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Biochemical Pharmacology 63 (2002) 2159–2167

Biochemical Pharmacology

Differences in caffeine and paraxanthine metabolism between human and murine CYP1A2

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Received 19 October 2001; accepted 27 March 2002

Abstract

For the characterisation of murine models of CYP1A2 mediated metabolism in humans we compared the metabolism of caffeine and paraxanthine in human liver microsomes (LM) (two samples) and in LM from CYP1A2-null and wild-type mice. Inhibition experiments were carried out with the quinolones norfloxacin and pefloxacin and the substrate, caffeine. Additionally, *in vivo* pharmacokinetics of paraxanthine was determined in CYP1A2-null and wild-type mice. All LM produced the primary metabolites of caffeine and paraxanthine. In human LM, the main metabolite of caffeine was paraxanthine (K_M 0.4 and 0.5 mmol L^{-1}). In wild-type and CYP1A2-null mice LM, the main caffeine metabolite was 1,3,7-trimethylurate, but formation was not saturable. Apparent K_M for paraxanthine formation from caffeine in wild-type and CYP1A2-null murine LM were 0.2 and 4.9 mmol L^{-1}, respectively. The main metabolite of paraxanthine was 1-methylxanthine in human (K_M 0.13 and 0.2 mmol L^{-1}) and in wild-type mice LM (K_M 0.53 mmol L^{-1}). In CYP1A2-null murine LM, the main paraxanthine metabolite was 7-methylxanthine. The quinolones competitively inhibited caffeine metabolism in human but not in wild-type or CYP1A2-null murine LM. No obvious differences were seen for blood pharmacokinetics and urinary metabolite excretion of paraxanthine between CYP1A2-null and wild-type mice. Thus, for paraxanthine, norfloxacin and pefloxacin interaction with CYP1A2 there were clear differences between mice and man. Our results suggest that an interspecies comparison is required for the metabolism of individual xenobiotics interacting with CYP1A2 prior to the use of mice models to predict its toxicity and/or pharmacological activity in man.

Keywords: Caffeine; Paraxanthine; Null mice; CYP1A2; Quinolones; Microsomes

1. Introduction

The cytochrome P450 enzyme CYP1A2 is an important enzyme in the metabolism of xenobiotics, such as drugs (e.g. theophylline, clozapine, and tacrine [1,2]) and food-borne procarcinogens (e.g. polycyclic aromatic hydrocarbons or imidazoquinoline derivatives [3,4]) in humans. Mice are often used as an *in vivo* model for human metabolism, toxicity and/or pharmacological activity of xenobiotics including those metabolised by CYP1A2. A prerequisite for this application is a sufficient degree of similarity in substrate and inhibitor characteristics with respect to this enzyme between the two species.

Murine and human CYP1A2 show about 72% amino acid sequence homology and also have catalytic activities in common [5,6]. For instance, caffeine metabolism in humans is primarily mediated by CYP1A2 in liver microsomes and is responsible for more than 90% of caffeine elimination *in vivo* [7–9]. Using mice lacking expression of CYP1A2 it was confirmed that also in this species 85% of caffeine clearance depends on CYP1A2 [10]. The initial objective of the present investigations was to further assess paraxanthine instead of caffeine as a specific probe for CYP1A2 in murine models, because urinary metabolic ratios based on paraxanthine metabolism provide better estimates of CYP1A2 activity in humans than those based on caffeine 3-demethylation [7]. To this end, we studied

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Abbreviations: 17X, paraxanthine; 13X, theophylline; 37X, theobromine; 137U, trimethylurate; 1X, 1-methylxanthine; 7X, 7-methylxanthine; 17U, 1,7-dimethylurate; CYP1A2, cytochrome P450 enzyme 1A2.

0006-2952/02/$ – see front matter
PII: S0006-2952(02)01019-5

the elimination of paraxanthine in CYP1A2-null and wild-type mice, but found no difference between the two mice lines. Additional studies with CYP1A2 specific inhibitors and liver microsomes of both species confirmed a difference in ligand binding between human and mice CYP1A2.

2. Materials and methods

2.1. Materials and animals

Caffeine, paraxanthine, theobromine, theophylline, and 1,7-dimethylurate were purchased from Sigma Chemicals and 1-methylxanthine, 7-methylxanthine, 1,3,7-trimethylurate, and 1-methylurate were purchased from Fluka Chemicals. Norfloxacin was from Merck and pefloxacin produced by Rhône–Poulenc–Rorer was a kind gift from Prof. Dr. U. Holzgrabe (Würzburg, Germany). All other chemicals were from commercial sources of suitable purity. Wild-type and CYP1A2-null mice (129/Sv and C57B1/6 strains) were produced as described [10,11]. Male mice aged 7–9 weeks were used for all experiments.

