Theophylline has no advantages over caffeine as a putative model drug for assessing CYP1A2 activity in humans

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Aims The cytochrome P4501A2 (CYP1A2) catalyses the metabolism of a number of clinically used drugs, and thus there is an interest in determining the activity of CYP1A2 in patients before treatment with CYP1A2 substrates. Caffeine is the most commonly used model drug to assess CYP1A2 function, but due to the complex metabolism of caffeine, there is a need for an alternative drug to use as an index of CYP1A2 activity. In this study the CYP1A2 substrate theophylline was tested as a possible alternative to caffeine as a model drug for CYP1A2.

Methods Twelve healthy volunteers ingested 200 mg of caffeine, and the caffeine metabolic ratios (CMR), $CMR_{urine} = (AFMU + 1MX + 1MU)/17DMU$ and $CMR_{plasma} = 17DMX/137TMX$ were determined 6 h after drug intake. After a period of about 2 months the volunteers ingested 257 mg theophylline and blood samples were drawn and urine was collected during the following 48 h. The oral and partial clearances of theophylline were calculated via *N*-demethylation and 8-hydroxylation. The theophylline metabolic ratios, 1MU/13DMX and 3MX/13DMX being evaluated as indices of CYP1A2 catalysed *N*-demethylation and 13DMU/13DMX as an index of partly CYP1A2 catalysed 8-hydroxylation, were estimated in 0–12 h, 0–24 h and 0–48 h urine samples, and in plasma and spot urine samples 6 h after the intake of theophylline.

Results The theophylline plasma ratios for the *N*-demethylation pathways correlated with the oral clearance of theophylline ($r_s = 0.881$-0.934, $P < 0.001$) and with the respective formation clearances of the metabolites ($r_s = 0.712$-0.925, $P < 0.05$). Furthermore, all of the theophylline plasma ratios correlated with the caffeine plasma ratio ($r_s = 0.645$-0.663, $P < 0.05$). None of the caffeine metabolic ratios and none of the 6 h urinary theophylline ratios correlated with the oral or the partial clearances of theophylline ($r_s = 0.042$-0.556, $P > 0.05$). The theophylline 0-12 h urine ratios correlated with the oral clearance of theophylline ($r_s = 0.677$-0.757, $P < 0.05$) and with the respective formation clearances of the metabolites ($r_s = 0.705$-0.750, $P < 0.05$). However, none of the theophylline urine ratios correlated with any of the caffeine metabolic ratios.

Conclusions In summary the theophylline 6 h plasma and 0–12 h urine ratios 1MU/13DMX and 3MX/13DMX, both reflecting *N*-demethylation seem to be predictors of the CYP1A2 mediated metabolism of theophylline, whereas only the plasma ratio correlated with the caffeine plasma 17DMX/137TMX ratio. Thus, it would appear that the plasma theophylline *N*-demethylation ratios are superior to the urine ratios as indices of CYP1A2 activity. However, because in some individuals the concentrations of theophylline metabolites in plasma were close to the limit of detection, it is concluded that theophylline does not have marked advantages over caffeine as a model drug for assessing CYP1A2 activity.

Keywords: CYP1A2, caffeine metabolic ratios, theophylline metabolic ratios

Introduction

Cytochrome P4501A2 enzyme (CYP1A2) is one of the major forms of this enzyme in the liver, accounting for about 15% of the total P450 content [1]. CYP1A2 catalyses the metabolism of many clinically used drugs such as theophylline and caffeine [2, 3], clozapine [4–6], imipramine [7], propranolol [8, 9] and tacrine [10].

Caffeine (1,3,7-trimethylxanthine, 137TMX) is the drug of choice for assessing CYP1A2 function in man [11–14], but the various caffeine metabolic ratios used seem to have shortcomings due to the very complex metabolism of caffeine, the involvement of other P450s, and the influence of urinary flow [14, 15].

Theophylline (1,3-dimethylxanthine, 13DMX) is closely related chemically to caffeine, but its metabolism is much

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less complex. Theophylline is metabolized in the liver predominantly by 8-hydroxylation to 1,3-dimethyluric acid (13DMU), which accounts for about half of the oral clearance of the drug [16, 17]. In addition, theophylline is *N*-demethylated to 3-methylxanthine (3MX) and 1-methylxanthine (1MX). The latter is oxidized very rapidly by xanthine oxidase [18] to 1-methyluric acid (1MU), which is the only *N*3-demethylation product detected in plasma and urine. CYP1A2 catalyses 80–90% of the *N*-demethylations and about 50% of the 8-hydroxylation of theophylline *in vitro* [19, 20]. CYP2E1 is responsible for the remainder of the 8-hydroxylation [20]. Hence, the two *N*-demethylation pathways of theophylline metabolism could be more precise indices of CYP1A2 activity in humans than using caffeine.

