



HAL
open science

Proposal of a new limited sampling strategy to predict CYP3A activity using a partial AUC of midazolam

Stephanie Katzenmaier, Christoph Markert, Gerd Mikus

► To cite this version:

Stephanie Katzenmaier, Christoph Markert, Gerd Mikus. Proposal of a new limited sampling strategy to predict CYP3A activity using a partial AUC of midazolam. *European Journal of Clinical Pharmacology*, 2010, 66 (11), pp.1137-1141. 10.1007/s00228-010-0878-2 . hal-00613129

HAL Id: hal-00613129

<https://hal.science/hal-00613129>

Submitted on 3 Aug 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



**Proposal of a new limited sampling strategy to predict
CYP3A activity using a partial AUC of midazolam**

Journal:	<i>European Journal of Clinical Pharmacology</i>
Manuscript ID:	EJCP-2010-0180.R1
Type of submission:	Original
Date Submitted by the Author:	20-Jul-2010
Complete List of Authors:	Katzenmaier, Stephanie; University of Heidelberg, Department of Clinical Pharmacology and Pharmacoepidemiology Markert, Christoph; University of Heidelberg, Department of Clinical Pharmacology and Pharmacoepidemiology Mikus, Gerd; University of Heidelberg, Department of Clinical Pharmacology and Pharmacoepidemiology

1
2
3 **Proposal of a new limited sampling strategy to predict CYP3A activity using a partial**
4
5 **AUC of midazolam**
6
7
8
9

10 Stephanie Katzenmaier , Christoph Markert, Gerd Mikus
11

12
13
14
15 Department of Clinical Pharmacology and Pharmacoepidemiology, University of Heidelberg,
16

17 Im Neuenheimer Feld 410, D-69120 Heidelberg, Germany
18
19

20
21
22 * Address for correspondence and reprint requests:
23

24 Gerd Mikus, MD
25

26
27 Department Clinical Pharmacology and Pharmacoepidemiology
28

29 University Hospital of Heidelberg
30

31 Im Neuenheimer Feld 410
32

33
34 D-69120 Heidelberg
35

36
37 Germany
38
39
40

41 Tel.: + 49 6221 56 39197, Fax: + 49 6221 56 4642
42

43 Email: gerd.mikus@med.uni-heidelberg.de
44
45
46
47

48 Word count: 2176
49

50 Word count abstract: 253
51
52
53
54
55
56
57
58
59
60

PURPOSE

Midazolam metabolic clearance to 1'-hydroxymidazolam is an accurate measure for CYP3A activity which requires extensive plasma and urine sampling. The objective of this study was to find a new limited sampling strategy (LSS) to predict midazolam metabolic clearance to 1'-hydroxymidazolam and subsequently CYP3A activity in an easy and reliable way, reducing costs and labour.

METHODS

The development of this LSS is based on data of an in vivo drug-drug interaction study carried out in our clinical research unit. Various partial AUCs of midazolam were calculated and correlated with metabolic clearance of midazolam to 1'-hydroxymidazolam by non-linear regression. The correlation with highest r^2 -values was chosen in order to predict the midazolam metabolic clearance. Statistical significance of this method was verified by calculating the 95% confidence interval of the differences (%) between predicted and measured metabolic clearance of midazolam to 1'-hydroxymidazolam.

RESULTS

The midazolam partial AUC from 2-4 hours after oral administration correlated best with metabolic clearance of midazolam to 1'-hydroxymidazolam with $r^2 = 0.9816$. This partial AUC comprised of four midazolam concentrations at 2, 2.5, 3 and 4 hours after oral administration of a midazolam solution. The 95% confidence interval of the differences between predicted metabolic clearance and measured metabolic clearance of midazolam to 1'-hydroxymidazolam was [-0.97 to +13.2].

CONCLUSION

1
2
3 The determination of the partial AUC using four plasma samples from 2-4 hours after oral
4
5 administration of midazolam as a solution is proposed to be an easy and reliable CYP3A
6
7 phenotyping measure which of course needs to be validated in prospective clinical trials.
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

Introduction

Cytochromes P (CYPs) 450 represent a multifaceted family of heme-containing enzymes belonging to the group of monooxygenases accountable for phase-I-metabolism. Mammalian cytochromes are membrane proteins taking an active part in biosynthesis and metabolism of many physiological active substances. Moreover these enzymes are capable of catalysing biotransformation and metabolism of substances of foreign origin like drugs and toxins [1].

Cytochrome P 450 3A (CYP3A) is the most abundant enzyme of CYPs in the liver and the gut, it metabolises approximately 50% of currently available drugs and accounts for 30% of the total CYP enzymes in hepatic microsomes. A large number of drugs interfere with CYP3A as they may act as inducers, substrates or inhibitors of this enzyme.

To determine the activity of CYP3A midazolam is often used as a probe drug for this enzyme [2]. Midazolam belongs to the benzodiazepines family and is frequently used for anaesthetic induction, narcotic premedication and sedation of patients prior to surgery or other invasive medical procedures. **It is extensively metabolised by CYP3A to its primary metabolite, 1'-hydroxymidazolam and to minor metabolites, 4'-hydroxymidazolam and 1, 4'-dihydroxymidazolam. Only 1 % of midazolam is eliminated unchanged in urine. Main urinary metabolite is 1'-hydroxymidazolamglucuronide, which amounts to 63-80 % of the administered dose within 24 hours. Conjugates of 4'-hydroxymidazolam and 1',4'-dihydroxymidazolam amount to 3 % and 1 % in urine [3].** Orally administered midazolam is used to assess the total - intestinal and hepatic - CYP3A activity.