2.2. In vitro studies using liver microsomes of humans and of wild-type and CYP1A2-null mice

2.2.1. Comparison of enzyme constants for primary metabolic steps of caffeine and paraxanthine

Metabolism of the two substrates (0, 100, 250, 625, 1563, 3907, and 4884 μmol L^{-1}) *in vitro* was determined in incubations using liver microsomes of two human donors (HLM1 and HLM2), of wild-type and of null mice. Microsomes were prepared by standard methods [12] and stored at −80°. Protein concentrations in microsomal suspensions were determined by the DC-Protein Assay-Kit (BIORAD laboratories Gmbh, Munich, Germany). Experimental conditions of incubations were as described previously [12]. Briefly, incubations were carried out in 0.1 mol L^{-1} potassium phosphate buffer pH 7.4, containing about 2 mg mL^{-1} microsomal protein, an NADPH-regenerating system, caffeine or paraxanthine and, in case of the inhibition studies (see below), the tested quinolones. Each incubation lasted 15 min and was stopped with 20% (w/v) trichloroacetic acid (final concentration 4% (w/v)) in which hydroxypropyltheophylline was added as an internal standard for HPLC analysis. Primary metabolites measured for the substrate caffeine (137X) were paraxanthine (17X), theophylline (13X), theobromine (37X), and trimethylurate (137U). Primary metabolites measured for paraxanthine were 1-methylxanthine (1X), 7-methylxanthine (7X), and 1,7-dimethylurate (17U). Enzyme constants K_M and V_{max} were estimated from metabolite formation rates at a given substrate concentration (caffeine or paraxanthine) by nonlinear regression analysis using an extended Michaelis–Menten equation [13], in which a second binding site not saturable at the substrate concentrations used [7] is described by a simplified term (Eq. (1)).

$$v = \frac{V_{max}[S]}{K_M + [S]} + a[S], \quad (1)$$

where v is the measured metabolite formation rate, V_{max} is the maximal metabolite formation rate, [S] is the substrate concentration, K_M is the Michaelis–Menten constant, a is the constant of proportionality.

2.2.2. Contribution of murine CYP1A2 to the metabolism of caffeine and paraxanthine

Metabolite formation rates of the two types of murine microsomes were compared to evaluate the contribution of murine CYP1A2 using the Eq. (2)

$$\text{percental contribution}_{CYP1A2} = \left(\frac{v_{\text{wild-type}} - v_{\text{CYP1A2-null}}}{v_{\text{wild-type}}}\right) \times 100, \quad (2)$$

where v is the measured metabolite formation rate.

2.2.3. Comparison of inhibitory effects of norfloxacin and pefloxacin on caffeine metabolism

The effects of the two quinolones (0, 50, 150, 500, and1000 μmol L^{-1}) on caffeine 3-demethylation were tested in microsomes of the two human donors and the two different murine microsome types. Caffeine concentrations used in inhibition studies were 0, 100, 250, 625, and 1563 μmol L^{-1} (for other experimental conditions, see Section 2.2.1). Enzyme kinetic parameters were estimated from the observed paraxanthine formation rate as described by Eq. (3). The main type of inhibition was defined as the type with the lower K_i value, indicating higher affinity to the enzyme [14].

$$v = \frac{V_{max}[S]}{K_M(1 + [I]/K_{i,c}) + [S](1 + [I]/K_{i,nc})} + a[S], \quad (3)$$

where v is the measured metabolite formation rate, V_{max} is the maximal metabolite formation rate, [S] is the substrate concentration, [I] is the inhibitor concentration, K_M is the Michaelis–Menten constant, $K_{i,c}$ and $K_{i,nc}$ are the inhibitor constants for competitive and noncompetitive inhibition, respectively, a is the constant of proportionality.

2.3. In vivo studies

2.3.1. Blood pharmacokinetics of paraxanthine in wild-type and CYP1A2-null mice

A dose of 1.8 mg kg^{-1} i.p. was given, and eight sequential blood samples were drawn periorbitally 0, 15, 30, 45, 60, 90, 150, and 250 min postdose in wild-type mice and 0, 15, 30, 45, 90, 150, 250, and 400 min postdose in null mice. Blood was collected in heparinised tubes and stored in −80° until analysis by HPLC. The rate constant for terminal elimination (k_{el}) was estimated by log-linear

regression of the last 3–4 data points of the concentration vs. time profile and converted to elimination half life ($t_{1/2}$) by $t_{1/2} = \ln(2)/k_{el}$. AUC values were calculated by the linear trapezoid rule for increasing concentrations and by the log-linear rule for falling concentrations, and extrapolation to infinity by adding the last quantificated concentration divided by k_{el}.

2.3.2. Urinary excretion of paraxanthine metabolites in wild-type and CYP1A2-null mice

In this study, 10 mg of paraxanthine per kilogram of body weight i.p. was administrated, and animals were hydrated with 3 mL of isotonic NaCl solution s.c. to safeguard sufficient urinary flow. Groups of five animals of the same genotype were held in one metabolic cage (Nalgene Company, Rochester, NY, USA) for 24 hr. Urine was collected and after the 24 hr the cages were rinsed with water and the diluted urine was stored in −80° until analysis by HPLC.