The purpose of this study was to evaluate the potential of theophylline as a model drug for assessing CYP1A2 function in man. To this end parameters of theophylline pharmacokinetics and metabolism in healthy volunteers were correlated with those of caffeine given on a separate occasion.

Methods

Twelve male volunteers with a median age of 23 years (range 22–30 years) participated in the study. All were non-smokers and had no known heart, liver, or kidney disease according to a clinical investigation, clinical chemical/haematological screening and ECG-test. They did not consume alcohol or drugs on a regular basis at the time of the study. The volunteers consented to participate in the study on the basis of written and verbal information, and the study was approved by the regional Ethics Committee of the counties of Vejle and Funen and by the Danish National Board of Health.

Study procedure

All volunteers performed a caffeine test. The subjects abstained from ingesting methylxanthine-containing foods, beverages and medication for at least 40 h before and 6 h after the intake of caffeine. Each subject ingested 200 mg caffeine (Nycomed DAK, Copenhagen, Denmark). Six hours later a 10 ml blood sample was drawn, and a spot urine sample was given in a conical tube containing 300 μl 1N HCl. After a period of about 2 months, the volunteers again abstained from ingesting methylxanthine-containing foods, beverages and medication for 3 days prior to and until the last blood sample was drawn. Before intake of theophylline, a blood sample was drawn and a 10 ml spot urine sample was given. The two samples were then analysed for the presence of caffeine metabolites. Each volunteer took a tablet of 300 mg of theophylline ethylenediamine (Teofylamin®, Nycomed DAK, Copenhagen, Denmark), containing 257 mg theophylline, and blood samples were drawn at 1, 2, 3, 4, 6, 8, 12, 24, 32, and 48 h. Urine was collected from 0 to 12 h, from 12 to 24 h and from 24 to 48 h. In addition, a 10 ml spot urine sample was obtained after 6 h. The blood samples were drawn via a heparinised intravenous catheter (Venflon®, Viggo Spectramed, Sweden) from 0 to 12 h and thereafter by venepuncture. Samples were transferred into EDTA containing tubes (Terumo, Belgium) and after centrifugation, plasma was separated and frozen at -20° C until analysis. Urine volumes were recorded and an aliquot of 10 ml was frozen at -20° C until analysis.

Chemicals and reagents

Theophylline (13DMX), 1-methylxanthine (1MX), 1,7-dimethylxanthine (17DMX), 1,7-dimethyluric acid (17DMU), and β-hydroxy-ethyltheophylline (internal standard) were purchased from Sigma (Missouri, USA), and caffeine (137TMX), 1-methyluric acid (1MU), 3-methylxanthine (3MX), and 1,3-dimethyluric acid (13DMU) were purchased from Fluka AG (Buchs, Switzerland). 5-acetylamino-6-formylamino-3-methyluracil (AFMU) was kindly donated by Mr Morten A. Kall, Roskilde University (Roskilde, Denmark). All chemicals were of h.p.l.c. grade and obtained from either Merck (Darmstadt, Germany) or Sigma. Water was purified by osmosis and distillation.

Analytical methods

Theophylline assay Plasma and urine were analysed for theophylline and metabolites by an h.p.l.c. method described in detail elsewhere [21]. For theophylline and the three metabolites the limits of detection were 0.1–0.2 μM in plasma and 1–2 μM in urine. The intra-assay and inter-assay coefficients of variation were $<6\%$ and $<9\%$, respectively, and the accuracy was within $\pm 10\%$ in both plasma and urine.

