Interaction of ibuprofen and probenecid with drug metabolizing enzyme phenotyping procedures using caffeine as the probe drug

Fatima Vrtic, Walter E. Haefeli,[1] Jürgen Drewe, Stephan Krähenbühl & Markus Wenk

Division of Clinical Pharmacology and Toxicology, Department of Internal Medicine, University Hospital, Basel, Switzerland, and [1]Department of Internal Medicine VI, Clinical Pharmacology and Pharmacoepidemiology, University Hospital, Heidelberg, Germany

Aims To examine the suspected inhibitory potential of the over-the-counter (OTC) drug ibuprofen on N-acetyltransferase 2 (NAT2) *in vitro* and *in vivo* and the possible implications for phenotyping procedures using caffeine as probe drug.

Methods We first studied the inhibitory effect of ibuprofen on NAT2 *in vitro*, using human liver cytosol and sulfamethazine as substrate. *In vivo* 15 fast and 15 slow acetylating healthy volunteers were treated with a single dose of ibuprofen (800 mg) orally and phenotyped for NAT2, CYP1A2, and xanthine oxidase (XO) with caffeine as probe drug before and during drug treatment. Because of unexpected *in vivo* results with ibuprofen this study was repeated in 20 healthy volunteers with probenecid, a model substrate of renal organic anion transport (OAT). For phenotyping tests a urine sample was collected 6 h after caffeine (200 mg) intake. The caffeine metabolites acetyl-6-formylamino-3-methyluracil (AFMU), 1-methylxanthine (1MX), 1-methyluric acid (1MU), and 1,7-dimethyluric acid (17MU) were quantified by HPLC, and the corresponding metabolic ratios for CYP1A2, NAT2, and XO were then calculated. Genotyping for NAT2 was performed with standard PCR-RFLP methods.

Results *In vitro*, with human liver cytosol an inhibition by ibuprofen of the acetylation of sulfamethazine with K_i values between 2.2 and 3.1 mM was observed. Surprisingly, *in vivo* a significant ($P < 0.001$) increase of the acetyl-6-formylamino-3-methyluracil/1-methylxanthine (AFMU/1MX) urinary ratio from 0.97 ± 0.16 to 1.08 ± 0.18 (95% CI on the difference 0.049, 0.170) was found, indicating an apparent elevation of NAT2 activity. In contrast, no change was observed for the ratios used for XO and CYP1A2. Because an induction of NAT2 could be excluded, an interaction of ibuprofen with the tubular secretion of some of the caffeine metabolites was assumed. To prove this assumption, the *in vivo* study was repeated with probenecid, a model substrate of the renal OAT system. Again, a prominent elevation of the AFMU/1MX ratio from 0.97 ± 0.21 to 1.53 ± 0.35 was found ($P < 0.002$; 95% CI on the difference 0.237, 0.876), but also the XO ratio 1MU/1MX was significantly ($P < 0.0001$) increased from 1.34 ± 0.09 to 2.24 ± 0.14 (95% CI on difference 0.735, 1.059) due to a reduction of 1MX excretion.

Conclusions Substrates of OAT interact with renal excretion of caffeine metabolites and may falsify NAT2 and XO phenotyping results. Other phenotyping procedures, which are based on urinary metabolic ratios, should also be validated in this respect, especially in patients under polymedication.

Keywords: caffeine metabolites, ibuprofen, N-acetyltransferase 2, organic anion transport, phenotyping

Introduction

Genetic polymorphism of N-acetyltransferase 2 (NAT2) results in the occurrence of slow and rapid acetylator phenotypes, who differ in the risk of side-effects of

Correspondence: Dr Markus Wenk, Division of Clinical Pharmacology and Toxicology, Department of Internal Medicine, University Hospital, CH-4031 Basel, Switzerland. Tel.: + 41 61 265 2395; Fax: + 41 61 265 5401; E-mail: markus-aloisius.wenk@unibas.ch.