2.3.3. HPLC analysis

Substrates and metabolites were determined by HPLC with UV detection at 278 nm [12]. For sample preparation, to 500 μL incubation volume, 5 mL of diisopropylether:2-propanole 70:30 (v/v), 50 μL of formic acid and 1 g of $NH_4H_2PO_4$ were added [15]. The organic phase was separated and dried. The residue was dissolved in 150 μL of mobile phase (10% methanol and 0.05% acetate), and 50 μL were injected onto a Beckman Ultrasphere ODS 5 μm (4.6 mm × 25 cm) column. Urine samples were prepared the same way. For blood samples, 20 μL were mixed with 5 μL of 10% acetic acid and 0.1 mL of acetonitrile containing hydroxypropytheophylline as an internal standard. The mixture was vortexed and centrifuged, and 80 μL of the supernatant was injected onto the column (see above). In each sample set, 12 samples with known metabolite concentrations were included for the calibration graph. For quantification, peak height ratios with the internal standard (hydroxypropyltheophylline) were used. These ratios were proportional to spiked concentrations of all substances measured from the lower limit of detection, which was defined as the concentration giving a peak height greater than three times the noise fluctuation of the chromatography baseline, up to 20 μmol L^{-1} ($r^2 > 0.998$). The measured values were only used for quantification when the variation coefficient did not exceed 15% for the determined concentration.

HPLC was performed on a Waters "LC Module 1 plus" (Waters, Eschborn, Germany) with the Waters "Millennium 3.05.01 Chromatographiemanager" software (Waters, Milford, USA). Caffeine, paraxanthine and their metabolites were eluted at a flow of 1.5 mL min^{-1} by a two solvent gradient from "Solvent A" (30% methanol, 0.05% acetic acid) and "Solvent B" (10% methanol, 0.05% acetic acid) combined using linear gradients. The final liquid phase contained 10% of methanol for 6 min, increasing up to 15% of methanol at 14 min and to 30% at 20 min. 30% of methanol was maintained for another 5 min. Afterwards the column was equilibrated for 15 min before the next injection.

3. Results

3.1. Caffeine metabolism in vitro

All liver microsomes used (HLM1 and HLM2, wild-type and null mice) were able to form the primary caffeine metabolites 17X, 13X, 37X, and 137U. Main metabolite at low substrate concentrations (250 μmol L^{-1} or less) was 17X in human liver microsomes and 137U in both murine liver microsomes, respectively (Fig. 1). Among demethylation pathways, 3-demethylation was the major pathway in all samples except in the CYP1A2-null mice.

Demethylating metabolic steps were mediated by both a high and a low affinity site. K_M of 3-demethylation (Table 1) were 0.40 and 0.55 mmol L^{-1} for HLM1 and HLM2, respectively. Apparent caffeine affinity for the 7-demethylation pathway was lower as shown by a much higher K_M value in the HLM2 sample and lack of enzyme saturation in HLM1. In contrast to demethylation pathways, formation of 137U was never saturable under the experimental conditions chosen.

In the mouse strains, K_M for 3-demethylation of caffeine was much lower in wild-type (0.23 mmol L^{-1}) compared to null mice liver microsomes (4.9 mmol L^{-1}). Absolute formation rates at 250 μmol L^{-1} caffeine concentrations were 3.3 times higher in wild-type than in null for 17X and 10 times higher for 37X in wild-type murine microsomes (Table 2). Accordingly, K_M for 1-demethylation was low in wild-type murine microsomes, but there was no saturation in CYP1A2-null mice. For the 7-demethylation pathway, wild-type murine microsomes also had a higher affinity than CYP1A2-null mice preparations (Table 1).

There was a clear difference in 8-hydroxylation rate of caffeine between wild-type and CYP1A2-null murine liver microsomes at higher substrate concentrations (larger than 250 μmol L^{-1} caffeine), with higher rates in wild-type (Fig. 1), but in both strains the formation rate of 137U could not be saturated at the substrate concentrations used.

As a result of these differences, the concentration dependency of the metabolic profile of caffeine showed differences between human and murine microsomes (Fig. 1). While human and wild-type mice samples showed increasing relative formation of 137U with increasing caffeine concentrations, in null mice the metabolic pattern was unchanged. The estimated participation of CYP1A2 in the microsomal metabolism of caffeine in wild-type mice is shown in Table 3. At low caffeine concentrations murine CYP1A2 played the main role in demethylations whereas

