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Mesalazine pharmacokinetics and NAT2 phenotype

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Abstract

Background Mesalazine undergoes extensive metabolism by *N*-acetylation. While there is some evidence for an involvement of *N*-acetyltransferase (NAT) type 1, a potential role of NAT type 2 (NAT2) in vivo has not been tested.

Methods In two studies in healthy young Caucasians, NAT2 phenotyping was carried out using a caffeine metabolic ratio in urine 4–6 h postdose. In study A, 1,000 mg mesalazine doses were given thrice daily for 5 days, and urine and blood samples were drawn during the last dosing interval. In study

B, a 1,000 mg single dose was given, and samples were taken for 48 h postdose. Pharmacokinetics of mesalazine and *N*-acetylmесalazine (LC-MS/MS) were calculated by noncompartmental methods.

Results NAT2 phenotype could be allocated unequivocally in 21 slow and 5 rapid acetylators in study A, and in 9 slow and 8 rapid acetylators in study B. Geometric mean (CV%) values in study A for slow [rapid] acetylators were as follows: mesalazine AUC 11.1 $\mu\text{g}/\text{mL}\cdot\text{h}$ (51%) [12.0 $\mu\text{g}/\text{mL}\cdot\text{h}$ (52%)], *N*-acetylmесalazine AUC 27.7 $\mu\text{g}/\text{mL}\cdot\text{h}$ (32%) [30.5 $\mu\text{g}/\text{mL}\cdot\text{h}$ (27%)], mesalazine Ae 8.53% (89%) [9.03% (52%)], *N*-acetylmесalazine Ae 31.4% (46%) [32.2 (41%)]. Values in study B were as follows: mesalazine AUC 3.45 $\mu\text{g}/\text{mL}\cdot\text{h}$ (113%) [2.36 $\mu\text{g}/\text{mL}\cdot\text{h}$ (87%)], *N*-acetylmесalazine AUC 21.3 $\mu\text{g}/\text{mL}\cdot\text{h}$ (29%) [18.0 $\mu\text{g}/\text{mL}\cdot\text{h}$ (39%)], mesalazine Ae 0.2% (256%) [0.1% (359%)], *N*-acetylmесalazine Ae 30.9% (44%) [18.1% (84%)]. Higher AUC and Ae values for mesalazine in steady state study indicate saturation of mesalazine metabolism. Statistics provided no evidence for a true difference in mesalazine pharmacokinetics between slow and rapid acetylators, and no significant correlation between NAT2 activity and any mesalazine pharmacokinetic parameter was found.

Conclusion NAT2 has no major role in human metabolism of mesalazine in vivo.

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Keywords Mesalazine · NAT2 · Genetic polymorphism ·
Caffeine · Phenotyping

Introduction

Mesalazine is an anti-inflammatory drug mainly used in the treatment of ulcerative colitis but also with some value in Crohn's disease. It undergoes extensive metabolism by

N-acetylation [1]. On average, concentrations of *N*-acetylmessalazine in plasma and *N*-acetylmessalazine amounts excreted in urine exceed those of the parent compound more than twofold. Two enzymes are known to primarily mediate *N*-acetylation in humans, *N*-acetyltransferase type 1 (NAT1) and type 2 (NAT2). However, currently it is not clear which enzyme plays the key role in *N*-acetylation of mesalazine. In the mouse (which has three NATs), all NATs were able to metabolize mesalazine in vitro, while murine NAT2 had the highest activity [2]. In an in vitro study with human enzymes, both NAT1 and NAT2 were able to mediate 4-aminosalicylic acid *N*-acetylation (mesalazine is 5-aminosalicylic acid), with NAT1 having a 131-fold higher intrinsic clearance than NAT2 [3].

Both human enzymes are expressed at high levels in the human intestine and in the liver [4]. *N*-acetylation of mesalazine is highly variable [5]. As both human NATs have genetic polymorphisms, this finding provides no information on the identity of the enzyme. In one study, pharmacokinetics of mesalazine and sulfapyridine, the two bacterial metabolites of sulfasalazine formed by reductive cleavage of the azo bond linking these two components, were determined. While acetylation of sulfapyridine, a known NAT2 substrate, was polymorphic, this could not be shown for mesalazine [6]. Mesalazine pharmacokinetics also did not co-segregate with NAT2 activity as assessed by sulfadimidine, but this study was conducted in six subjects only [7]. Furthermore, under saturation conditions for mesalazine, no relationship of ex vivo mucosal *N*-acetylation of mesalazine to systemic *N*-acetylation of sulfapyridine following systemic administration of sulfasalazine was found [8]. On the other hand, in two studies there was also no difference with regard to mesalazine tolerability and therapeutic response between NAT1 genotypes [9, 10].