Caffeine assay Urine were analysed for AFMU, 1MU, 1MX and 17DMU by a previously published h.p.l.c. method [22]. For the four metabolites, the detection limit was 1–2 μM. The intra-assay and inter-assay coefficients of variation was $<3\%$ and $<7\%$ respectively, and the accuracy was within $\pm 3\%$. Plasma was analysed for caffeine and 17DMX by modifications of the theophylline assay [21]. Briefly, 500 μl plasma was acidified to pH 4 by addition of 1N HCl and extracted with 5 ml ethylacetate/2-propanol (90 : 10 w/w). The organic phase was evaporated to dryness and the residue was reconstituted in the mobile phase, which consisted of 0.01 M acetate buffer, pH 4.0, and methanol (91 : 9 w/w). An aliquot of 40 μl was injected onto the h.p.l.c. system (Merck-Hitachi, Tokyo, Japan). The detection limit was 0.2 μM for 17DMX and 0.6 μM for caffeine. The intra-assay and inter-assay coefficients of variation was $<8\%$ and $<8\%$ respectively, and the accuracy was within $\pm 13\%$.

Pharmacokinetic analyses

Caffeine In the 10 ml spot urine sample, a caffeine metabolic ratio (CMR_{urine}) was calculated according to Campbell [11]:

$$CMR_{urine} = (AFMU + 1MX + 1MU)/17DMU \quad \text{(equation 1)}$$

In plasma, a caffeine metabolic ratio (CMR_{plasma}) was

calculated according to Fuhr & Rost [14]:

$$CMR_{plasma} = 17DMX/137TMX \quad \text{(equation 2)}$$

Theophylline For theophylline the following pharmacokinetic parameters were calculated. The oral theophylline clearance:

$$CL_{13DMX} = Dose/AUC_{13DMX(oral)} \quad \text{(equation 3)}$$

$AUC_{13DMX(oral)}$ is the area under the plasma concentration time curve calculated by the trapezoidal rule with extrapolation from the last measurable concentration to infinity. Complete absorption of theophylline from the intestine was assumed [16].

The partial theophylline clearances were calculated as:

$$CL_{13DMX \rightarrow 1MU} = 1MU/AUC_{13DMX(0,48h)} \quad \text{(equation 4)}$$

$$CL_{13DMX \rightarrow 3MX} = 3MX/AUC_{13DMX(0,48h)} \quad \text{(equation 5)}$$

$$CL_{13DMX \rightarrow 13DMU} = 13DMU/AUC_{13DMX(0,48h)} \quad \text{(equation 6)}$$

where the numerator is the number of moles of the metabolite recovered in urine from 0–48 h and $AUC_{13DMX(0,48h)}$ is the corresponding AUC of theophylline.

Equations 4–6 are based on the assumption that the rate of metabolite formation equals the rate of appearance in urine. The partial clearance via *N*3-demethylation is calculated as the formation of the end product, 1MU, because the primary product, 1MX, is neither detectable in urine nor plasma. The data on theophylline pharmacokinetics have been reported in more detail in an earlier publication [17].

The following theophylline metabolic ratios were calculated in all urine and plasma samples collected:

1MU/13DMX (equation 7)

3MX/13DMX (equation 8)

13DMU/13DMX (equation 9)

The theophylline urine and plasma ratios were compared with the oral and partial clearances of theophylline, and with the urine and plasma caffeine metabolic ratios using the Spearman's rank correlation test. A P value less than 0.05 was considered statistically significant. The statistical analysis was carried out using the Medstat program package, version 2.1 (Astra Group, Albertslund, Denmark, 1988).

Results

No caffeine metabolites were detected in the plasma samples from the 12 volunteers given before intake of theophylline. Small quantities of 1MU, 3MX or other interfering substances were found in some pre-dose urine samples, but their contribution to metabolite recovery following dosing with theophylline was negligible.

The median (range) oral clearance of theophylline was 80 ml min^{-1} (53–137 ml min^{-1}), in the 12 volunteers studied. The partial clearances were 17 ml min^{-1} (8.3–36 ml min^{-1}), 8.9 ml min^{-1} (5.0–16 ml min^{-1}) and 21 ml min^{-1} (13–38 ml min^{-1}) for the formation of 1MU, 3MX and 13DMU, respectively. The theophylline metabolic ratios (medians (range)) in the 0-12 h urine collection, in the spot urine samples and in plasma 6 h after theophylline intake were 2.3 (1.0–4.2), 2.1 (1.3–3.6), 0.055 (0.029–0.12) for 1MU/13DMX, 1.3 (0.56–2.0), 0.97 (0.77–1.7), 0.40 for 3MX/13DMX, 2.9 (2.0–5.2), 3.2 (2.5–5.1) and 0.068 (0.042–0.13) for 13DMU/13DMX), respectively. The caffeine metabolic ratios were 5.4 (3.6–9.5) for CMR_{urine} and 0.71 (0.44–1.0) for CMR_{plasma}.