Usually, CYP3A activity is tested before and during treatment with a potential inducer or inhibitor. To assess the time course of inhibition or induction frequent midazolam testing would be required. The most predictive and reliable parameter to assess CYP3A activity is the metabolic clearance to 1'-hydroxymidazolam [4]. Therefore, a full pharmacokinetic profile ($AUC_{0-\infty}$) and the urinary excretion of 1'-hydroxymidazolam and its glucuronide over at least 24 hours after single dose administration are required. Hence the use of midazolam as a probe

1
2
3 drug to determine the time course of CYP3A activity is very limited. A useful strategy for the
4
5 clinical practice of CYP3A4 phenotyping might be the reduction of the number of plasma
6
7 samples with still accurate determination of CYP3A activity. It will then be possible to obtain
8
9 CYP3A activity over many days and assess the time course of drug drug interactions.
10
11

12 We here propose a new limited sampling strategy to predict the midazolam metabolic
13
14 clearance to 1'-hydroxymidazolam and subsequently CYP3A-activity in an easy and reliable
15
16 way using much less plasma samples and no urine sampling. Limited sampling is a useful
17
18 strategy to simplify proceedings and reduce costs and labour. This is relevant for the clinical
19
20 application of CYP3A phenotyping.
21
22
23
24
25
26
27
28

29 **Methods**

30
31 The development of this LSS is based on data of an in vivo drug-drug interaction study
32
33 carried out at the clinical research unit of the Department of Clinical Pharmacology and
34
35 Pharmacoepidemiology, University of Heidelberg. In this study twelve participants were
36
37 included. The protocol was approved by the competent authority (BfArM, Germany) and the
38
39 Ethics Committee of the University of Heidelberg, written informed consent was obtained
40
41 from each subject prior to study-related procedures. On the first occasion they received
42
43 midazolam in order to determine the baseline metabolic clearance of midazolam to 1'-
44
45 hydroxymidazolam. Thereafter efavirenz (400 mg p.o. once daily) was administered over 14
46
47 days to study the inducing effect of efavirenz on CYP3A. Midazolam was given orally on day
48
49 1 and 14 of this period. **After an overnight fast 3 mg midazolam (Dormicum® Roche**
50
51 **5mg/5ml, solution for injection or infusion) were administered in 100 ml tap water. 4 hours**
52
53 **thereafter a standard meal was provided for each subject.** Blood samples were taken after
54
55 midazolam at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 hours and urine was collected
56
57 completely over 24 hours. Concentrations of midazolam and 1'-hydroxymidazolam in plasma
58
59
60

1
2
3 and urine were **quantified** by high performance liquid chromatography coupled to tandem
4
5 mass spectrometry after modification of a published method [5] as described elsewhere [6].
6
7 The LC/MS/MS system (Thermo Fisher, Dreieich, Germany) consisted of a Surveyor Plus
8
9 HPLC System and a triple stage quadrupole mass spectrometer (TSQ 7000 with API-2 ion
10
11 source and performance kit). For quantification of the drugs a Phenomenex Max RP column
12
13 (150 x 2 mm, 4 μ m, Phenomenex, Aschaffenburg, Germany) with integrated guard column
14
15 was used at 40°C for chromatographic separation. The bioanalytical method was validated
16
17 according to the recommendations of the FDA in the range of 0.61-460 nmol/L for midazolam
18
19 and 0.59-439 nmol/L for 1'-hydroxymidazolam in plasma and 1.53-153 nmol/L for
20
21 midazolam and 73.2-7315 nmol/L for 1'-hydroxymidazolam in urine. Lower limits of
22
23 quantification for midazolam and 1'-hydroxymidazolam in plasma were 0.61 and 0.59 nmol/l,
24
25 respectively, (with accuracy/precision of -4.2%/5.0% and +11.6%/4.6%, respectively); lower
26
27 limit of quantification in urine was 1.53 nmol/l for midazolam and 72.2 nmol/l for 1'-
28
29 hydroxymidazolam (accuracy/precision of +8.0%/7.9% and +3.8%/7.1%, respectively).
30
31 Metabolic clearance of midazolam to 1'-hydroxymidazolam was calculated as the amount of
32
33 total (conjugated + unconjugated) 1'-hydroxymidazolam excreted in urine (0-24 h) divided by
34
35 midazolam AUC_{0-∞}.
36
37 In order to predict metabolic clearance of midazolam to 1'-hydroxymidazolam various partial
38
39 midazolam AUCs were calculated and correlated with the metabolic clearance of midazolam
40
41 to 1'-hydroxymidazolam. Stepwise multiple regressions were used to identify optimal time
42
43 points and intervals of midazolam plasma concentrations, respectively. Standard
44
45 pharmacokinetic parameter and partial AUCs (1-2 hours, 1-3 hours, 1-4 hours, 2-4 hours, and
46
47 1-6 hours) after oral administration of midazolam were calculated by noncompartmental
48
49 analysis using WinNonlin 5.2 (Pharsight Corp., Mountain View, CA, USA). Midazolam
50
51 concentrations were expressed per mg dose of midazolam. Non-linear regressions were
52
53 performed using Prism 5.03 (GraphPad Software Inc., La Jolla, CA, USA).
54
55
56
57
58
59
60

From the following equations [7] we derived a formula to calculate the metabolic clearance of midazolam to 1'-hydroxymidazolam using a partial AUC of midazolam.

Clearance is defined as the removal of substance from blood, expressed as volume per time.

$$\frac{dA_e}{dt} = k_{10} \cdot A = k_{10} \cdot V \cdot C = \lambda_z \cdot V_z \cdot C = CL \cdot C \quad (1)$$

A_e (total eliminated drug), k (elimination rate constant first order), A (amount of drug), V (volume of distribution), C (concentration), λ (reaction rate constant first order), CL (clearance), t (time after medication)

The total eliminated drug is consistent with the integral of equation 1

$$A_{e_{\infty}} = CL \int_0^{\infty} C \cdot dt = CL \cdot AUC \quad (2)$$

$A_{e_{\infty}}$ (total eliminated drug until time point of infinity), AUC (area under the curve)

which is also equal to systemically available dose ($F \times D$)

$$A_{e_{\infty}} = CL \cdot AUC = F \cdot D \quad (3)$$

F (bioavailability), D (dose)

$$AUC = \frac{F}{CL} \cdot D \quad (4)$$

Clearance and AUC are inversely related, and almost no midazolam is excreted unchanged in urine. Hence, clearance can be replaced by metabolic clearance. By means of non-linear regression of various partial AUCs and metabolic clearance of midazolam to 1'-hydroxymidazolam (Prism 5.03) the factor A was calculated:

$$partialAUC = \frac{A}{CL_{met}} \quad (5)$$

Using the best fit (highest r^2) the prediction of the metabolic clearance of midazolam to 1'-hydroxymidazolam from this partial AUC is done by:

$$CL_{met-predicted} = \frac{A}{partialAUC} \quad (6)$$

In order to verify the statistical significance of this predictive method the 95% confidence interval of the differences (%) between predicted metabolic clearance and measured metabolic clearance of midazolam to 1'-hydroxymidazolam was constructed. Precision and bias were assessed by the Bland-Altman method [8].