certain drugs and in the development of malignancies [1–4]. Phenotyping of this polymorphism is routinely performed by ingesting a single oral dose of caffeine as probe drug and the subsequent determination of the ratio of caffeine metabolites in urine [5, 6]. A good agreement between phenotype and genotype has been observed in most studies, especially with healthy volunteers [7]. However, in some studies with severely ill patients being treated with many drugs, discrepancies have been observed between NAT2 phenotype and genotype [8, 9], for example by the unexplained predominance of slow acetylators in patients with AIDS [10]. We assumed that drug interactions might account for these discrepant observations [11], especially in patients taking over-the-counter (OTC) drugs, which are often used in an uncontrolled fashion. Indeed, recently we found a significant inhibition of NAT2 activity by the OTC drug acetaminophen [12]. The nonsteroidal anti-inflammatory agent ibuprofen is another widely used OTC drug. Although ibuprofen is not a substrate of NAT2, the former may cause a noncompetitive enzyme inhibition at high drug concentrations, as observed *in vitro* [13, 14]. Thus, clinically significant inhibition of NAT2 by ibuprofen is possible.

Our primary aim was to investigate the interaction potential of ibuprofen with NAT2 *in vitro* and *in vivo*, using sulfamethazine and caffeine, respectively. A discrepancy between our *in vitro* and *in vivo* results led to an additional *in vivo* experiment replacing ibuprofen by probenecid. We suspected that ibuprofen, a known substrate of the renal organic anion transport (OAT) [15], might interfere with the renal excretion of the caffeine metabolites.

Subjects and methods

Materials

Ibuprofen tablets were obtained from Knoll (Liestal, Switzerland) and, probenecid tablets were from Merck, Sharp & Dome-Chibret (Glattbrugg, Switzerland). Acetyl-6-formylamino-3-methyluracil (AFMU) was generously supplied by Nestec (Vevey, Switzerland), and methylxanthines and uric acids were from Fluka (Buchs, Switzerland). Ibuprofen for analytical purposes, flurbiprofen, and sulfamethazine were from Sigma (Buchs, Switzerland) and N-acetylsulfamethazine from INC (Costa Mesa, CA, USA). All solvents and buffer substances were purchased from Merck (Darmstadt, Germany) and were of analytical grade. Caffeine capsules for caffeine tests were prepared by the Institute of Hospital Pharmacy, University Hospital (Basel, Switzerland). Double distilled water was used for the preparation of all solutions.

Protocols

In vitro *N-acetyltransferase 2 assay*

Inhibition of NAT2 in liver cytosol by ibuprofen was investigated using genotyped human liver tissue from one fast and one slow acetylator donor prepared as described previously [11]. NAT2 activity was measured by monitoring the formation of N-acetyl-sulfamethazine from sulfamethazine by HPLC [16]. Substrate concentrations were 10, 30, and 90 μM, covering the previously determined K_m values for the slow (23 μM) and fast (73 μM) acetylator liver specimens. Ibuprofen was added to the reaction solution to give final concentrations of 0, 0.27, 1.35, and 2.7 mM, corresponding to the range of therapeutic to toxic concentrations [24]. The reaction was stopped with 10 μl 15% $HClO_4$, and the precipitated proteins were removed by centrifugation for 3 min at 30 000 *g*. An aliquot of 50 μl of the supernatant was directly injected onto the HPLC column. All incubations were carried out in triplicate.

In vivo *ibuprofen study*

Subjects

After approval had been obtained from the Ethics Committee of the University Hospital, Basel, Switzerland, 15 previously genotyped fast and 15 slow acetylating healthy adult volunteers (mean age 34 years, 18 females, 12 males, seven smokers) were enrolled in the study with ibuprofen (part 1 of the study) after giving written informed consent. The subjects were not taking any medication from the week before until the end of the study, with the exception of four females who were taking oral contraceptives. All participating volunteers were healthy and had normal liver and kidney function. Individuals with known adverse drug reactions to ibuprofen or probenecid, or known caffeine intolerance were excluded from the study.