In summary, current evidence for a major role of NAT1 in acetylation of mesalazine in vivo is partially contradictory, and the evidence suggesting that NAT2 has no major role originates from studies with relevant methodological limitations. Thus, the objective of the present evaluation is to assess the role of NAT2 in the *N*-acetylation of mesalazine in humans in vivo by comparison of mesalazine pharmacokinetics to NAT2 phenotype based on a validated caffeine-based NAT2 metric.

Volunteers and methods

A total of 28 and 18 young female and male Caucasian volunteers were included in two separate studies A and B, respectively. The studies were approved by the Ethics Committee of the University of Cologne. Each study participant provided written informed consent prior to inclusion in the study. Participants were considered to be

healthy based on a screening examination including physical examination, medical history, vital signs, ECG recording, clinical chemistry tests, urinalysis, and drug and alcohol tests. Smokers could be included if they did not smoke more than 10 cigarettes per day.

In both studies, NAT2 phenotyping was carried out using a urine sample collected 4–6 h after a 150 mg caffeine test. Caffeine metabolites were quantified by LC-MS/MS, and the molar ratio of caffeine metabolites (AFMU+AAMU)/(AFMU+AAMU+1X+1U) (for abbreviations, see legend to Fig. 1) was used as a validated NAT2 metric [11]. Based on maximum log likelihood analysis, individuals with a ratio below 0.23 were classified as slow acetylators, those with values above 0.26 were considered as rapid acetylators (Fig. 1). Those with intermediate values were rated as “unclassified.”

Individuals were studied under standardized conditions. Alcohol and grapefruit products were prohibited before and during the study. The volunteers had to remain in the clinical pharmacology ward from the evening prior to dosing on the profiling days until the last sample was taken.

In study A, no food intake was allowed from 8 h before until 2 h after each morning administration, from 2 h before until 2 h after each afternoon administration and from 3.5 h before until 8 h after each late evening administration, with the exception of a snack given 10 min prior to each administration of the study medication. Standardized meals were given 12 h prior to and 2 and 5 h after the last mesalazine dose on the profiling day. In study B, subjects fasted from 10 h prior to mesalazine administration until 6 h thereafter, and they remained in a supine position for 6 h postdose. Meals were given 6, 9, 12, and 16 h after mesalazine administration. The generally allowed fluid intake was 1.8–2.0 l daily. In both studies, no fluid intake

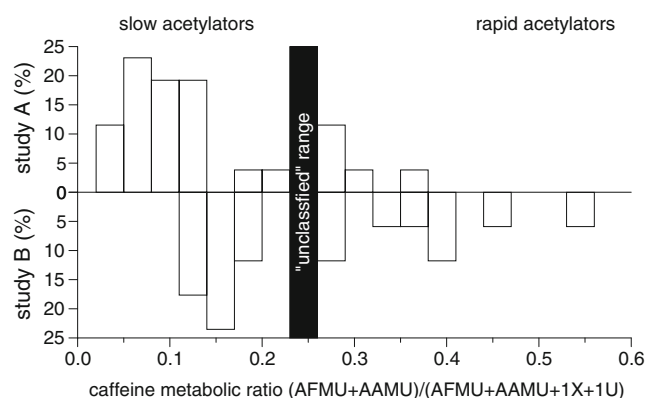


Fig. 1 Relative frequency distribution of NAT2 activity. Individuals with a caffeine metabolic ratio below 0.23 were classified as slow acetylators, those with a ratio above 0.26 as rapid acetylators, and those in between as “unclassified.” 1X 1-Methylxanthine, 1U 1-methyluric acid, AFMU 5-acetylamino-6-formylamino-3-methyluracil, AAMU 5-acetylamino-6-amino-3-methyluracil