The two caffeine metabolic ratios correlated highly with each other ($r_s = 0.874$, $P < 0.001$). Table 1 shows the correlations between the oral and partial theophylline clearances, and the caffeine metabolic ratios or theophylline metabolic ratios in both plasma and urine. Surprisingly, neither of the caffeine metabolic ratios showed statistically significant correlations with the oral clearance of theophylline or the partial formation clearances to its three metabolites. Furthermore, none of the theophylline metabolic ratios measured in a 6 h spot urine sample correlated with the oral or partial clearances of theophylline. In contrast, all of theophylline 0–12 h urine metabolic ratios correlated well with the oral and partial clearances of theophylline (r_s = 0.677–0.757, $P < 0.05$). Similar correlation between theophylline clearances and theophylline metabolic ratios based on 0–24 h and 0–48 h collections were obtained (data not shown). The plasma theophylline metabolic ratios for 1MU and 3MX formation, but not for 13DMU formation, correlated with the oral clearance of theophylline ($r_s = 0.934$, $P < 0.001$ and $r_s = 0.881$, $P < 0.001$, respectively). All three theophylline metabolic ratios in plasma correlated with the respective formation clearances of the three metabolites (Table 1). Table 2 shows the correlations between the caffeine metabolic ratios and the theophylline metabolic ratios determined in both plasma and urine. The theophylline ratios for 1MU and 3MX formation determined in plasma correlated with the plasma caffeine ratio ($r_s = 0.663$, $P < 0.05$ and 0.645, $P < 0.05$, respectively), whereas the theophylline metabolic ratios determined in urine did not correlate with either of the caffeine metabolic ratios.

Discussion

Caffeine metabolism is the present gold standard for assessing CYP1A2 function [11–14]. A recent study in healthy volunteers demonstrated, that 17DMX/137TMX, was the caffeine ratio that showed the best reflection of changes in CYP1A2 activity when the enzyme was influenced by the chemical inhibitor, fluvoxamine [23]. This ratio also showed the highest correlation with CYP1A2 immunoreactivity in liver samples from patients undergoing hepatectomy [24]. A recent study concluded that the caffeine urinary ratio, (AFMU + 1MU + 1MX)/17DMU, was a valid measure of CYP1A2 activity [25], whereas a theoretical study concluded that none of the caffeine urinary ratios used as markers of CYP1A2 activity are specific for the enzyme, but considered the plasma ratio to be the most robust caffeine based marker of CYP1A2 activity [26]. However, owing to the complex

Table 1 The correlations between the oral and partial clearances of theophylline and the various caffeine and theophylline metabolic ratios in urine and plasma from 12 healthy volunteers.

	r_s-values			
	CL_{oral}	$CL_{13DMX\rightarrow1MU}$	$CL_{13DMX\rightarrow3MX}$	$CL_{13DMX\rightarrow13DMU}$
Caffeine ratios				
(AFMU + 1MU + 1MX)/17DMU	0.425	0.423	0.003	0.339
17DMX/137TMX	0.556	0.535	0.178	0.458
Theophylline ratios in urine				
0–12 h collection				
1MU/13DMX	0.757**	0.748**	—	—
3MX/13DMX	0.677*	—	0.705*	—
13DMU/13DMX	0.687*	—	—	0.750**
6 h spot sample				
1MU/13DMX	0.257	0.343	—	—
3MX/13DMX	0.068	—	0.042	—
13DMU/13DMX	0.061	—	—	0.086
Theophylline ratios in plasma				
1MU/13DMX	0.934***	0.925***	—	—
3MX/13DMX	0.881***	—	0.712*	—
13DMU/13DMX	0.472	—	—	0.822**

*Significance level (two-tailed test) $P<0.05$. **Significance level (two-tailed test) $P<0.01$. ***Significance level (two-tailed test) $P<0.001$.