In this open study all 30 subjects abstained from methylxanthine-containing food and beverages from 12 h before until the end of the study. Phenotyping for NAT2 was performed with caffeine as the probe drug at the first (baseline) and the second day (treatment with ibuprofen). The subjects received a capsule of 200 mg caffeine p.o. and a spot urine sample was collected after 6 h in a 10-ml tube containing 200 mg of ascorbic acid and frozen at -20°C until analysis. On the following day (day 2) the subjects received 800 mg of ibuprofen (Brufen®) orally 2 h prior to caffeine administration, and again a 6-h spot urine sample was collected for phenotyping. On day 2 a venous blood sample was drawn 2 h after intake of ibuprofen for analysis of the latter used as a measure of compliance.

In vivo *probenecid study*

The protocol was identical to that for the study with ibuprofen, except that only 20 out of the 30 volunteers took part (mean age 32 years, 14 females and six males, 10 genotyped fast and 10 slow acetylators). The interval between the two studies was 5 months. On the day after phenotyping with caffeine for the baseline enzyme activities (day 2) the subjects received probenecid (Benemid®) tablets (2 × 500 mg) p.o. 3 h prior to caffeine intake (200 mg p.o.).

Analytical procedures

Determination of caffeine metabolites in urine

The pH of thawed urine was adjusted to less than 3.5 with acetic acid and an aliquot of 200 µl was vortexed for 5 s with 25 µl of internal standard solution containing 1,9-dimethyluric acid (1.2 mg l^{-1}) and 135 mg ammonium sulphate. After extraction with 6 ml chloroform:isopropanol (95:5, v/v) the organic phase was evaporated to dryness at 30°C under N_2. The residue was dissolved in 750 µl of 10% methanol in 0.05% acetic acid and the following caffeine metabolites were analysed by HPLC: acetyl-6-formylamino-3-methyluracil (AFMU), 1-methylxanthine (1MX), 1-methyluric acid (1MU), and 1,7-dimethyluric acid (17MU). Caffeine metabolites were separated by HPLC on a Superspher® 100 RP-18 column (LiChroCART® 250-4; Merck, Dietikon, Switzerland). The solvent used consisted of a ternary gradient of phase A (6% methanol), phase B (6% acetonitrile), and phase C (25% acetonitrile), all in 0.05% acetic acid. The settings for the gradient were as follows: 0–10 min 100% A; 10–14 min 50% A and 50% B; 14–20 min 35% A, 35% B, and 30% C; 20–30 min 100% C; re-equilibration with A from 30 to 50 min. The flow was 0.86 ml min^{-1} at a temperature of 32°C, the injection volume was 15 µl, and the diode array detector was set at 280 nm. The within-assay coefficients of variation (CV) for AFMU, 1MU, 1MX, 17MU, and 17MX in the concentration range between 60 and 170 µmol l^{-1} were 1.4, 1.0, 1.5, 2.0 and 2.2%, respectively. The corresponding between-assay CVs were 2.5, 3.8, 9.0, 4.3, and 8.9%, respectively. The chromatogram did not show any interfering peaks following analysis of samples from subjects who had taken ibuprofen or probenecid.

Quantification of ibuprofen in serum

Ibuprofen was measured in serum after extraction using the HPLC method described by Geisslinger *et al.* [19]. Briefly, a 500-µl serum aliquot was acidified by adding 100 µl of 2 M hydrochloric acid. The compounds of interest were extracted into 5 ml of ice-cold hexane-diethyl ether (8:2, v/v) containing 20.5 µM flurbiprofen as internal standard. After centrifugation for 5 min at 1500 ***g***, 4.5 ml of the organic layer were removed and evaporated to dryness under a gentle stream of dry nitrogen. The residue was dissolved in 500 µl of mobile phase and analysed by HPLC with u.v. detection at 220 nm. Ibuprofen was separated on a Superspher® RP-18e column (LiChroCART® 250-4; Merck, Dietikon, Switzerland). The mobile phase consisted of methanol–water (65:35, v/v), containing 1 ml l^{-1} of concentrated phosphoric acid. The flow was maintained at 0.75 ml min^{-1} at a temperature of 40°C. A five-point standard curve was constructed for ibuprofen concentrations between 7.3 and 730 µM. The retention times were 13.7 min for ibuprofen and 10.2 min for flurbiprofen. The detection limit was 0.45 µM for ibuprofen. Within- and between-assay coefficients of variation for ibuprofen at a concentration of 368 µmol l^{-1} were 2.9%, and 3.6%, respectively.