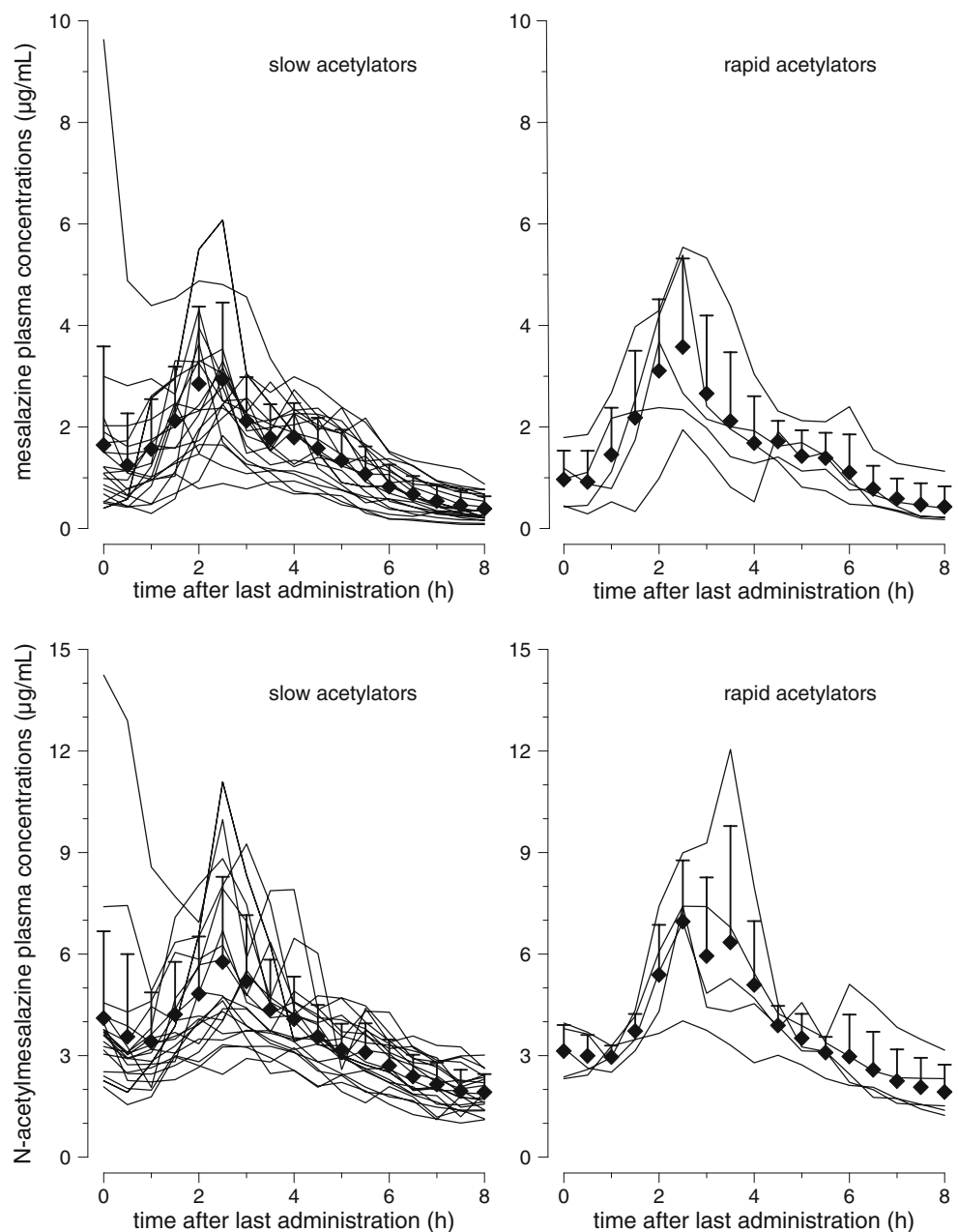
additional to that provided for drug intake (180 ml) was allowed from 2 h before to 2 h after drug intake. Thereafter, 120 mg of water was administered every hour.

Mesalazine doses were 1,000 mg given as two 500-mg commercial prolonged release tablets (Pentasa). In the multiple-dose study A, this 1,000 mg dose was given thrice daily for 5 days, and urine samples (0–4 and 4–8 h postdose) and 17 blood samples were drawn in the last dosing interval. In the 1,000 mg single-dose study B, 21 blood samples were taken over 48 h postdose, and urine was collected 0–4, 4–8, 8–12, 12–24, and 24–48 h postdose.

Plasma and urine samples of mesalazine and *N*-acetylmesalazine were analyzed by validated LC-MS/MS assays.