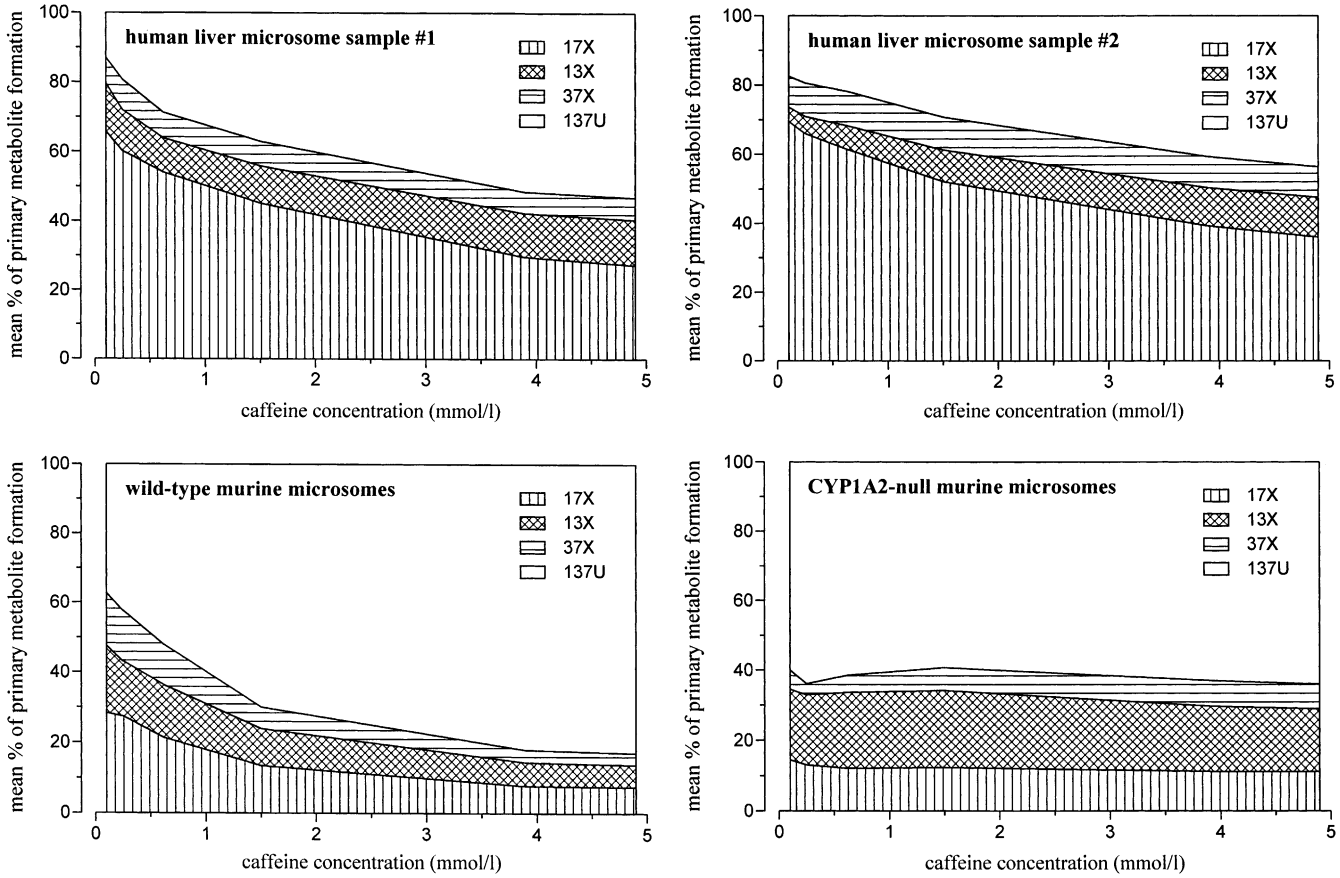

Fig. 1. Caffeine metabolic profiles for the various microsomal probes depending on substrate concentration. (For identification of metabolites, see legend to Table 1).

Table 1
Apparent enzyme kinetic parameters K_M (mmol L^{-1}) and V_{max} (pmol min^{-1} mg $protein^{-1}$) for the conversion of caffeine to the primary metabolites by microsomes of two human donors (HLM1 and HLM2), wild-type mice (WT) and CYP1A2-null mice (KO) for the high affinity component (point estimate ± SE)

Type of microsomes		17X formation	13X formation	37X formation	137U formation
HLM1	K_M	0.40 ± 0.13	n.c.	0.47 ± 0.18	n.c.
	V_{max}	140 ± 20	n.c.	100 ± 20	n.c.
HLM2	K_M	0.55 ± 0.05	8.9 ± 3.0	6.4 ± 3.5	n.c.
	V_{max}	770 ± 20	640 ± 150	120 ± 40	n.c.
WT	K_M	0.23 ± 0.11	1.0 ± 0.7	0.36 ± 0.14	n.c.
	V_{max}	110 ± 20	140 ± 70	70 ± 10	n.c.
KO	K_M	4.9 ± 0.97	3.8 ± 0.7	n.c.	n.c.
	V_{max}	320 ± 40	470 ± 50	n.c.	n.c.