Determination of creatinine in urine

Creatinine in all urine samples was measured by an enzymatic F DAOS method (Wako Chemicals, Neuss, Germany) on a Hitachi 917 clinical chemistry analyser.

Genotyping for NAT2

A 5-ml EDTA blood sample was drawn from each subject for genomic DNA isolation by column extraction with the QIAamp® DNA blood kit (Qiagen, Basel, Switzerland). *NAT2* was analysed by PCR-RFLP for the wild-type allele *NAT2*4* and the most common alleles *NAT2*5*, *NAT2*6*, *NAT2*7* and *NAT2*14*, which account for almost all of the slow acetylator phenotypes in Caucasians [20, 21].

Data analysis

For the determination of the kinetic constants (K_m, V_{max}) data were fitted by nonlinear regression to the Michaelis–Menten equation using the GraphPad Prism® 3.0 program (GraphPad Software, Inc. San Diego, CA, USA).

The inhibition constant (K_i) of ibuprofen in liver cytosol was calculated on the basis of a Dixon Plot with a kinetic software package (EKI 1.0; Institute of Physiological Chemistry, University of Tübingen, Germany) using means from triplicate determinations. The estimate of the apparent K_i values and the nature of inhibition were obtained from Dixon plots, where the apparent K_i was given by the intersection point of the linear regression lines for data sets of 1/v against the concentration of inhibitor.

Statistical analysis was performed using the SPSS software package (SPSS, Chicago, IL, USA). A nonparametric Wilcoxon test for paired data was used to analyse changes in metabolic ratios before and after drug ingestion and for the creatinine-corrected urinary metabolite concentrations. A two-sided P-value <0.05 was consid-

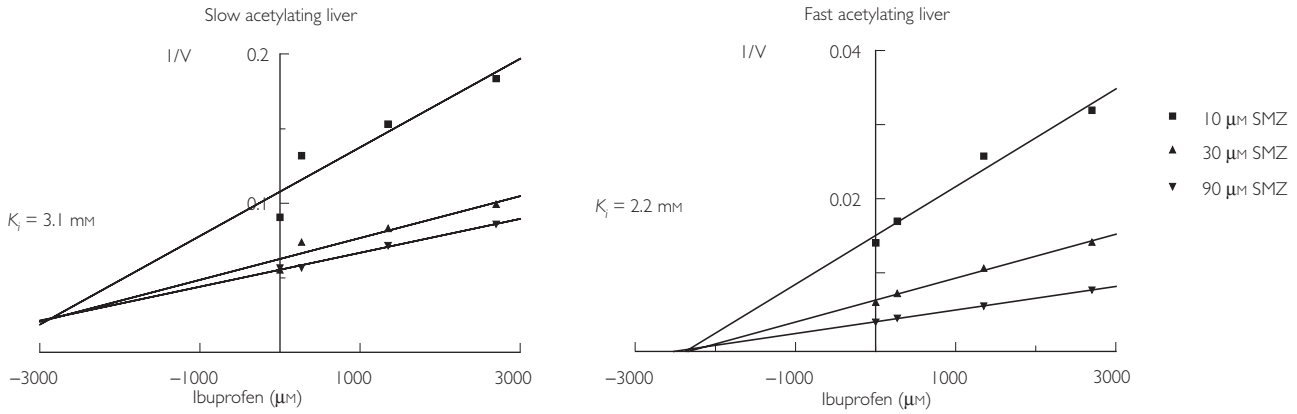

Figure 1 Dixon plot showing inhibition of sulfamethazine (SMZ) acetylation by ibuprofen human liver homogenates (for one fast (left) and one slow (right) acetylator). V is expressed as pmol min^{-1} mg^{-1} protein.

ered to be significant. Data are presented as mean ± standard error of the mean (s.e.m.).