Results

All partial AUCs / mg midazolam of 1-2 hours, 1-3 hours, 1-4 hours, 2-4 hours and 1-6 hours after oral medication correlated with metabolic clearance of midazolam to 1'-hydroxymidazolam. The full plasma concentration time profile of midazolam is shown in Figure 1, correlation results in Table 1. The correlation of partial AUC from 2-4 hours with the measured metabolic clearance of midazolam to 1'-hydroxymidazolam showed the highest r^2 -value ($r^2 = 0.9816$; Figure 2). This partial AUC comprises of four blood samples at 2, 2.5, 3 and 4 hours after oral midazolam administration. The use of a further reduced number of plasma samples to calculate partial AUC results in weaker correlations (partial AUC of 2 – 4 hr with three blood samples at 2, 3, 4 hr: $r^2 = 0.9062$, partial AUC of 2 – 4 hr with two blood samples at 2, 4 hr: $r^2 = 0.8795$).

Related to this non-linear regression the factor was calculated as $A = 5656$. Therefore, the following formula predicts the metabolic clearance of midazolam to 1'-hydroxymidazolam and CYP3A activity, respectively:

$$CL_{met-predicted} = \frac{5656}{AUC_{2-4}} \quad (7)$$

The midazolam concentrations to calculate partial AUC have to be expressed per mg dose of midazolam.

There is a strong relationship between the measured and predicted metabolic clearance of midazolam to 1'-hydroxymidazolam. Using linear regression, regression coefficient was 0.98, $p < 0.0001$. The 95% confidence interval of the differences between predicted metabolic

1
2
3 clearance and measured metabolic clearance of midazolam to 1'-hydroxymidazolam [-0.97 %
4 to +13.2 %] showed no significant difference. There was good precision and low bias of the
5 predicted metabolic clearance of midazolam to 1'-hydroxymidazolam (Figure 3). **In contrast**
6 **the 95% confidence interval of the differences between predicted metabolite clearance and**
7 **measured metabolic clearance of midazolam to 1'-hydroxymidazolam using this partial AUC**
8 **and minor plasma samples showed a significant difference (95% confidence interval using**
9 **three blood samples (2, 3, 4 hr):[+18.11% to +31.68%], 95% confidence interval using two**
10 **blood samples (2, 4 hr): [+13.33% to +28.71%]).**
11
12
13
14
15
16
17
18
19
20
21
22
23
24

25 Discussion

26
27 The aim of this investigation was to find a useful strategy which predicts CYP3A activity in
28 an easy and reliable way and facilitates the clinical application of CYP3A phenotyping and
29 the determination of the time course of drug drug interactions. LSS has already been proven
30 to be an effective tool in monitoring the safety and efficacy of chemotherapeutic agents by
31 minimising the number of blood samples necessary to evaluate AUC [9]. It was first
32 introduced in 1987 in order to minimise the number of plasma samples for measuring AUC of
33 vinblastine. With only two time points, Ratain and Vogelzang were able to estimate total
34 AUC using stepwise multiple regression analysis [10]. Since then various LSSs have been
35 investigated for many drugs in order to simplify sampling procedures and minimise costs.
36
37 Regarding midazolam and the activity of CYP3A Rogers et al. found that neither single
38 plasma concentrations of the metabolite 1-hydroxy-midazolam nor the ratio of 1-hydroxy-
39 midazolam and midazolam can be used for predicting midazolam clearance in healthy
40 subjects [11]. Another study was performed to investigate limitations of using a single post-
41 dose midazolam concentration to predict CYP3A-mediated drug-drug interactions. The
42 purpose was to assess the ability of one single midazolam concentration for predicting
43 midazolam AUC in the presence and absence of CYP3A modulation by Gingko biloba
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 inducing CYP3A. The study showed that optimal midazolam sampling times to predict AUC
4
5 were different before or after Gingko biloba [12]. This approach is not useful for prospective
6
7 clinical trials where CYP3A activity is to be determined. Another strategy is the use of saliva
8
9 for CYP3A phenotyping using midazolam [10]. This method however requires numerous
10
11 samples over at least 6 hours and a very sensitive analytical method due to the very low
12
13 concentrations in saliva [13].
14

15
16
17 Midazolam pharmacokinetics is well known und understood. Primary metabolic pathway is
18
19 the formation of 1'-hydroxymidazolam via CYP3A which is subsequently conjugated and
20
21 excreted in urine and accounts for >70% of the oral dose administered [4]. Hence,
22
23 quantification of this pathway by the metabolic clearance of midazolam to 1'-
24
25 hydroxymidazolam is the most suitable parameter for CYP3A activity. We investigated the
26
27 correlations between various partial midazolam AUCs and metabolic clearance of midazolam
28
29 to 1'-hydroxymidazolam in order to find a reliable method to predict midazolam metabolic
30
31 clearance. When midazolam is administered as an oral solution the best results were obtained
32
33 using the midazolam partial AUC from 2 to 4 hours comprising of four plasma samples at 2,
34
35 2.5, 3 and 4 hr after oral administration of midazolam. Resorption and distribution is fast after
36
37 an oral solution and seems not to be influencing the prediction of CYP3A activity when using
38
39 plasma samples from 2-4 hours after midazolam administration. The prediction of the
40
41 metabolic clearance of midazolam to 1'-hydroxymidazolam is only valid for the study
42
43 conditions described above and for healthy volunteers without any conditions which could
44
45 modify the absorption, distribution, metabolism or excretion of drugs. However, this method
46
47 might also be applied to patients with limited metabolism caused by liver diseases in order to
48
49 identify drug-drug interactions. The alteration of CYP3A activity by the addition of a drug
50
51 should also be valid in these patients with the limitation that the prediction of the metabolic
52
53 clearance might not be accurate.
54
55
56
57
58
59
60