17X, paraxanthine; 13X, theophylline; 37X, theobromine; 137U, trimethylurate; n.c., not calculated (because of missing saturation, no enzyme kinetic parameters could be estimated).

Table 2
Formation rates of primary metabolites of caffeine (pmol min^{-1} mg $protein^{-1}$) by microsomes of two human donors (HLM1 and HLM2), wild-type mice (WT) and CYP1A2-null mice (KO) at a substrate concentration of 250 μmol L^{-1} (mean of N = 4 ± SD)

Type of microsomes	17X formation	13X formation	37X formation	137U formation
HLM1	58 ± 6.5	11 ± 3	8.4 ± 1.8	19 ± 6
HLM2	242 ± 12.4	18 ± 1.4	35.3 ± 2.4	71 ± 5.6
WT	64 ± 6.8	37 ± 2.8	33.7 ± 5.6	99 ± 17
KO	19 ± 5.2	29 ± 2.8	3.3 ± 0.5	94 ± 21

For identification of metabolites, see legend to Table 1.

Table 3
Estimated percental contribution of CYP1A2 to the microsomal formation of primary metabolites in wild-type mice calculated according to Eq. (2)

Substrate concentration (μmol L^{-1})	CYP1A2 contribution to formation of caffeine metabolites in mice (%)				CYP1A2 contribution to formation of paraxanthine metabolites in mice (%)	
	17X	37X	13X	137U	1X	7X
100	80	86	60	0	80	25
250	70	86	20	4	71	13
625	56	67	0	8	60	37
1563	53	45	0	56	49	26
3907	43	20	0	70	34	26
4884	49	29	2	74	33	24

For identification of metabolites, see legends to Tables 1 and 4.

at higher concentrations it mediated the major part of 8-hydroxylation.

3.2. Paraxanthine metabolism in vitro

All microsome samples were able to form the three main metabolites of paraxanthine 1X, 7X, and 17U. Because of lack of saturation with the substrate concentrations used, enzyme kinetics were only estimated for the 7-demethylation pathway of paraxanthine. K_M values for the 7-demethylation were 0.13 and 0.20 mmol L^{-1} for human HLM1 and HLM2 and 0.53 mmol L^{-1} for wild-type murine liver microsomes, respectively. In contrast, there was no high affinity site for 1X formation in CYP1A2-null mice microsomes, and formation rate of 1X at 250 μmol L^{-1} of paraxanthine was 3.6-fold lower than in wild-type (Table 4).

Formation rate of 7X at 250 μmol L^{-1} of paraxanthine reached from 23 to 131 pmol min^{-1} mg $protein^{-1}$ for the different microsomal probes and was not saturable at the substrate concentrations used (Table 4). Main metabolite of paraxanthine was 1X in wild-type murine microsomes and in both HLM1 and HLM2 microsomes, whereas the main metabolite of paraxanthine in CYP1A2-null was 7X. For technical reasons (peak interference) 17U formation by murine liver microsomes could not be quantificated. While the major role of murine CYP1A2 for 1X formation decreased with increasing paraxanthine concentrations, CYP1A2 made only a smaller and concentration independent contribution to 7X production (Table 3).

Table 4
Formation rates of primary metabolites of paraxanthine (pmol min^{-1} mg $protein^{-1}$) by microsomes of two human donors (HLM1 and HLM2), wild-type mice (WT) and CYP1A2-null mice (KO) at a substrate concentration of 250 μmol L^{-1} (mean of N = 4 ± SD)

Type of microsomes	1X formation	7X formation	17U formation
HLM1	66 ± 30	43 ± 19	136 ± 32
HLM2	305 ± 88	131 ± 66	234 ± 164
WT	36 ± 11	27 ± 7	n.q.
KO	10 ± 2	23 ± 9	n.q.

1X, 1-methylxanthine; 7X, 7-methylxanthine; 17U, 1,7-dimethylurate; n.q., no quantification for technical reasons.

3.3. Inhibition of caffeine metabolism in vitro

Nonlinear regression using Eq. (3) showed that both pefloxacin and norfloxacin were mainly competitive type inhibitors of human caffeine 3-demethylation. The inhibition constants for the competitive binding site of the two quinolones ($K_{i,c}$) was 0.11 and 0.10 mmol L^{-1} for norfloxacin and 1.42 and 0.91 mmol L^{-1} for pefloxacin, respectively. $K_{i,nc}$ exceeded 2 mmol L^{-1} in all cases. In contrast, norfloxacin and pefloxacin showed no relevant inhibition of caffeine 3-demethylation in incubations with microsomes from wild-type or CYP1A2-null murine liver microsomes (Fig. 2).