The molar ratios AFMU/1MX and AFMU/(AFMU +1MX +1MU) were used for NAT2 phenotyping. For the AFMU/1MX ratio an antimode of 0.55 was used to discriminate slow from fast acetylators [7].

Results

As shown in Figure 1, using ibuprofen inhibited the acetylation of the NAT2 substrate sulfamethazine in an apparent noncompetitive fashion in the liver cytosol from the slow acetylator (K_i 3.1 mM) and competitively in that from the fast acetylator (K_i 2.2 mM).

The effect of ibuprofen on markers of NAT2 activity *in vivo* in 30 previously genotyped healthy volunteers is shown in Figure 2 and Table 1. The serum concentration of ibuprofen 2 h after drug intake was 209 ± 9 μmol l^{-1}. In contrast to the *in vitro* ibuprofen, administration increased the AFMU/1MX ratio significantly in both slow and fast acetylators, suggesting a higher activity of NAT2. A similar trend was found for the ratio AFMU/(AFMU +1MX +1MU), another *in vivo* marker of NAT2 activity [18], which reached statistical significance in fast acetylators. Fast and slow acetylators were well separated by both phenotyping ratios, and a 100% correlation between phenotype and genotype was found before and during ibuprofen treatment. In contrast to the metabolic ratios reflecting NAT2 activity, the ratios 1MU/1MX (a marker for xanthine oxidase activity) and (AFMU +1MX +1MU)/17MU (a marker for CYP1A2 activity) were not significantly affected by treatment with ibuprofen (Table 1).

Because 1MX and 1MU are at least partially eliminated by renal tubular secretion [22] and because ibuprofen and its metabolites can interfere with renal secretion of drugs [23], the effect of ibuprofen on the renal elimination of IMX and IMU was investigated after normalization with creatinine concentrations to correct for urine dilution (Table 2). Ibuprofen had a tendency to increase the renal excretion of AFMU and to decrease the renal excretion of 1MX and 17MU.

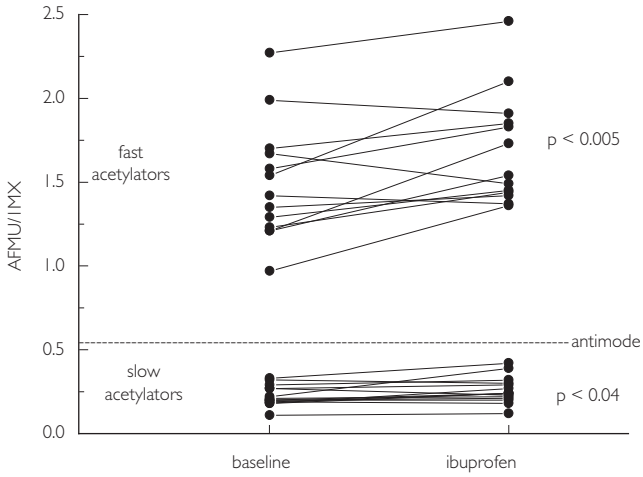

Figure 2 AFMU/1MX ratios in urine in 15 fast and 15 slow acetylators before (baseline) and during treatment with ibruprofen. An antimode of 0.55 separates fast from slow acetylators.

To evaluate the role of the OAT system in the renal elimination of AFMU, 1MU, and 1MX *in vivo*, we performed a study with probenecid, a substrate and inhibitor of the OAT system. As shown in Table 2, probenecid caused a large and significant ($P < 0.0001$) reduction of the excretion of 1MX. Correspondingly, the AFMU/1MX ratio also increased significantly, in both slow and fast metabolizers (Figure 3 and Table 3). The alternative ratio AFMU/(AFMU +1MU +1MX) increased slightly in subjects treated with probenecid, but this reached statistical significance only in slow acetylators ($P = 0.048$). The ratio (AFMU +1MX +1MU)/17MU

Table 1 Effect of ibuprofen on different metabolic caffeine ratios commonly used for NAT2 phenotyping (a, b), and as indices of CYP1A2 (c), and XO activity (d) in 15 healthy fast and 15 slow acetylators.