For mesalazine, plasma samples (0.1 mL) were precipitated by addition of 200 μ L of acetonitrile containing the internal standard. After thorough mixing, the samples were centrifuged for 10 min at 3,280 *g* at approximately +4°C, and the supernatant was diluted (1:4) with ammonium acetate buffer. Then 60 μ L of each sample was chromatographed on a reversed-phase column (Nucleosil 100–5 Protect 1), eluted with an isocratic solvent system consisting of ammonium acetate (pH 3.5) buffer and acetonitrile (75/25, v/v), and monitored by LC-MS/MS with an SRM method as follows: precursor \rightarrow product ion for mesalazine m/z 152 \rightarrow m/z 108 and m/z 266 \rightarrow m/z 223 for internal standard; all analyses were in negative mode. Under these

Fig. 2 Individual and mean \pm SD mesalazine (*upper panel*) and *N*-acetylmessalazine (*lower panel*) concentration vs. time profiles in the last dosage interval following multiple-dose administration of 1,000 mg mesalazine thrice daily for 5 days (study A)



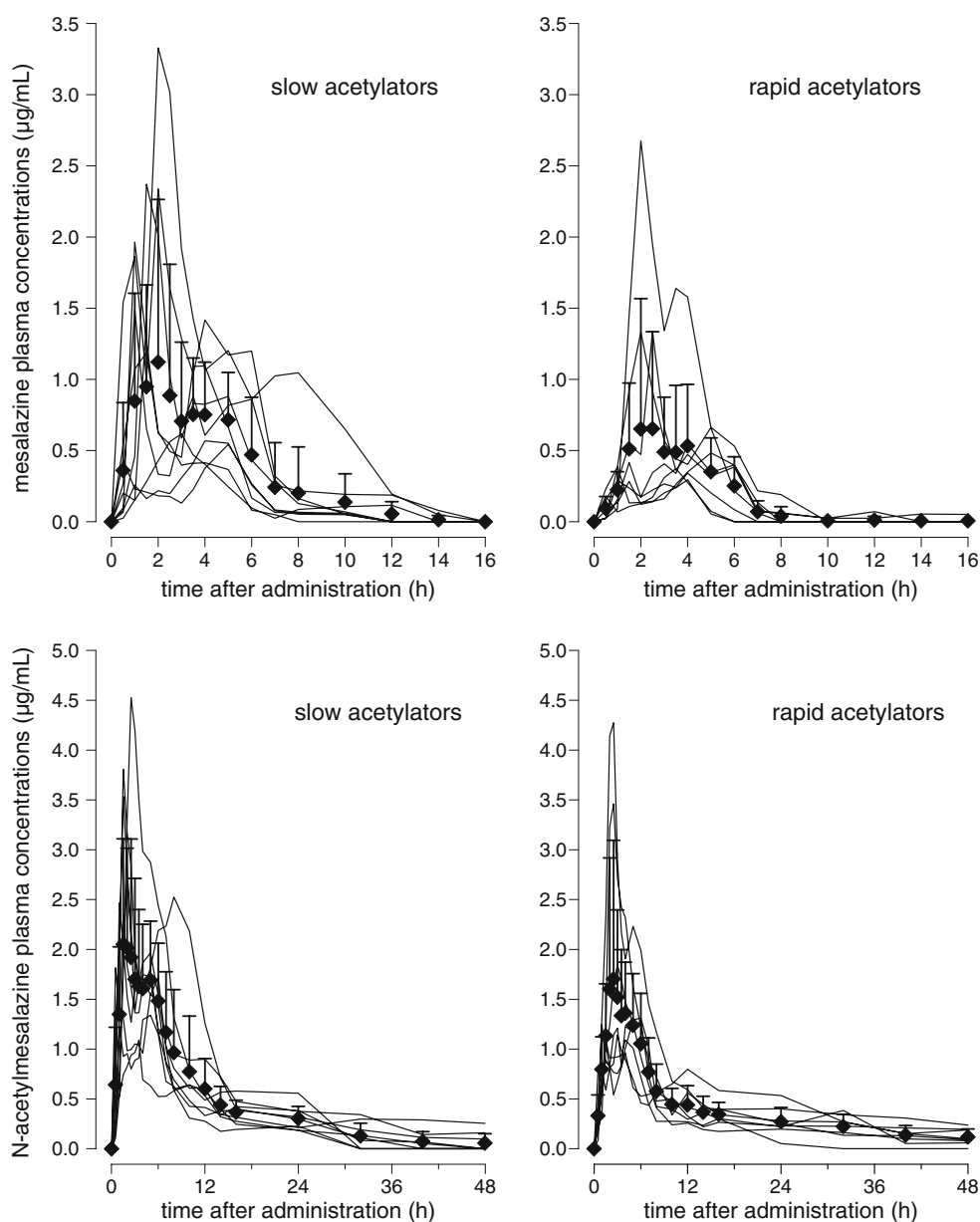
conditions mesalazine and the internal standard were eluted after approximately 1.5 and 1.6 min, respectively. The MacQuan software (version 1.6, PE Sciex, Thornhill, Ontario, Canada, 1991-1998) was used for evaluation of chromatograms. Urine samples were thawed and stored for 20 min at 37°C. Then 0.02 mL of each sample was diluted using 1.98 mL of mobile phase containing the internal standard. After thorough mixing, the samples were stored for 10 min at 37°C, and 25 μ L of each sample was chromatographed as described above. There was no interference observed in plasma or urine for mesalazine or the internal standard.