1
2
3 Using four plasma samples to determine partial AUC instead of one single plasma
4
5 concentration minimises errors in prediction of CYP3A activity due to imprecisions of the
6
7 analytical measurements.
8

9
10 General prediction of CYP3A-activity has to comprise a full range of metabolic activity,
11
12 including induction, inhibition and normal state of enzyme activity. Although this LSS solely
13
14 is derived from induced and normal CYP3A activity, it is clear from Figure 2 that inhibition
15
16 of CYP3A activity will result in high partial AUCs and very low metabolic clearance
17
18 approaching zero when there is no metabolic activity.
19
20
21
22
23

24 **Conclusion**

25
26 We propose a new LSS to predict midazolam metabolic clearance to 1'-hydroxymidazolam
27
28 and thus CYP3A activity using a full pharmacokinetic dataset from an interaction study. The
29
30 determination of the partial AUC from 2-4 hours after oral administration of midazolam
31
32 predicts CYP3A4 activity with high precision and will enable the assessment of the time
33
34 course of drug drug interactions and the clinical application of CYP3A4 phenotyping.
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

REFERENCES

1. Myasoedova, K.N., New findings in studies of cytochromes P450. *Biochemistry (Mosc)*, 2008. 73(9): p. 965-9.
2. Greenblatt, D.J., et al., Inhibition of oral midazolam clearance by boosting doses of ritonavir, and by 4,4-dimethyl-benziso-(2H)-selenazine (ALT-2074), an experimental catalytic mimic of glutathione oxidase. *Br J Clin Pharmacol*, 2009. 68(6): p. 920-7.
3. Streetman, D.S., J.S. Bertino, Jr., and A.N. Nafziger, Phenotyping of drug-metabolizing enzymes in adults: a review of in-vivo cytochrome P450 phenotyping probes. *Pharmacogenetics*, 2000. 10(3): p. 187-216.
4. Thummel, K.E., et al., Oral first-pass elimination of midazolam involves both gastrointestinal and hepatic CYP3A-mediated metabolism. *Clin Pharmacol Ther*, 1996. 59(5): p. 491-502.
5. Quintela, O., et al., A sensitive, rapid and specific determination of midazolam in human plasma and saliva by liquid chromatography/electrospray mass spectrometry. *Rapid Commun Mass Spectrom*, 2004. 18(24): p. 2976-82.
6. Hafner, V., et al., Effect of simultaneous induction and inhibition of CYP3A by St John's Wort and ritonavir on CYP3A activity. *Clin Pharmacol Ther*, 2010. 87(2): p. 191-6.
7. Langguth, P., G. Fricker, and H. Wunderli-Allenspach, *Biopharmazie*. 2004, Weinheim: WILEY-VCH Verlag GmbH & Co.KGaA.
8. Chaobal, H.N. and E.D. Kharasch, Single-point sampling for assessment of constitutive, induced, and inhibited cytochrome P450 3A activity with alfentanil or midazolam. *Clin Pharmacol Ther*, 2005. 78(5): p. 529-39.
9. Egorin, M.J., et al., A limited sampling strategy for cyclophosphamide pharmacokinetics. *Cancer Res*, 1989. 49(11): p. 3129-33.
10. Ratain, M.J. and N.J. Vogelzang, Limited sampling model for vinblastine pharmacokinetics. *Cancer Treat Rep*, 1987. 71(10): p. 935-9.
11. Rogers, J.F., et al., Single plasma concentrations of 1'-hydroxymidazolam or the ratio of 1'-hydroxymidazolam:midazolam do not predict midazolam clearance in healthy subjects. *J Clin Pharmacol*, 2002. 42(10): p. 1079-82.
12. Penzak, S.R., et al., Limitations of using a single postdose midazolam concentration to predict CYP3A-mediated drug interactions. *J Clin Pharmacol*, 2008. 48(6): p. 671-80.

- 1
2
3 13. Link, B., et al., Pharmacokinetics of intravenous and oral midazolam in plasma and
4 saliva in humans: usefulness of saliva as matrix for CYP3A phenotyping. Br J Clin
5 Pharmacol, 2008. 66(4): p. 473-84.
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

Table 1: Non linear regression of different midazolam partial AUCs / mg midazolam and measured metabolic clearance of midazolam to 1'-hydroxymidazolam (r^2 – coefficient of correlation).

partial AUC / mg MDZ	r^2
1 – 2 hr	0.8471
1 – 3 hr	0.8427
1 – 4 hr	0.8558
1 – 6 hr	0.8657
2 – 4 hr (2, 4 hr)	0.8795
2 – 4 hr (2, 3, 4 hr)	0.9062
2 – 4 hr (2, 2.5, 3, 4 hr)	0.9816

1
2
3 **Figure legends:**
4
5
6
7

8 Figure 1: Mean \pm SD (n=12) plasma concentration– time course of midazolam after oral
9 administration of 3 mg midazolam alone (●) and during efavirenz treatment (■)
10
11
12

13
14
15 Figure 2: Non-linear regression of midazolam AUC₂₋₄ and measured metabolic clearance of
16 midazolam to 1'-hydroxymidazolam
17
18

19
20
21
22 Figure 3: Bland-Altman analysis of predicted metabolic clearance and measured metabolic
23 clearance of midazolam to 1'-hydroxymidazolam
24
25

26 $CL_{\text{met-diff}}$ = difference of $CL_{\text{met-predicted}}$ and $CL_{\text{met-measured}}$
27
28