	AFMU/1MX (a)	*P-value*	*AFMU/(AFMU + 1MX + 1MU) (b)*	*P-value*	*(AFMU + 1MX + 1MU)/17MU (c)*	*P-value*	*1MU/1MX (d)*	*P-value*
All volunteers (n = 30)								
Baseline value	0.97 ± 0.16		0.25 ± 0.03		3.83 ± 0.27		1.11 ± 0.05	
During ibuprofen	1.08 ± 0.18	< 0.001	0.27 ± 0.03	< 0.04	4.00 ± 0.34	0.52	1.21 ± 0.05	0.26
95% CI	0.049, 0.170		0.0019, 0.0248		–0.550, 0.471		–0.028, 0.222	
Fast acetylators (n = 15)								
Baseline value	1.72 ± 0.18		0.40 ± 0.02		4.01 ± 1.19		1.28 ± 0.32	
During ibuprofen	1.91 ± 0.17	< 0.005	0.43 ± 0.02	0.13	4.34 ± 2.07	0.80	1.34 ± 0.25	0.56
95% CI	0.083, 0.291		–0.0012, 0.0378		–0.454, 0.984		–0.102, 0.295	
Slow acetylators (n = 15)								
Baseline value	0.22 ± 0.02		0.10 ± 0.01		3.66 ± 1.73		0.95 ± 0.15	
During ibuprofen	0.26 ± 0.02	< 0.03	0.12 ± 0.01	0.27	3.51 ± 1.65	0.45	1.09 ± 0.25	0.36
95% CI	0.0053, 0.0593		–0.0044, 0.0213		–1.06, 0.37		–0.062, 0.257	

Data are given as mean ± s.e.m. with 95% CI on differences. AFMU, Acetyl-amino-6-formylamino-3-methyluracil; 1MX, 1-methylxanthine; 1MU, 1-methyluric acid.

Table 2 Effect of ibuprofen and probenecid on urinary concentrations of acetyl-amino-6-formylamino-3-methyluracil (AFMU), 1-methylxanthine (1MX), 1-methyluric acid (1MU), and 1,7-dimethyluric acid (17MU) normalized to creatinine concentration.

	AFMU	*P-value*	*1MX*	*P-value*	*1MU*	*P-value*	*17MU*	*P-value*
Ibuprofen (n = 30)								
Baseline value	10.8 ± 1.6		15.2 ± 1.9		16.7 ± 2.0		10.2 ± 0.9	
During ibuprofen	11.9 ± 2.1	0.40	14.6 ± 2.0	0.49	16.8 ± 2.1	0.98	8.9 ± 1.3	0.09
95% CI	–0.69, 3.03		–2.64, 1.42		–2.68, 2.89		–3.18, 0.51	
Probenecid (n = 20)								
Baseline value	10.9 ± 2.1		14.9 ± 1.9		19.6 ± 2.8		11.5 ± 1.3	
During probenecid	9.1 ± 1.6	0.08	9.6 ± 1.6	< 0.0005	21.0 ± 3.9	0.69	10.5 ± 1.2	0.27
95% CI	–3.51, –0.08		–7.35, –3.31		–3.25, 6.07		–3.61, 1.69	

Data are expressed as μmol l^{-1} of caffeine metabolite per mmol l^{-1} creatinine (mean ± s.e.m.) with 95% CI on differences.

was not affected by probenicid (Table 3). No subject reported an adverse reaction during any part of the study.