Weighted linear regression (1/concentration) was performed for calibration in human plasma and urine. Linearity of the calibration could be proven in human plasma between

0.0493 and 4.89 μ g/mL (study A and B) and in human urine between 0.953 and 97.9 μ g/mL for study A and between 0.251 and 60.1 μ g/mL for study B. Lower limits of quantification were identical with the lowest calibration levels. The interday precision of mesalazine in human plasma ranged from 6.0 to 8.3% with an analytical recovery between 99.3 and 102.2%. The interday precision of the spiked quality control standards of mesalazine in human urine ranged from 6.4 to 14.3% with an analytical recovery between 97.6 and 107.5% for study B.

For *N*-acetylmесalazine preparation of the human plasma and urine samples, see mesalazine. Thereafter, human plasma (35 μ L) and urine (25 μ L) samples were chromatographed on a reversed-phase column (Waters Symmetry Shield C-8), eluted with an isocratic solvent system consisting

Fig. 3 Individual and mean \pm SD mesalazine (*upper panel*) and *N*-acetylmесalazine (*lower panel*) concentration vs. time profiles following administration of a 1,000 mg mesalazine single dose (study B)



of ammonium acetate (pH 3.5) buffer and acetonitrile (70/30, v/v) and monitored by LC-MS/MS with an SRM method as follows: precursor → product ion for *N*-acetylmесalazine m/z 194 → m/z 150 and m/z 266 → m/z 223 for internal standard; all analyses were in negative mode. Under these conditions *N*-acetylmесalazine and the internal standard were eluted after approximately 1.4 and 0.9 min, respectively. The

MacQuan software (version 1.6, PE Sciex, Thornhill, Ontario, Canada, 1991-1998) was used for evaluation of chromatograms. There was no interference observed in plasma or urine for *N*-acetylmесalazine or the internal standard. Weighted linear regression (1/concentration) was performed for calibration. Linearity of the calibration could be proven in human plasma between 0.0497 and 4.94 μg/mL

Table 1 Pharmacokinetic parameters of mesalazine [arithmetic means±SD or geometric means (% CV)] and the respective comparison for rapid acetylators relative to slow acetylators [difference in arithmetic

means or ratio of geometric means (95% CI)] following oral administration of 1,000-mg doses