3.3.1. Blood pharmacokinetics of paraxanthine in the mice

Elimination from blood of three CYP1A2-null mice was compared to that of four wild-type mice. CYP1A2-null mice were not impaired in their ability to eliminate paraxanthine (Fig. 3). The apparent terminal half life ($t_{1/2}$) in CYP1A2-null mice (90 ± 43 min, mean ± SD) was similar to that in wild-type mice (60 ± 20 min), the areas under the concentration vs. time profile were almost identical in the two mouse strains (knock-out: 769 ± 133 μmol L^{-1} min; wild-type: 763 ± 62 μmol L^{-1} min). Likewise, the concentration at $t = 15$ min were similar for the two strains, suggesting that there was no relevant strain difference in the volume of distribution relative to body weight (Fig. 3).

3.3.2. Urinary excretion of paraxanthine metabolites in the mice

All demethylated and hydroxylated metabolites were found in urine of both mouse strains. About 33% of the paraxanthine dose administrated was recovered in the urine of wild-type mice and 45% in the urine of null mice (Fig. 4). There was no obvious difference in metabolite pattern between the mouse strains.

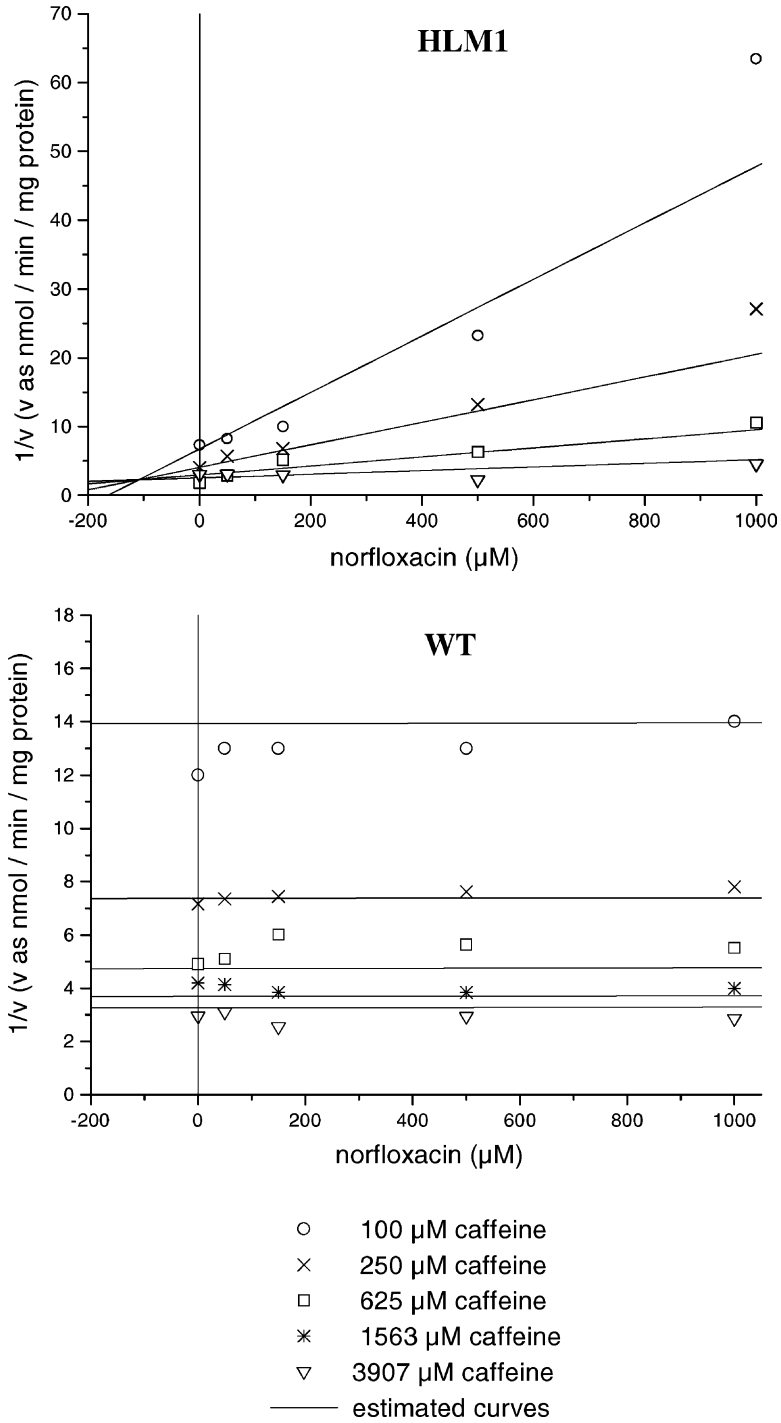

Fig. 2. Typical Dixon plots describing the effects of norfloxacin on caffeine 3-demethylation in human liver microsomes (sample no. 1, HLM1) and in murine wild-type microsomes (WT). Estimated curves were obtained using Eq. (3) (see Section 2).

4. Discussion

In the *in vitro* studies, we used human liver microsomes from only two donors. This does not take into account the pronounced interindividual variability in phase I metabolism of xenobiotics in general. However, as there are no genetic variations with functional relevance known for human CYP1A2 and absolute expression and activity was not important for our studies, this small number was considered sufficient. In contrast, for the inbred mice strains no relevant variability is expected.