Discussion

Our study shows that ibuprofen inhibits NAT2 *in vitro* but leads to an apparent increase of NAT2 activity *in vivo*. The *in vitro* inhibition of NAT2 activity in the liver of a slow and a fast acetylator is in agreement with studies by Chung *et al*. [13, 14] of the *in vitro* inhibition of other forms of NAT. The K_i values obtained (2.2 and 3.1 mM in the livers from the slow and fast acetylators, respectively) are clearly above the maximum serum concentrations C_{max} of 0.1–0.4 mM, after therapeutic doses of ibuprofen in humans [24], suggesting that after normal ibuprofen doses no change in human NAT2 activity occurs, but not excluding the possibility [25]. In contrast, we observed *in vivo* an apparent elevation in NAT2 activity, if we applied the urinary ratio of the caffeine metabolites using the AFMU/1MX ratio, a common index for this enzyme. The alternative NAT2 ratio, AFMU/(AFMU +1MU +1MX), was affected only in fast acetylators, and the ratios for XO (1MU/1MX) and CYP1A2 ((AFMU +1MU +1MX)/17MU) showed no change during treatment with ibuprofen. Because NAT2 is thought to be not inducible and induction would be unlikely to occur within hours after treatment, we assumed an effect of ibuprofen on the renal excretion of some of the caffeine metabolites. Ibuprofen and other NSAIDs are known to inhibit the OAT system [15]. Although the knowledge on the mechanisms of the renal excretion of caffeine metabolites is limited, related compounds such as xanthine and uric acid are known substrates of OAT [26]. In addition, it has been shown that the renal clearance of 1MX and 1MU is higher than the glomerular filtration rate, suggesting that renal secretory

Table 3 Effect of probenecid on the different metabolic caffeine ratios commonly used for NAT2 phenotyping (a, b), and as indices of CYP1A2 (c), and XO (d) in 10 healthy fast and 10 slow acetylators.

	AFMU/1MX (a)	*P-value*	*AFMU/(AFMU + 1MX + 1MU) (b)*	*P-value*	*(AFMU + 1MX + 1MU)/17MU (c)*	*P-value*	*1 MU/1MX (d)*	*P-value*
All volunteers (n = *30)*								
Baseline value	0.97 ± 0.21		0.24 ± 0.04		4.82 ± 0.33		1.34 ± 0.09	
During probenecid	1.53 ± 0.35	< 0.002	0.26 ± 0.04	0.054	3.83 ± 0.37	0.25	2.24 ± 0.14	< 0.0001
95% CI	0.237, 0.876		−0.0012, 0.0303		−0.801, 0.309		0.74, 1.06	
Fast acetylators (n = *15)*								
Baseline value	1.74 ± 0.21		0.40 ± 0.02		3.88 ± 1.33		1.52 ± 0.44	
During probenecid	2.73 ± 0.42	< 0.004	0.42 ± 0.03	0.33	3.95 ± 1.58	0.77	2.52 ± 0.60	< 0.002
95% CI	0.47, 1.51		−0.014, 0.046		−0.644, 0.775		0.75, 1.26	
Slow acetylators (n = *15)*								
Baseline value	0.21 ± 0.18		0.09 ± 0.08		4.28 ± 1.68		1.17 ± 0.31	
During probenecid	0.33 ± 0.04	< 0.004	0.10 ± 0.01	< 0.05	3.70 ± 1.83	0.19	1.96 ± 0.48	< 0.002
95% CI	0.063, 0.189		0.0007, 0.0250		−1.40, 0.29		0.604, 0.189	

Data are given as mean ± s.e.m. with 95% CI on differences. AFMU, Acetyl-amino-6-formylamino-3-methyluracil; 1MX, 1-methylxanthine; 1MU, 1-methyuric acid.

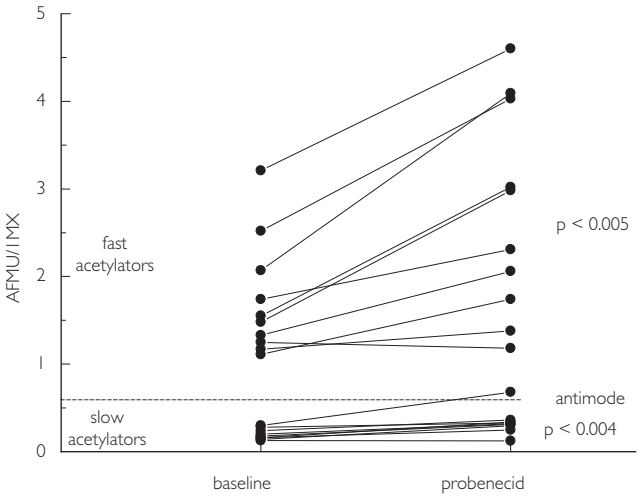

Figure 3 AFMU/1MX ratios in urine in 10 fast and 10 slow acetylators before (baseline) and during treatment with probenecid. An antimode of 0.55 separates fast from slow acetylators.