Table 2 The correlations between the various caffeine and theophylline metabolic ratios in urine and plasma from 12 healthy volunteers.

	r_s-values	
	Caffeine metabolic ratios	
	(AFMU + 1MU − 1MX)/17DMU (urine)	*17DMX/137TMX (plasma)*
Theophylline ratios in urine		
0–12 h collection		
1MU/13DMX	0.423	0.441
3MX/13DMX	0.129	0.096
13DMU/13DMX	0.323	0.366
6 h spot sample		
1MU/13DMX	0.385	0.399
3MX/13DMX	0.488	0.290
13DMU/13DMX	0.327	0.196
Theophylline ratios in plasma		
1MU/13DMX	0.446	0.663*
3MX/13DMX	0.544	0.645*
13DMU/13DMX	0.273	0.472

*Significance level (two-tailed test) $P<0.05$.

metabolism of caffeine, there is a need for a better model drug for assessing CYP1A2 activity. Theophylline has a much simpler metabolism pattern than caffeine and we have shown that it is metabolized predominantly by CYP1A2 [17, 20]. *In vivo* the oral clearance of theophylline was reduced by 70% and the partial demethylation clearances by as much as 90% during concomitant intake of the potent CYP1A2 inhibitor fluvoxamine [17, 27]. These results are in very good agreement with the results of an *in vitro* study [20].

The theophylline pharmacokinetic data from the control phase of the fluvoxamine interaction study by Rasmussen *et al.* [17] were used in the present work demonstrating potent inhibition of theophylline metabolism by fluvoxamine. The theophylline metabolic ratios measured in plasma and in a 0–12 h urine samples were good predictors of the oral and partial clearances of theophylline, and thus it can be concluded that the theophylline metabolic ratios do reflect CYP1A2 activity. The two caffeine metabolic ratios, CMR_{urine} and CMR_{plasma}, were highly intercorrelated, but neither of them showed statistically significant correlations with the oral clearance or the partial formation clearances of theophylline. The somewhat higher r_s values (Table 1) suggest that the theophylline metabolic plasma ratios are slightly better biomarkers of CYP1A2 than their urine counterparts. Thus, the CMR_{plasma} correlated with the two demethylation ratios for theophylline in plasma but not with any of the urine ratios (Table 2). The poor correlation

between caffeine and theophylline metabolism observed in this study could be explained by a number of factors, such as the small number of subjects studied ($n=12$), and by the relatively low variability in CYP1A2 activity within this sample. Thus, it can be estimated that a sample size of 12 volunteers gives the study a power of only 48%, when a $r_s=0.5$ is obtained, and that increasing the power of the study to 80% would require a sample size of 42 volunteers. Furthermore, the caffeine and theophylline testing were carried out 2 months apart, but data on the constancy over time of CYP1A2 activity within an individual are scanty. It is well known that CYP1A2 is induced by smoking [28–30], broccoli and charcoal-broiled meat [31–33], omeprazole [34, 35], griseofulvin [17] and inhibited by several drugs [27, 36]. Thus, it is possible that the poor correlation obtained between caffeine and theophylline metabolism in part is due to a time-dependent intraindividual variation in CYP1A2.

Previous results on caffeine metabolism from our research group [22, 23], support the view that caffeine serves as a good model drug for assessing CYP1A2 activity in populations, but that it is not an accurate predictor of CYP1A2 activities in individuals. This is probably due to its complex metabolic pattern and the involvement of additional enzymes in its metabolism. Furthermore, the presence of endogenous xanthines in both urine and plasma impedes the analysis of caffeine and its metabolites.

None of the theophylline metabolic ratios measured in a 6 h spot urine sample correlated to the theophylline clearances. This lack of correlation could be explained by the dependency of urinary flow in the excretion of theophylline [37], which seems to disqualify theophylline ratios measured in spot urine samples as CYP1A2 markers. However the theophylline ratios measured in the 0–12 h urine sampling do seem to reflect CYP1A2 activity well, but the collecting of 12 h samples, can be inconvenient in certain situations. The theophylline plasma ratios for the CYP1A2 catalysed *N*-demethylations (1MU/13DMX and 3MX/13DMX) correlated with the respective *N*-demethylation clearances, with the oral clearance of theophylline and also with the caffeine plasma ratio. Thus, the 1MU/13DMX and 3MX/13DMX ratios measured in plasma 6 h after the intake of a single dose of theophylline, seem to be the most convenient way to predict the CYP1A2 mediated metabolism of theophylline. However, the metabolites of theophylline are present in plasma in very low concentrations, due to their high elimination clearances. Thus, in individuals with low CYP1A2 activity plasma metabolites would be undetectable. A further disadvantage of using theophylline as a probe drug for CYP1A2 is that it is far more toxic than caffeine.

It must be concluded that theophylline does not have marked advantages compared with caffeine as a model drug for CYP1A2 activity. There is still a strong need for the evaluation of additional metabolic probes for CYP1A2 function in humans.

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