29 $CL_{\text{met-avg}}$ = average of $CL_{\text{met-predicted}}$ and $CL_{\text{met-measured}}$
30
31

32 Solid line = mean; Dashed line = 1.96*SD
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 1:

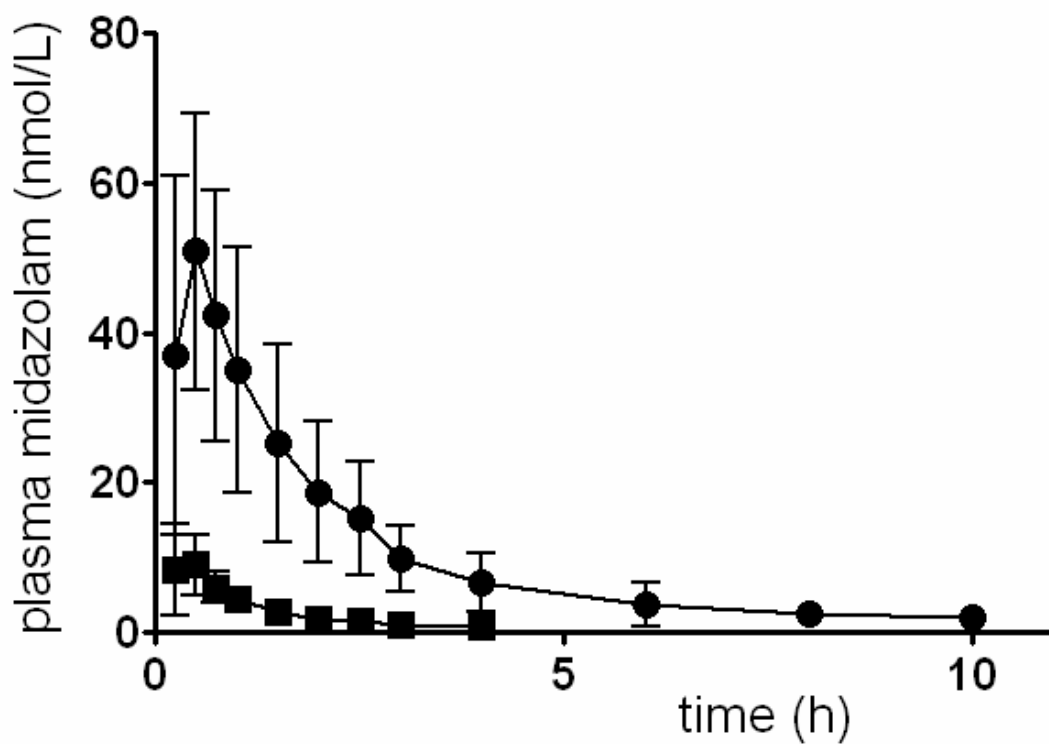


Figure 2:

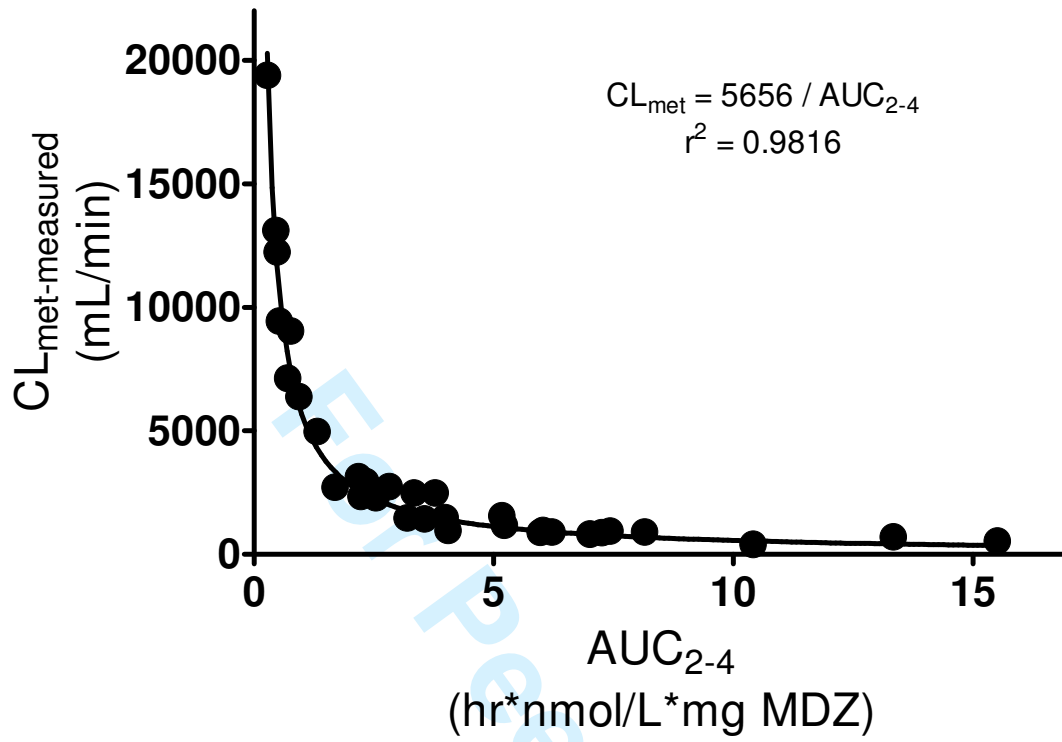
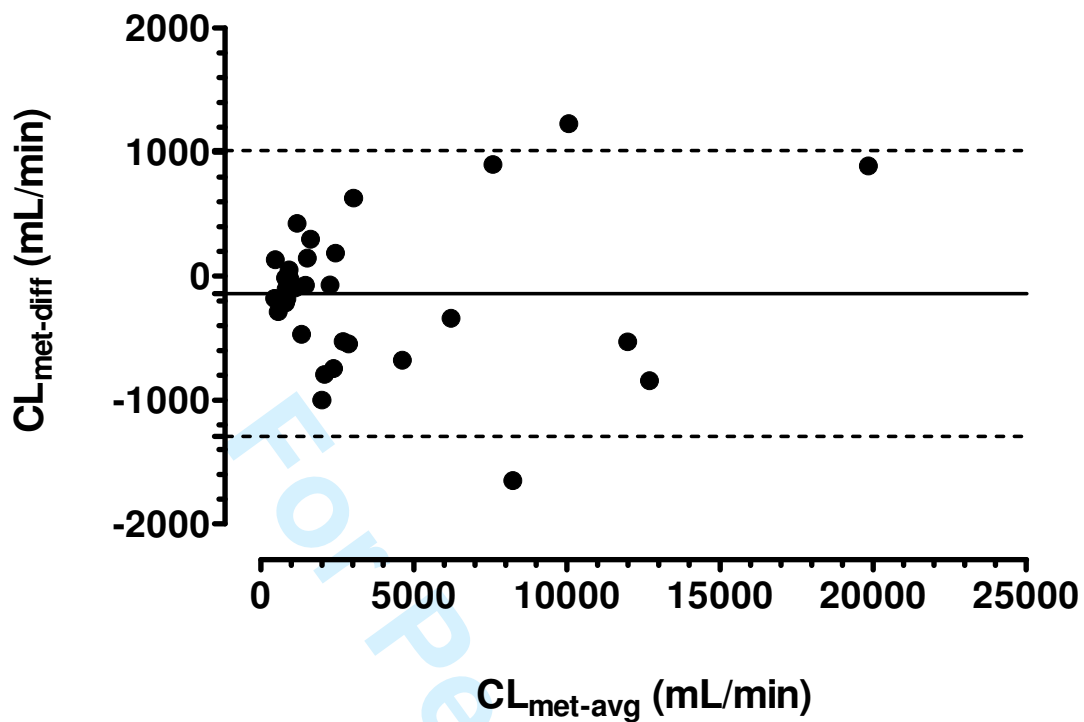


