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To the editor:

Recently, in *Clinical Pharmacology and Therapeutics*, Kharasch et al [1] published a study on the role of CYP3A5 polymorphisms in the pharmacokinetics and pharmacodynamics of the CYP3A probe drugs alfentanil and midazolam, both CYP3A4 and CYP3A5 substrates. There was no relevant impact of *CYP3A5* alleles **3, *6* and **7* on systemic or apparent oral clearances of alfentanil and midazolam, although these confer decreased CYP3A5 expression and activity.

We assessed data on midazolam pharmacokinetics following semi-simultaneous administration of oral and i.v. midazolam, allowing for separate assessment of intestinal and hepatic CYP3A activity, from 59 healthy male volunteers and 30 HIV infected patients participating in six previously performed phenotyping studies (see **Table 1**) and related them to the main CYP3A5 polymorphism *CYP3A5*3*.

All studies were approved by the ethics committee of the Medical Faculty of the University of Cologne and conducted in accordance with the Declaration of Helsinki, all participants gave their written informed consent. In all studies, oral and i.v. midazolam was given as a part of a phenotyping cocktail, whereas the other cocktail components varied between the respective studies (see **Table 1**). While in study A, the cocktail was given only once, in studies B - F (interaction studies), phenotyping was performed in two study periods, respectively, with and without co-medication.

*CYP3A5*3* genotyping was done via LightCycler® melting curve analysis (Primer R5'-TAgTTgTAGgACACACAgCAACC, Primer F5' - TTTgCCTCTTTgTACTTCTTCATC, Sensor 5'- gAgCTTTTTgTCTTTCAATATCTCT—FL, Anchor 5'- LC Red640-CCCTgTTTggACCACATTACCCTT—PH). Midazolam in plasma was quantified by LC-MS/MS [2]

Total clearance (Cl) and intestinal availability (Fi) of midazolam, used as metrics of hepatic and intestinal CYP3A activity, respectively, were assessed by population analysis using

NONMEM software (V version 1.1, NONMEM Project Group, University of California at San Francisco, 1998). A two compartment model gave the best fit allowing for determination of Cl and oral bioavailability, whereas for calculation of Fi an additional equation was implemented [3].

Results of the pharmacokinetic evaluation sorted by study and *CYP3A5* genotype are shown in **Table 1.** *CYP3A5* genotype was not a significant covariate neither for hepatic nor for intestinal CYP3A activity.

Our results, derived upon a single phenotyping session with sequential midazolam administration allowing for a separate evaluation of both intestinal and hepatic CYP3A activity, which are independently regulated, confirm and expand the data by Kharasch et al. The clear inconsistency with respect to in vitro data showing a relevant effect of *CYP3A5* polymorphism on midazolam pharmacokinetics would suggest that, irrespective of *CYP3A5* genotype, the overall CYP3A5 plus 3A4 protein expression is relatively constant in both intestine and liver, and that relevant differences between *CYP3A5* genotypes in vivo may occur only for substances which are metabolized more specifically by CYP3A5.

Conflict of interest

The authors declared no conflict of interest

References

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Table 1

Characteristics of the phenotyping studies, results of *CYP3A5*3* genotyping, and respective midazolam (M) pharmacokinetics.

Pheno-typing study	Administration of midazolam (M) *(other cocktail components[a])*	study population	Age [years]	BMI [kg/m^2]	*CYP3A5*3* genotype	Total clearance of M [L/h]	Intestinal availability of M [-]
A	1 mg i.v., then 1.5 mg orally 1.5 hours thereafter *(Caff., Tol., Meph., Dex., Chlorz., Dig., Amox., Nic.)*	10 healthy male subjects	30.9 (25-40)	24.6 (21.2-27.0)	*1/3 N=2	22.60	0.84
					*3/3 N=8	22.74 (21.23-24.24)	0.91 (0.77-1.04)
B	2 mg **orally**, then 1 mg **i.v.** 4 hours thereafter *(Caff., Tol., Meph., Dex.)*	16 healthy male subjects	29.3 (23-42)	23.5 (21.5–26.6)	*1/1* N=1	27.30	0.70
					1/3 N=4	28.18 (23.02-33.33)	0.48 (0.40-0.56)
					3/3 N=11	26.93 (23.82-30.04)	0.59 (0.54-0.64)
C	2 mg **orally**, then 1 mg **i.v.** 4 hours thereafter *(Caff., Tol., Meph., Dex., Dig.)*	12 healthy male subjects	38.0 (24-49)	24.5 (20.0–29.3)	*1/3* N=1	19.46	0.25
					3/3 N=11	16.07 (14.29-17.84)	0.45 (0.36-0.54)
D	2 mg **orally**, then 1 mg **i.v.** 4 hours thereafter *(Caff., Tol., Meph., Dex.)*	10 healthy male subjects	29.7 (22-43)	24.1 (20.4–27.8)	*1/3* N=2	21.80	0.40
					3/3 N=8	19.64 (16.57-22.68)	0.49 (0.38-0.60)
E	1.5 mg **orally**, then 1 mg **i.v.** 4 hours thereafter *(Dex., Dig.)*	11 healthy male subjects	30.6 (18-48)	22.3 (20.2– 5.2)	*3/3* N=11	27.47 (21.38-33.57)	0.75 (0.63-0.88)
F	1.5 mg **orally**, then 1 mg **i.v.** 4 hours thereafter *(Dex., Dig.)*	30 HIV-positive patients (23 male and 7 female)	39.9 (25-60)	23.5 (16.4–36.7)	*1/1* N=5	22.66 (17.00-28.32)	0.89 (0.60-1.17)
					1/3 N=8	24.33 (19.85-28.80)	1.13 (0.90-1.35)
					3/3 N=17	26.06 (22.99-29.13)	1.10 (0.95-1.26)
Total		89 subjects (69 healthy male subjects and 30 HIV-positive patients)	34.4 (18-60)	23.7 (16.4–36.7)	*1/1* N=6	23.43 (18.17-28.70)	0.86 (0.58-1.13)
					1/3 N=17	24.45 (21.32-27.57)	0.80 (0.64-0.97)
					3/3 N=66	23.60 (22.00-25.18)	0.75 (0.67-0.84)

Demographic data (determined at the screening examination) are given as mean and (range); pharmacokinetics is given as mean and (95% confidence interval). For studies with two

periods (B - F), the pharmacokinetic parameters were calculated in the periods without co-medication. Confidence interval was calculated if N>3

[a]Cocktail components: Caff. – caffeine, Tol. - tolbutamide, Meph. - mephenytoin, Dex. - dextromethorphan, Chlorz. - chlorzoxazone, Dig. - digoxin, Amox. - amoxicillin, Nic. - nicotine