-1}\,kg^{-1}$) and time between caffeine dosing and sampling (h) as independent variables. Lines in the 3D space represent the estimated ratios 3, 4, 5, 6 and 7 h after test dose intake, obtained using equation (5). Measured values are shown as triangles (plasma ratios) or circles (saliva ratios), which are connected to the corresponding estimate for easier allocation in the space. The length of the connecting line is the residual of estimation.

between 'poor metabolizers' and 'extensive metabolizers' seems to be compatible with this limited data set.

Discussion

The partial metabolic clearance of caffeine by N3-demethylation was suggested as the 'gold standard' for CYP1A2 phenotyping with caffeine (Kalow & Tang, 1993), but could not be determined retrospectively in the clinical studies used to validate the paraxanthine/caffeine ratios in plasma and saliva. Therefore, systemic caffeine clearance, calculated from plasma concentrations, was used as the reference for CYP1A2 phenotyping. The absolute values, which were not presented, resembled those described in many previous studies on caffeine pharmacokinetics; smokers had a higher clearance than nonsmokers in studies II, III and IV, and those patients with impaired liver function in study III had a low caffeine clearance.

The parameter showing the highest correlation coefficients in comparison with the systemic clearance was the caffeine clearance derived from caffeine saliva concentrations. The elimination process can be accurately described by this parameter because saliva can be seen as an ultrafiltrate of plasma, and caffeine binding to plasma proteins is of minor importance (Campbell *et al.*, 1987, and references therein). The high correlation between systemic and saliva clearance in our study is in good accordance to numerous previous studies (Fuhr *et al.*, 1993, and references therein). They equivocally showed that saliva samples are valid substitutes to replace plasma in determining caffeine pharmacokinetics. Therefore, it is surprising that saliva samples have not been used more often for CYP1A2 phenotyping. This valid non-invasive parameter should have attracted more attention. To our knowledge, there is no report concerning CYP1A2 activity *in vivo*, which is solely based on the use of saliva samples without additional plasma determinations.

The correlations between caffeine clearance and the caffeine breath test (study IV) were lower than those reported (Kalow & Tang, 1993). However, instead of 2 h the breath test was performed for 8 h in study IV.

Urinary metabolite ratios showed the lowest correlation coefficients. Those urinary metabolite ratios comprising only the caffeine 3-demethylation (Kadlubar *et al.*, 1990; Butler *et al.*, 1992) more closely correlated with the caffeine clearance than

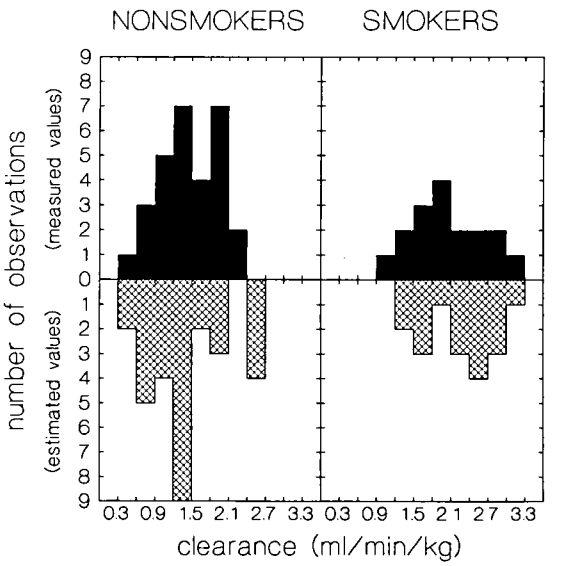

Fig. 2. Histogram of caffeine clearance distribution in healthy subjects without concomitant intake of other compounds. The upper row are clearance values derived directly from the plasma concentration time profile, the lower row clearance values are calculated from the paraxathine/caffeine ratio in plasma 5 or 6 h after caffeine intake using equation (5). Values were available for 29 nonsmokers and 17 smokers (Tobacco consumption 14 ± 7 cigarettes per day [mean $\pm$ SD].)