Figure 3:



1
2
3 **Proposal of a new limited sampling strategy to predict CYP3A activity using a partial**
4
5 **AUC of midazolam**
6
7
8
9

10 Stephanie Katzenmaier , Christoph Markert, Gerd Mikus
11

12
13
14
15 Department of Clinical Pharmacology and Pharmacoepidemiology, University of Heidelberg,
16

17 Im Neuenheimer Feld 410, D-69120 Heidelberg, Germany
18
19

20
21
22 * Address for correspondence and reprint requests:
23

24 Gerd Mikus, MD
25

26 Department Clinical Pharmacology and Pharmacoepidemiology
27

28 University Hospital of Heidelberg
29

30 Im Neuenheimer Feld 410
31

32 D-69120 Heidelberg
33

34 Germany
35
36
37
38
39
40

41 Tel.: + 49 6221 56 39197, Fax: + 49 6221 56 4642
42

43 Email: gerd.mikus@med.uni-heidelberg.de
44
45
46
47

48 Word count: 2176
49

50 Word count abstract: 253
51
52
53
54
55
56
57
58
59
60

PURPOSE

Midazolam metabolic clearance to 1'-hydroxymidazolam is an accurate measure for CYP3A activity which requires extensive plasma and urine sampling. The objective of this study was to find a new limited sampling strategy (LSS) to predict midazolam metabolic clearance to 1'-hydroxymidazolam and subsequently CYP3A activity in an easy and reliable way, reducing costs and labour.

METHODS

The development of this LSS is based on data of an in vivo drug-drug interaction study carried out in our clinical research unit. Various partial AUCs of midazolam were calculated and correlated with metabolic clearance of midazolam to 1'-hydroxymidazolam by non-linear regression. The correlation with highest r^2 -values was chosen in order to predict the midazolam metabolic clearance. Statistical significance of this method was verified by calculating the 95% confidence interval of the differences (%) between predicted and measured metabolic clearance of midazolam to 1'-hydroxymidazolam.

RESULTS

The midazolam partial AUC from 2-4 hours after oral administration correlated best with metabolic clearance of midazolam to 1'-hydroxymidazolam with $r^2 = 0.9816$. This partial AUC comprised of four midazolam concentrations at 2, 2.5, 3 and 4 hours after oral administration of a midazolam solution. The 95% confidence interval of the differences between predicted metabolic clearance and measured metabolic clearance of midazolam to 1'-hydroxymidazolam was [-0.97 to +13.2].

CONCLUSION

1
2
3 The determination of the partial AUC using four plasma samples from 2-4 hours after oral
4 administration of midazolam as a solution is proposed to be an easy and reliable CYP3A
5
6
7
8 phenotyping measure which of course needs to be validated in prospective clinical trials.
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

Introduction

Cytochromes P (CYPs) 450 represent a multifaceted family of heme-containing enzymes belonging to the group of monooxygenases accountable for phase-I-metabolism. Mammalian cytochromes are membrane proteins taking an active part in biosynthesis and metabolism of many physiological active substances. Moreover these enzymes are capable of catalysing biotransformation and metabolism of substances of foreign origin like drugs and toxins [1].

Cytochrome P 450 3A (CYP3A) is the most abundant enzyme of CYPs in the liver and the gut, it metabolises approximately 50% of currently available drugs and accounts for 30% of the total CYP enzymes in hepatic microsomes. A large number of drugs interfere with CYP3A as they may act as inducers, substrates or inhibitors of this enzyme.

To determine the activity of CYP3A midazolam is often used as a probe drug for this enzyme [2]. Midazolam belongs to the benzodiazepines family and is frequently used for anaesthetic induction, narcotic premedication and sedation of patients prior to surgery or other invasive medical procedures. It is extensively metabolised by CYP3A to its primary metabolite, 1'-hydroxymidazolam and to minor metabolites, 4'-hydroxymidazolam and 1, 4'-dihydroxymidazolam. Only 1 % of midazolam is eliminated unchanged in urine. Main urinary metabolite is 1'-hydroxymidazolamglucuronide, which amounts to 63-80 % of the administered dose within 24 hours. Conjugates of 4'-hydroxymidazolam and 1',4'-dihydroxymidazolam amount to 3 % and 1 % in urine [3]. Orally administered midazolam is used to assess the total - intestinal and hepatic - CYP3A activity.