Parameter	Point estimates and metric of variability		Comparison rapid vs. slow acetylators
Study A (multiple dose)			
Acetylator phenotype	Slow	Rapid	-
Number classifiable	21	5	n.a.
NAT2 metric ^a	0.086 (58%)	0.299 (13%)**	3.49 (2.26–5.39)
Sex (<i>n</i> male/female)	16/5	3/2	n.a.
Age (years)	26.0±4.3	27.8±2.0	1.8 (-2.3 to 6.0)
Body weight (kg)	73.0±10.8	72.4±10.1	-0.7 (-11.7 to 10.3)
Body height (cm)	179±8	175±10	-4 (-13 to 5)
Mесalazine AUC _{τ(0–8 h)} (μg/mL·h)	11.1 (51%)	12.0 (52%)	1.08 (0.71–1.65)
<i>N</i> -acetylmесalazine AUC _{τ(0–8 h)} (μg/mL·h)	27.7 (32%)	30.5 (27%)	1.10 (0.83–1.46)
AUC _{τ(0–8 h)} ratio <i>N</i> -acetylmесalazine/mесalazine	2.49 (28%)	2.54 (38%)	1.02 (0.78–1.33)
Mесalazine C _{ss,max} (μg/mL)	3.00 (57%)	3.48 (60%)	1.16 (0.73–1.85)
<i>N</i> -acetylmесalazine C _{ss,max} (μg/mL)	5.71 (51%)	7.13 (47%)	1.25 (0.82–1.90)
C _{ss,max} ratio <i>N</i> -[mesalazine/mесalazine	1.91 (35%)	2.05 (48%)	1.07 (0.77–1.49)
Mесalazine Ae _{0–8 h} (% of dose)	8.53 (89%)	9.03 (52%)	1.06 (0.57–1.97)
<i>N</i> -acetylmесalazine Ae _{0–8 h} (% of dose)	31.4 (46%)	32.2 (41%)	1.03 (0.70–1.51)
Ae _{0–8 h} ratio <i>N</i> -acetylmесalazine/mесalazine	3.68 (74%)	3.57 (32%)	0.97 (0.57–1.65)
Mесalazine t _{ss,max} (h)	2.15±0.57	2.30±0.27	0.15 (-0.40 to 0.70)
<i>N</i> -acetylmесalazine t _{ss,max} (h)	2.69±0.75	2.70±0.45	0.01 (-0.72 to 0.73)
Study B (single dose)			
Acetylator phenotype	Slow	Rapid	-
Number classifiable	9	8	n.a.
NAT2 metric ^a	0.150 (18%)	0.370 (28%)**	2.46 (1.98–3.05)
Sex (<i>n</i> male/female)	5/4	7/1	n.a.
Age (years)	25.2±2.5	26.6±4.2	1.4 (-2.1 to 4.9)
Body weight (kg)	74.4±14.1	74.1±4.8	-0.3 (-11.8 to 10.9)
Body height (cm)	179±8	179±6	0 (-8 to 7)
Mесalazine AUC _{0–48 h} (μg/mL·h)	3.45 (113%)	2.36 (87%)	0.68 (0.33–1.41)
<i>N</i> -acetylmесalazine AUC _{0–48 h} (μg/mL·h)	21.3 (29%)	18.0 (39%)	0.84 (0.62–1.14)
AUC _{0–48 h} ratio <i>N</i> -acetylmесalazine/mесalazine	6.18 (112%)	7.63 (69%)	1.23 (0.63–2.43)
Mесalazine C _{max} (μg/mL)	0.81 (104%)	0.82 (147)	1.01 (0.44–2.32)
<i>N</i> -acetylmесalazine C _{max} (μg/mL)	2.40 (61%)	1.67 (73%)	0.69 (0.41–1.18)
C _{max} ratio <i>N</i> -acetylmесalazine/mесalazine	2.95 (110%)	2.03 (89%)	0.69 (0.34–1.42)
mесalazine Ae _{0–48 h} (% of dose)	0.2 (256%)	0.1 (359%)	0.42 (0.10–1.80)
<i>N</i> -acetylmесalazine Ae _{0–48 h} (% of dose)	30.9 (44%)	18.1 (84%)*	0.59 (0.35–0.98)
Ae _{0–48 h} ratio <i>N</i> -acetylmесalazine/mесalazine	138 (259%)	190 (308%)	1.38 (0.34–5.52)
Mесalazine t _{max} (h)	3.39±1.64	2.70±1.51	-0.69 (-2.33 to 0.94)
<i>N</i> -acetylmесalazine t _{max} (h)	2.89±2.33	3.25±1.79	0.36 (-1.81 to 2.53)
Mесalazine half value duration (h)	3.60±1.60	2.22±1.25	-1.39 (-2.88 to 0.11)
<i>N</i> -acetylmесalazine half value duration (h)	6.10±2.89	6.65±3.85	0.54 (-2.95 to 4.03)

P*<0.05, *P*<0.00001

^aUrinary caffeine metabolic ratio (5-acetylamino-6-formylamino-3-methyluracil + 5-acetylamino-6-amino-3-methyluracil)/(5-acetylamino-6-formylamino-3-methyluracil + 5-acetylamino-6-amino-3-methyluracil + 1-methylxanthine + 1-methyluric acid)

(study A and B) and in human urine between 0.956 and 98.2 $\mu\text{g}/\text{mL}$ for study A and between 2.00 and 100 $\mu\text{g}/\text{mL}$ for study B. Lower limits of quantification were identical with the lowest calibration levels. The inter-day precision of *N*-acetylmessalazine in human plasma ranged from 6.3 to 9.1% with an analytical recovery between 98.3 and 103.4%. The inter-day precision of the spiked quality control standards of *N*-acetylmessalazine in human urine ranged from 2.4 to 10.4% with an analytical recovery between 95.3 and 111.0%.