ratios based on paraxanthine 7-demethylation products (Campbell *et al.*, 1987; Kalow & Tang, 1991). However, sampling of urine in our studies (see Table 1) was different to the procedures recommended recently (Kalow & Tang, 1993). Optimal results are expected when urine sampling is carried out 8 h or more after intake of the test dose for paraxanthine 7-demethylation based ratios and between 4 and 5 h after intake for caffeine 3-demethylation based ratios. This might explain the less valid correlations obtained for urinary ratios in our studies with even differing sampling periods than the r values between 0.7 and 0.9 (Butler *et al.*, 1992; Kalow & Tang, 1993) after standardization against systemic caffeine clearance. But the results of these authors are different. Additionally, quantitation of some of the polar metabolites of caffeine in urine by reversed phase HPLC is related to a variety of major problems like interference with endogenous substances and each other, instability, incomplete extraction, and altered performance after change of the HPLC column or of the liquid phase. Therefore, urinary metabolite ratios do not appear as methods easily reproducible by other investigators. Results from study IV indicate that these methods indeed indicate CYP1A2 activity, but exhibit a higher variability than plasma clearance (Rost & Roots, 1994).

The paraxanthine/caffeine ratios in plasma and in saliva better reflected the systemic caffeine clearance than did urinary ratios in ours as well as in previously published studies by other investigators (see above). The increase in correlation coefficients for the paraxanthine/caffeine ratios with postdose time might be explained by a decreasing influence of dietary intake of caffeine many hours prior to the test. The data presented here were derived from studies without control of overnight caffeine abstinence. Many predose samples contained caffeine and paraxanthine (most of them less than 0.5 mg l^{-1} of each).

The close correlations between paraxanthine/caffeine ratios and systemic caffeine clearance provides further evidence that this parameter is a valid alternative for monitoring CYP1A2 activity. Reproducibility within one caffeine test is documented by a coefficient of variation of approximately 15% for clearance estimated from the paraxanthine/caffeine ratios. Reproducibility between different days, which was not determined here, should also be satisfying due to the correlation with caffeine clearance which itself was reported to have a coefficient of variation of approximately 15% between days (Balogh *et al.*, 1992/3). By using equation (5), it seems possible to estimate caffeine clearance from any single plasma or saliva sample 3–7 (preferably 5–7) h postdose more precisely than by urinary ratios or by the breath test. As a practical advantage, the use of the paraxanthine/caffeine ratios in saliva or plasma implies a considerable reduction of time, money, and equipment needed for CYP1A2 phenotyping. Because the saliva ratio is a non-invasive parameter of CYP1A2 activity, it is most suitable for epidemiological studies.

Although only a small number of subjects was available, a similar polymodal distribution of CYP1A2 activity obtained from plasma clearance and from paraxanthine/caffeine ratios (Fig. 2) supports the use of the latter in epidemiology and is in accordance with Butler *et al.* (1992). The log-normal distribution reported by Kalow & Tang (1991) may be due to inclusion of smokers and nonsmokers and of women with and without oral contraceptives. Both, plasma and saliva paraxanthine/caffeine ratios are suitable screening methods in drug interaction studies.

References

Armitage P, Berry G. Statistical methods in medical research. Oxford: Blackwell Scientific Publications, 1987: 305–307.

Balogh A, Harder S, Vollandt R, Staib AH. Intra-individual variability of caffeine elimination in healthy subjects. *Int J Clin Pharmacol Ther Toxicol* 1992: **30**, 383–387; Erratum: 1993: **31**, 208.

Botsch S, Gautler JC, Beaune P, Eichelbaum M, Kroemer HK. Identification and characterization of the cytochrome P450 enzymes involved in N-dealkylation of propafenone: Molecular base for interaction potential and variable disposition of active metabolites. *Mol Pharmacol* 1993: **43**, 120–126.

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.

Campbell ME, Spielberg SP, Kalow W. A urinary metabolite ratio that reflects systemic caffeine clearance. *Clin Pharmacol Ther* 1987: **42**, 157–165.

Fuhr U, Doehmer J, Battula N, Wölfel C, Kudla C, Keita Y, Staib AH. Biotransformation of caffeine and theophylline in mammalian cell lines genetically engineered for expression of single cytochrome P450 enzymes. *Biochem Pharmacol* 1992: **43**, 225–235.

Fuhr U, Klittich K, Staib AH. Inhibitory effect of grapefruit juice and the active component naringenin on CYP1A2 dependent metabolism of caffeine in man. *Br J Clin Pharmacol* 1993a: **35**, 431–436.