Usually, CYP3A activity is tested before and during treatment with a potential inducer or inhibitor. To assess the time course of inhibition or induction frequent midazolam testing would be required. The most predictive and reliable parameter to assess CYP3A activity is the metabolic clearance to 1'-hydroxymidazolam [4]. Therefore, a full pharmacokinetic profile ($AUC_{0-\infty}$) and the urinary excretion of 1'-hydroxymidazolam and its glucuronide over at least 24 hours after single dose administration are required. Hence the use of midazolam as a probe

1
2
3 drug to determine the time course of CYP3A activity is very limited. A useful strategy for the
4
5 clinical practice of CYP3A4 phenotyping might be the reduction of the number of plasma
6
7 samples with still accurate determination of CYP3A activity. It will then be possible to obtain
8
9 CYP3A activity over many days and assess the time course of drug drug interactions.
10
11
12 We here propose a new limited sampling strategy to predict the midazolam metabolic
13
14 clearance to 1'-hydroxymidazolam and subsequently CYP3A-activity in an easy and reliable
15
16 way using much less plasma samples and no urine sampling. Limited sampling is a useful
17
18 strategy to simplify proceedings and reduce costs and labour. This is relevant for the clinical
19
20 application of CYP3A phenotyping.
21
22
23
24
25
26
27
28

29 **Methods**

30
31 The development of this LSS is based on data of an in vivo drug-drug interaction study
32
33 carried out at the clinical research unit of the Department of Clinical Pharmacology and
34
35 Pharmacoepidemiology, University of Heidelberg. In this study twelve participants were
36
37 included. The protocol was approved by the competent authority (BfArM, Germany) and the
38
39 Ethics Committee of the University of Heidelberg, written informed consent was obtained
40
41 from each subject prior to study-related procedures. On the first occasion they received
42
43 midazolam in order to determine the baseline metabolic clearance of midazolam to 1'-
44
45 hydroxymidazolam. Thereafter efavirenz (400 mg p.o. once daily) was administered over 14
46
47 days to study the inducing effect of efavirenz on CYP3A. Midazolam was given orally on day
48
49 1 and 14 of this period. After an overnight fast 3 mg midazolam (Dormicum[®] Roche
50
51 5mg/5ml, solution for injection or infusion) were administered in 100 ml tap water. 4 hours
52
53 thereafter a standard meal was provided for each subject. Blood samples were taken after
54
55 midazolam at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 hours and urine was collected
56
57 completely over 24 hours. Concentrations of midazolam and 1'-hydroxymidazolam in plasma
58
59
60

1
2
3 and urine were quantified by high performance liquid chromatography coupled to tandem
4
5 mass spectrometry after modification of a published method [5] as described elsewhere [6].
6
7 The LC/MS/MS system (Thermo Fisher, Dreieich, Germany) consisted of a Surveyor Plus
8
9 HPLC System and a triple stage quadrupole mass spectrometer (TSQ 7000 with API-2 ion
10
11 source and performance kit). For quantification of the drugs a Phenomenex Max RP column
12
13 (150 x 2 mm, 4 μ m, Phenomenex, Aschaffenburg, Germany) with integrated guard column
14
15 was used at 40°C for chromatographic separation. The bioanalytical method was validated
16
17 according to the recommendations of the FDA in the range of 0.61-460 nmol/L for midazolam
18
19 and 0.59-439 nmol/L for 1'-hydroxymidazolam in plasma and 1.53-153 nmol/L for
20
21 midazolam and 73.2-7315 nmol/L for 1'-hydroxymidazolam in urine. Lower limits of
22
23 quantification for midazolam and 1'-hydroxymidazolam in plasma were 0.61 and 0.59 nmol/l,
24
25 respectively, (with accuracy/precision of -4.2%/5.0% and +11.6%/4.6%, respectively); lower
26
27 limit of quantification in urine was 1.53 nmol/l for midazolam and 72.2 nmol/l for 1'-
28
29 hydroxymidazolam (accuracy/precision of +8.0%/7.9% and +3.8%/7.1%, respectively).
30
31 Metabolic clearance of midazolam to 1'-hydroxymidazolam was calculated as the amount of
32
33 total (conjugated + unconjugated) 1'-hydroxymidazolam excreted in urine (0-24 h) divided by
34
35 midazolam AUC_{0-∞}.
36
37 In order to predict metabolic clearance of midazolam to 1'-hydroxymidazolam various partial
38
39 midazolam AUCs were calculated and correlated with the metabolic clearance of midazolam
40
41 to 1'-hydroxymidazolam. Stepwise multiple regressions were used to identify optimal time
42
43 points and intervals of midazolam plasma concentrations, respectively. Standard
44
45 pharmacokinetic parameter and partial AUCs (1-2 hours, 1-3 hours, 1-4 hours, 2-4 hours, and
46
47 1-6 hours) after oral administration of midazolam were calculated by noncompartmental
48
49 analysis using WinNonlin 5.2 (Pharsight Corp., Mountain View, CA, USA). Midazolam
50
51 concentrations were expressed per mg dose of midazolam. Non-linear regressions were
52
53 performed using Prism 5.03 (GraphPad Software Inc., La Jolla, CA, USA).
54
55
56
57
58
59
60

From the following equations [7] we derived a formula to calculate the metabolic clearance of midazolam to 1'-hydroxymidazolam using a partial AUC of midazolam.