In urinary recovery studies in mice, we did not try to quantificate the main human paraxanthine metabolite, 5-acetyl-6-formylamino-3-methyluracil (AFMU), because in studies with labelled caffeine it has been shown conclusively that this metabolite is not present in mice [16].

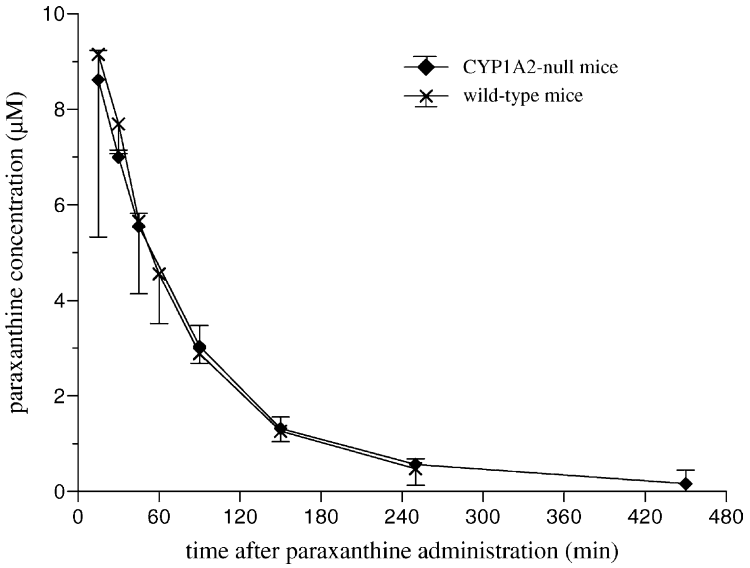

Fig. 3. Whole blood paraxanthine concentration vs. time profiles in CYP1A2-null mice (N = 3) and wild-type mice (N = 4) after i.p. doses of 1.8 mg kg^{-1}. Blood samples were drawn suborbitally and analysed by HPLC. Mean ± SD are given.

4.1. Caffeine metabolism

The much higher apparent K_M value for 3-demethylation of caffeine in CYP1A2-null mice microsomes compared to both wild-type murine and human liver preparations demonstrate that CYP1A2 is the high affinity binding site for this pathway in the two species. The human enzyme kinetic parameters obtained in this study correspond to published studies with human liver microsomes or other human *in vitro* systems [11,15,17–20]. Our data also correspond to the metabolic pattern observed *in vivo* [21] and in mammalian cell lines expressing isolated human CYP1A2 [2], except for a larger fraction of the 8-hydroxylated product of caffeine, 1,3,7-trimethylurate, formed in human liver microsomes (see below).

Concerning mice, the results of our *in vitro* studies confirm the important role of CYP1A2 in murine metabolism of caffeine as shown by *in vivo* studies using CYP1A2-null mice [10], where CYP1A2 mediated 87% of caffeine clearance. Murine CYP1A2 seemed to be partially responsible for all demethylations of caffeine *in vitro*, while playing a major role in 3-demethylation in humans [2,8,9]. In contrast to human metabolism, the overall major metabolite of caffeine in murine liver microsomes even at low caffeine concentrations was 137U (Fig. 1). However, this metabolite was mainly formed by enzymes other than murine CYP1A2 (Table 3), as this metabolite was present in large amounts also in the CYP1A2-null mice. Likewise, formation of 137U in human liver microsomes is also only in part attributable to CYP1A2. In agreement with our data, it was repeatedly reported that the contribution of the 8-hydroxylation pathway to overall primary caffeine metabolism in human liver microsomes is much greater than *in vivo* [21,22]. It appears that this phenomenon can be explained by the low affinity of caffeine to human CYP1A2 for the formation of 137U and the lower caffeine concentrations *in vivo* compared to those required for microsomal incubations. Indeed, isolated CYP1A2 does not form 137U to a large extent [2], suggesting that in human liver microsomes CYP1A2 is not the enzyme mainly responsible for the formation of 137U. It appears that CYP3A enzymes account for this reaction, albeit with a low affinity [17].

Thus, regarding 137U formation as an *in vitro* "artefact" presumably caused by the presence of enzymes other than CYP1A2 in both human and murine microsomal preparations, our data show that both human and murine CYP1A2 have close similarities in the primary metabolic steps of caffeine.