Fuhr U, Engelhardt R, Sachs M, Rost KL, Schymanski P, Huschka C, Liermann D, Wissing H, Staib AH. Human CYP1A2 activity: relationship between caffeine clearance *in vivo* and its 3-demethylation *in vitro*. In: Gulbenkian Institute of Science: Abstract book of the 8th International Conference on Cytochrome P450. Lisbon: 1993b: 202.

Gu L, Gonzalez FJ, Kalow W, Tang BK. Biotransformation of caffeine, paraxanthine, theobromine and theophylline by c-DNA expressed human CYP1A2 and CYP2E1. *Pharmacogenetics* 1992: **2**, 73–77.

Guengerich FP. Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chem Res Toxicol* 1991: **4**, 391–407.

Imaoka S, Enomoto K, Oda Y, Asada A, Fujimori M, Shimada T, Fujita S, Guengerich FP, Funae Y. Lidocaine metabolism by human cytochrome P-450s purified from hepatic microsomes: Comparison of those with rat hepatic cytochrome P-450s. *J Pharmacol Exp Ther* 1990: **55**, 1385–1391.

Kadlubar FF, Talaska G, Butler MA, Teitel CH, Hassengill JP, Lang NP. Determination of carcinogenic arylamine *N*-oxidation phenotype in humans by analysis of caffeine urinary metabolites. In: Mendelsohn ML, Albertini RJ, eds. Mutation and the environment. Part B: Metabolism, testing methods, and chromosomes. New York: John Wiley & Sons, 1990: 107–114.

Kalow W, Tang BK. Use of caffeine metabolite ratios to explore CYP1A2 and xanthine oxidase activities. *Clin Pharmacol Ther* 1991: **50**, 508–519.

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

Kitada M, Taneda M, Ohta K, Nagashima K, Itahashi K, Kamataki T. Metabolic activation of aflatoxin B_1 and 2-amino-3-methylimidazo[4,5-f]-quinoline by human adult and fetal liver. *Cancer Res* 1990: **50**, 2641–2645.

Kroemer HK, Gautier JC, Beaune P, Henderson C, Wolf CR, Eichelbaum M. Identification of P450 enzymes involved in the metabolism of verapamil in humans. *Arch Pharmacol* 1993: **348**, 332–337.

Lambert GH, Kotake AN, Schoeller D. The CO_2 breath tests as monitors of the cytochrome P450 dependent mixed function monooxygenase system. In: MacLeod SM, Okey AB, Spielberg SP, eds. Developmental pharmacology. New York: Alan R Liss, 1983: 119–145.

Lambrecht RW, Sinclair PR, Gorman N, Sinclair JF. Uroporphyrinogen oxidation catalysed by reconstituted cytochrome P4501A2. *Arch Biochem Biophys* 1992: **294**, 504–510.

Lemoine A, Gautier JC, Azoulay D, Kiffel L, Belloc C, Guengerich FP, Maurel P, Beaune P, Leroux JP. 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.

Rost KL, Roots I. Accelerated caffeine metabolism after omeprazole treatment is indicated by urinary metabolite ratios – coincidence with plasma clearance and breath test. *Clin Pharmacol Ther* 1994: in press.

Sesardic D, Boobis AR, Edwards RJ, Davies DS. A form of cytochrome P450 in man, orthologous to form d in rat, catalyses the *O*-deethylation of phenacetin and is inducible by cigarette smoking. *Br J Clin Pharmacol* 1988: **26**, 363–372.

Shimada T, Iwasaki M, Martin MV, Guengerich FP. Human liver microsomal cytochrome P-450 enzymes involved in the bioactivation of procarcinogens detected by *umu* gene response in *salmonella typhimurium* TA 1535/pSK1002. *Cancer Res* 1989: **49**, 3218–3228.

Tanaka E, Ishikawa A, Yamamoto Y, Osada A, Tsuji K, Fukao K, Misawa S, Iwasaki Y. A simple useful method for the determination of hepatic function in patients with liver cirrhosis using caffeine and its three major dimethylmetabolites. *Int J Clin Pharmacol Ther Toxicol* 1992: **30**, 336–341.

Tassaneeyakul W, Mohamed Z, Birkett DJ, McManus ME, Verones ME, Tukey RH, Quattrochi LC, Gonzalez FJ, Miners JO. Caffeine as a probe for human cytochromes P450: validation using cDNA-expression, immunoinhibition and microsomal kinetic and inhibitor techniques. *Pharmacogenetics* 1992: **2**, 173–178.