mechanisms are involved in their elimination [22], whereas AFMU is thought to be reabsorbed in the kidney [27]. Therefore, we hypothesized that a drug interaction between ibuprofen and caffeine metabolites at the level of the renal OAT system might best explain the results of our *in vivo* study. Such an interaction might have masked any inhibition of NAT2 activity by ibuprofen.

In order to test this hypothesis, we studied the effect of probenecid, a model substrate and inhibitor of the renal OAT system, on caffeine metabolic ratios. A significant elevation of the AFMU/1MX ratio was found, which led to the misclassification of one slow metabolizer. Apparent drug-induced conversion of slow to fast acetylators has not been reported previously [12, 28, 29]. In contrast to the study with ibuprofen, the xanthine oxidase marker ratio 1MU/1MX was almost doubled during probenecid administration. Because caffeine phenotyping was performed using an established protocol [5, 6], only spot urine samples were collected. This precludes precise determination of the cumulative amount of excreted metabolites. To estimate indirectly which of the caffeine metabolites were most affected by probenicid, the urinary concentrations were normalized to urinary creatinine concentrations. Whereas probenecid and ibuprofen significantly decreased the renal excretion of 1MX, the latter increased renal excretion of AFMU. However, the changes did not reach statistical significance. The interpretation of these data is difficult, because creatinine also undergoes tubular secretion, which may be impaired by probenecid and ibuprofen or its metabolites.

The OAT system is a complex family of related transporters with different substrate specificities [31–33]. Thus, it is possible that different OAT subtypes (OAT1–OAT4) are involved in the transport of probenecid, ibuprofen, and caffeine metabolites, respectively, which may explain some of the findings of the present study. In addition, a large number of other anionic drug transporting proteins are present in the kidney, including sodium/phosphate cotransporter type I (NPT1), multidrug resistance proteins (MRP1–MRP6), a subfamily within the ATP-binding cassette (ABC) transporter superfamily, and organic anion transporting polypeptides (OATPs) [33]. However, the molecular identity and exact localization in renal tissue of some of these transporters is still elusive.

Probenecid and to a lesser extent ibuprofen are potential inhibitors of most of these transporters [33, 35, 36], although ibuprofen is not efficiently transported by OAT1 [35] or OAT3 [36]. Considering the very large number of known endogenous and exogenous substrates of the OAT family [26, 31, 33], the potential for interactions with probe drugs used for enzyme phenotyping is considerable, especially in patients receiving polymedication. However, it is not yet clear to what extent these interactions are responsible for the reported discrepancies between phenotype and genotype [8–10].

As demonstrated previously [17], the present study indicates that the alternative ratio for NAT2 phenotyping AFMU/(AFMU +1MU +1MX) is less affected by drug interactions than the more commonly used ratio AFMU/1MX.

Genotyping could circumvent problems associated with phenotyping. However, the latter remains the method of choice for defining functional enzyme activity in patients. However, the majority of probe drugs for phenotyping have been evaluated in healthy volunteers or in well-defined patient populations and the number of safe and validated test procedures is still limited. This is especially true for phenotyping cocktails [39] because the potential for drug interactions increases with the addition of each probe drug.

In conclusion, this study indicates that the NAT2 phenotyping procedure using caffeine as a probe drug can be influenced by concomitant intake of probenecid or the widely used OTC drug ibuprofen and, therefore, probably by other substrates of the renal OAT and/or other transport systems. This drug interaction might also affect the determination of xanthine oxidase activity, whereas the urinary ratio used as a marker of CYP1A2 activity was not significantly affected by either drug. It remains to be determined whether other phenotyping procedures employing urinary metabolic ratios are affected by interactions involving renal excretion. Phenotyping procedures based on urinary ratios should be interpreted very carefully, especially in patients receiving multiple drug therapy.

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