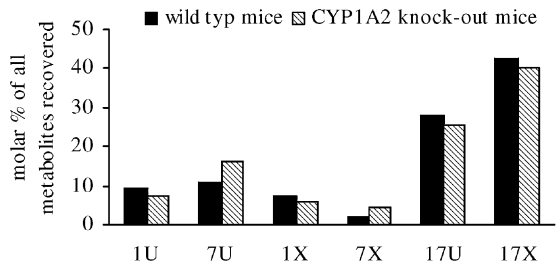

Fig. 4. Urinary excretion of paraxanthine metabolites (molar% of all metabolites recovered) after an i.p. 10 mg kg^{-1} dose to wild-type and CYP1A2-null mice (pooled samples of N = 5 male animals). Urine was collected for 24 hr and analysed by HPLC. Recovery for the sum of metabolites was 32.8% of the dose in wild-type mice and 45.2% of the dose in null mice, respectively. (For identification of metabolites, see legends to Tables 1 and 4).

4.2. Paraxanthine metabolism

It has been proposed that in man paraxanthine is metabolised by CYP1A2 to an unknown unstable intermediate

with open ring structure [23,24]. *In vivo*, this intermediate is acetylated by *N*-acetyltransferase type 2 (NAT2) to form AFMU, or the ring is closed to form 1-methylxanthine. From urinary recovery studies in man, it is known that 7-demethylation is the main primary *in vivo* elimination pathway of paraxanthine accounting for 67% of total clearance [25]. Accordingly, in studies with cDNA-expressed human enzymes, CYP1A2 was able to metabolise paraxanthine, and the main product was 1-methylxanthine [19]. *In vivo*, urinary metabolic ratios reflecting paraxanthine metabolism in man significantly correlated to hepatic CYP1A2 activity and content [7], further supporting the relevance of this enzyme in elimination of paraxanthine. Our *in vitro* data of human microsomal paraxanthine metabolism confirmed the important role of the 7-demethylation pathway.

In mice, our results show that enzymes additional to CYP1A2 must be involved in paraxanthine metabolism. CYP1A2 was responsible for the majority of 7-demethylation of paraxanthine in murine microsomes, but not for the major fraction of 1-demethylation (Table 3). The high affinity site of 1X formation was not found in CYP1A2-null mice, which shows the important role of CYP1A2 for 7-demethylation also in mice.

However, the *in vitro* findings did not correspond to *in vivo* studies as there was no difference in elimination of paraxanthine between CYP1A2-null and wild-type mice (Figs. 3 and 4). Although *in vitro* data suggest an important role of murine CYP1A2 in paraxanthine 7-demethylation, there was no obvious difference between the two mouse strains in excretion of 7-demethylated products (1-methylurate and 1-methylxanthine) in urine (Fig. 4). The presence of 1-methylxanthine reported in the urine of CYP1A2-null mice treated with caffeine [10] supports the important role of enzymes other than CYP1A2 to 7-demethylation of paraxanthine *in vivo*. As no parameters determining pharmacokinetics of paraxanthine differed between null and wild-type mice except the lack of expression of CYP1A2 [10], it must be concluded that *in vivo* paraxanthine is not metabolised by murine CYP1A2 to a relevant extent. This is in contrast to the situation found in humans.

4.3. *In vitro inhibition*

As clinical relevant interactions of quinolones and drugs have been reported repeatedly [26–28], an animal model enabling the study of such interactions would be of interest. The quinolones used here are known to cause relevant inhibition of human CYP1A2 dependent metabolism [29–34]. Inhibitory effect of norfloxacin or pefloxacin (500 μM) reached 55.7 and 22.0% in incubations of human liver microsomes with the substrate caffeine (500 μM), respectively [15], and *in vivo* pefloxacin caused a 2-fold decrease of caffeine clearance in man [14]. It is supposed that the inhibitory effect of pefloxacin is caused at least in part by norfloxacin, which is formed by a CYP1A2 mediated 4-demethylation [14]. The known inhibitory effects of these quinolones on human CYP1A2 were confirmed by our results. In contrast, in murine hepatic microsomes quinolones did not exert an inhibition of caffeine 3-demethylation. A similar species difference with respect to CYP1A2 inhibition was reported for furafylline. This methylxanthine derivative is a highly potent and selective inhibitor of human CYP1A2, but the affinity of furafylline was 1000-fold lower to rat CYP1A2 [33], and furafylline had also a much weaker inhibitory effect on murine CYP1A2 [35] as compared to its effect on the human enzyme.

5. Conclusion

It was shown previously that null mice are an useful model to examine metabolic pathways and to attribute these to specific enzymes [36–39]. Null mice also proved to be a suitable model for the investigation of consequences of metabolic activation of procarcinogens [40]. Thus, it is not deniable that murine models are important for the understanding of metabolism of xenobiotics and related carcinogenesis in humans. But extrapolation of data obtained with murine models may be inaccurate in certain cases as shown in this and former studies [41]. We showed that for some low affinity ligands to CYP1A2, namely paraxanthine, norfloxacin, and pefloxacin, binding to the enzyme and metabolic consequences has clear differences between mice and man. These results suggest that an interspecies comparison is required for the metabolism of the individual xenobiotic interacting with CYP1A2 prior to the use of mice models to predict its toxicity and/or pharmacological activity in man.

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