Pharmacokinetic parameters were calculated by non-compartmental methods (WinNonlin 2.0, Pharsight, Palo Alto, CA, U.S.A.). Statistical evaluations are considered as descriptive. Mesalazine pharmacokinetics were compared between acetylator groups by the *t*-test for independent samples. The relationship of mesalazine kinetics to NAT2 activity was further examined by linear regression (SPSS 15.0 for Windows, SPSS, Chicago, IL, U.S.A.). For metabolic ratios, AUC, C_{max} , and A_e , a multiplicative model was assumed, and geometric means instead of arithmetic means and ratios instead of differences were calculated by ln-transformation of the data prior to analysis and respective back-transformation thereafter.

Results

The frequency distribution of NAT2 activities is shown in Fig. 1. Overall, 30 rapid and 13 slow acetylators were identified. Concentration vs. time profiles for both mesalazine and *N*-acetylmessalazine are depicted in Figs. 2 and 3. Irregular profiles and persisting low concentrations of mesalazine in the final part of some profiles precluded a reliable calculation of apparent elimination half-lives; thus, these are not reported, and extrapolation of AUC was not done.

Concentration vs. time plots are presented separately for slow and rapid acetylators in the multiple-dose study

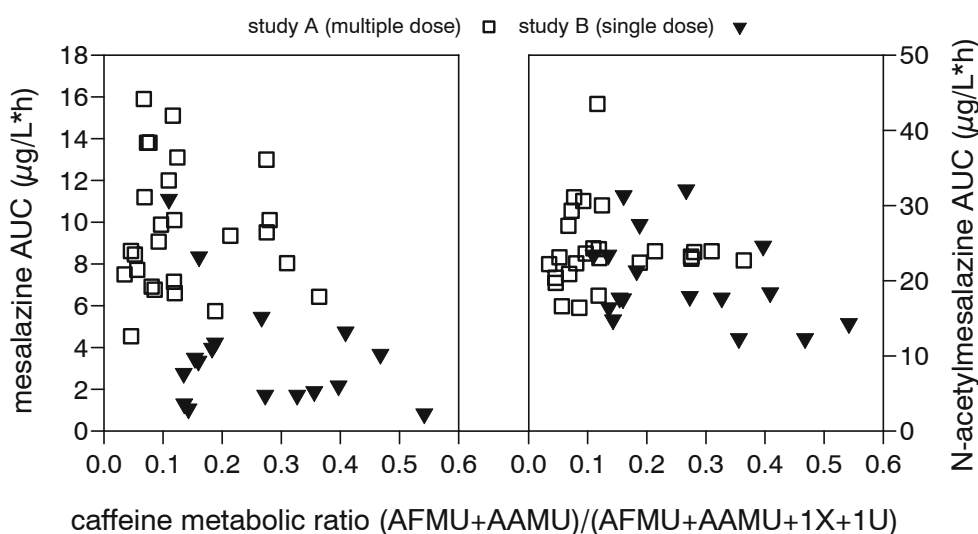
(Fig. 2) and the single-dose study (Fig. 3). Main pharmacokinetic parameters with relevance for the rate and extent of metabolism are presented in Table 1. There was pronounced interindividual variability in the profiles and the respective parameters, which was even higher in the single-dose study. Considerably higher AUC values for mesalazine in the steady-state study indicate saturation of mesalazine metabolism. This is also reflected in the fact that less than 0.3% of a mesalazine dose was excreted unchanged in the single-dose study while roughly 9% of the doses were excreted unchanged in the multiple-dose study (Table 1).

There is no evidence for a true difference in mesalazine pharmacokinetics between slow and rapid acetylators. The plots do not show clear differences between NAT2 phenotypes (Figs. 2, 3). For only 1 parameter out of 25 was a statistically significant difference ($P < 0.05$) observed, and this one does not support a more rapid metabolism of mesalazine in NAT2 rapid acetylators. There was no overlapping of 95% CIs for the comparison between acetylator phenotypes between the NAT2 metric and any mesalazine parameter in the multiple-dose study, and only marginal overlapping for a few parameters in the single-dose study (Table 1). Also, there was no significant correlation between NAT2 phenotype and any of the mesalazine pharmacokinetic parameters (for AUC values, see Fig. 4).

Discussion

In this evaluation, pharmacokinetics of mesalazine were compared between slow and rapid acetylators for NAT2 both in a single-dose and a multiple-dose study as acetylation is saturated during chronic administration. Our results do not provide evidence for a major role for NAT2 in the metabolism of mesalazine in vivo.

Fig. 4 Relationship of mesalazine and *N*-acetylmessalazine AUC to NAT2 activity. There was no significant correlation of AUC values calculated separately for studies A and B and the caffeine NAT2 metabolic ratio. *1X* 1-Methylxanthine, *1U* 1-methyluric acid, *AFMU* 5-acetylamino-6-formylamino-3-methyluracil, *AAMU* 5-acetylamino-6-amino-3-methyluracil



Traditionally, NAT2 phenotypes have been described as “slow” or “rapid” acetylators. Depending on the number of *NAT2* alleles coding for a highly active enzyme variant (i.e., 0, 1 or 2, respectively), however, three groups of individuals with respect to acetylation rate of individual NAT2 substrates may be discerned [12], although statistical analysis usually fails to identify more than two groups based on pharmacokinetic parameters. In the present study, 30% of classifiable individuals were allocated to the slow acetylator phenotype, which is less than the 50% of Caucasians reported to have two low-activity alleles [13]. Thus, the allocation to “slow” and “rapid” acetylator phenotype may represent some ambiguity with regard to *NAT2* genotype. However, the caffeine metabolic ratio used here as the NAT2 metric has been validated extensively [11 and references therein]. From 4 h after dosing on, this ratio remained unchanged for at least another 12 h and thus is robust against deviations in urine sampling [14]. Therefore, the results of NAT2 phenotyping are considered reliable from both a scientific and technical point of view and, as for any valid phenotyping procedure, may provide information beyond genotyping [15]. It should be mentioned that the metabolic caffeine ratio has been shown to reflect the activity of hepatic NAT2 [16], but activity differences between NAT2 genotypes most probably also apply to intestinal tissue [17].

Pharmacokinetic parameters of mesalazine observed here are similar to those reported previously, including saturation of acetylation during chronic administration [1, 18]. Because of the short elimination half-life of mesalazine of clearly less than 3 h [1, 7], a prolonged release preparation is needed for maintaining plasma concentrations during a dosing interval, but also for direct delivery to any intestinal site of action. Mesalazine release from this formulation has been reported to occur throughout the gastrointestinal tract [19]. It has a bioavailability of approximately 30% [18], which is lower than that of gastroresistant tablets and may in part be caused by a more extensive intestinal first-pass metabolism that is saturable [7] (see also Table 1). Indeed, *in vitro* studies showed that homogenates of colonic mucosa [8] and more specifically the cytosol of human colonic epithelial cells are able to mediate mesalazine *N*-acetylation [20].

The current evaluation was done within the framework of bioequivalence studies, which had not been powered explicitly to assess the role of NAT2 phenotype in mesalazine pharmacokinetics. Because of the limited number of individuals and the pronounced interindividual variation in mesalazine pharmacokinetics, we were not able to exclude an effect of *NAT2* genotype on mesalazine acetylation *in vivo*. However, the 95% CIs for the comparison between phenotypes clearly showed that if there were any differences in mesalazine kinetics between acetylator phenotypes, they

would be much smaller than differences in enzyme activity. In contrast, for the known NAT2 substrate isoniazid, pharmacokinetic differences fully reflect differences in enzyme activity [12]. Thus, our data show that NAT2 has no major role—if any—in human metabolism of mesalazine *in vivo*. As *NAT2* (and *NAT1*) polymorphisms also have no influence on inflammatory bowel disease itself [21], there is no rationale to consider NAT2 activity in the treatment with mesalazine.

It is still desirable to identify sources of variability in mesalazine pharmacokinetics other than NAT2 polymorphism. Obviously, intestinal liberation rate of the drug from the preparation may contribute. Furthermore, there is a clear role of bacterial NAT in overall *N*-acetylation of mesalazine, although its magnitude is controversial [22, 23]. Also, the reported lack of differences in mesalazine response and tolerability between *NAT1* genotypes [9, 10] does not exclude differences in mesalazine pharmacokinetics because differences in activity in NAT1 variants are limited [24, 25] and pharmacodynamic parameters are less sensitive than pharmacokinetic parameters. Finally, in patients the degree of intestinal inflammation affects mesalazine pharmacokinetics [26]. Further research is needed to improve our understanding of the fate of mesalazine in the human body in order to assess a rationale for individual dose optimization, with the chance to improve the risk/benefit ratio of this drug and maybe also to reduce the risk for rare adverse effects such as interstitial nephritis [27].

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