Clearance is defined as the removal of substance from blood, expressed as volume per time.

$$\frac{dA_e}{dt} = k_{10} \cdot A = k_{10} \cdot V \cdot C = \lambda_z \cdot V_z \cdot C = CL \cdot C \quad (1)$$

A_e (total eliminated drug), k (elimination rate constant first order), A (amount of drug), V (volume of distribution), C (concentration), λ (reaction rate constant first order), CL (clearance), t (time after medication)

The total eliminated drug is consistent with the integral of equation 1

$$A_{e_{\infty}} = CL \int_0^{\infty} C \cdot dt = CL \cdot AUC \quad (2)$$

$A_{e_{\infty}}$ (total eliminated drug until time point of infinity), AUC (area under the curve)

which is also equal to systemically available dose ($F \times D$)

$$A_{e_{\infty}} = CL \cdot AUC = F \cdot D \quad (3)$$

F (bioavailability), D (dose)

$$AUC = \frac{F}{CL} \cdot D \quad (4)$$

Clearance and AUC are inversely related, and almost no midazolam is excreted unchanged in urine. Hence, clearance can be replaced by metabolic clearance. By means of non-linear regression of various partial AUCs and metabolic clearance of midazolam to 1'-hydroxymidazolam (Prism 5.03) the factor A was calculated:

$$partialAUC = \frac{A}{CL_{met}} \quad (5)$$

Using the best fit (highest r^2) the prediction of the metabolic clearance of midazolam to 1'-hydroxymidazolam from this partial AUC is done by:

$$CL_{met-predicted} = \frac{A}{partialAUC} \quad (6)$$

1
2
3 In order to verify the statistical significance of this predictive method the 95% confidence
4 interval of the differences (%) between predicted metabolic clearance and measured metabolic
5 clearance of midazolam to 1'-hydroxymidazolam was constructed. Precision and bias were
6
7
8
9
10 assessed by the Bland-Altman method [8].
11

12 13 14 15 **Results**

16
17 All partial AUCs / mg midazolam of 1-2 hours, 1-3 hours, 1-4 hours, 2-4 hours and 1-6 hours
18 after oral medication correlated with metabolic clearance of midazolam to 1'-
19 hydroxymidazolam. The full plasma concentration time profile of midazolam is shown in
20 Figure 1, correlation results in Table 1. The correlation of partial AUC from 2-4 hours with
21
22 the measured metabolic clearance of midazolam to 1'-hydroxymidazolam showed the highest
23
24 r^2 -value ($r^2 = 0.9816$; Figure 2). This partial AUC comprises of four blood samples at 2, 2.5, 3
25 and 4 hours after oral midazolam administration. The use of a further reduced number of
26
27 plasma samples to calculate partial AUC results in weaker correlations (partial AUC of 2 – 4
28
29 hr with three blood samples at 2, 3, 4 hr: $r^2 = 0.9062$, partial AUC of 2 – 4 hr with two blood
30
31 samples at 2, 4 hr: $r^2 = 0.8795$).
32
33
34
35
36
37
38
39
40

41 Related to this non-linear regression the factor was calculated as $A = 5656$. Therefore, the
42
43 following formula predicts the metabolic clearance of midazolam to 1'-hydroxymidazolam
44
45 and CYP3A activity, respectively:
46
47

$$48 \quad CL_{met-predicted} = \frac{5656}{AUC_{2-4}} \quad (7)$$

49
50
51

52 The midazolam concentrations to calculate partial AUC have to be expressed per mg dose of
53
54 midazolam.
55

56
57 There is a strong relationship between the measured and predicted metabolic clearance of
58
59 midazolam to 1'-hydroxymidazolam. Using linear regression, regression coefficient was 0.98,
60
 $p < 0.0001$. The 95% confidence interval of the differences between predicted metabolic

1
2
3 clearance and measured metabolic clearance of midazolam to 1'-hydroxymidazolam [-0.97 %
4 to +13.2 %] showed no significant difference. There was good precision and low bias of the
5 predicted metabolic clearance of midazolam to 1'-hydroxymidazolam (Figure 3). In contrast
6 the 95% confidence interval of the differences between predicted metabolite clearance and
7 measured metabolic clearance of midazolam to 1'-hydroxymidazolam using this partial AUC
8 and minor plasma samples showed a significant difference (95% confidence interval using
9 three blood samples (2, 3, 4 hr): [+18.11% to +31.68%], 95% confidence interval using two
10 blood samples (2, 4 hr): [+13.33% to +28.71%]).

21 Discussion

22
23
24
25
26
27 The aim of this investigation was to find a useful strategy which predicts CYP3A activity in
28 an easy and reliable way and facilitates the clinical application of CYP3A phenotyping and
29 the determination of the time course of drug drug interactions. LSS has already been proven
30 to be an effective tool in monitoring the safety and efficacy of chemotherapeutic agents by
31 minimising the number of blood samples necessary to evaluate AUC [9]. It was first
32 introduced in 1987 in order to minimise the number of plasma samples for measuring AUC of
33 vinblastine. With only two time points, Ratain and Vogelzang were able to estimate total
34 AUC using stepwise multiple regression analysis [10]. Since then various LSSs have been
35 investigated for many drugs in order to simplify sampling procedures and minimise costs.
36
37 Regarding midazolam and the activity of CYP3A Rogers et al. found that neither single
38 plasma concentrations of the metabolite 1-hydroxy-midazolam nor the ratio of 1-hydroxy-
39 midazolam and midazolam can be used for predicting midazolam clearance in healthy
40 subjects [11]. Another study was performed to investigate limitations of using a single post-
41 dose midazolam concentration to predict CYP3A-mediated drug-drug interactions. The
42 purpose was to assess the ability of one single midazolam concentration for predicting
43 midazolam AUC in the presence and absence of CYP3A modulation by Gingko biloba

1
2
3 inducing CYP3A. The study showed that optimal midazolam sampling times to predict AUC
4 were different before or after Gingko biloba [12]. This approach is not useful for prospective
5 clinical trials where CYP3A activity is to be determined. Another strategy is the use of saliva
6 for CYP3A phenotyping using midazolam [10]. This method however requires numerous
7 samples over at least 6 hours and a very sensitive analytical method due to the very low
8 concentrations in saliva [13].
9

10
11 Midazolam pharmacokinetics is well known and understood. Primary metabolic pathway is
12 the formation of 1'-hydroxymidazolam via CYP3A which is subsequently conjugated and
13 excreted in urine and accounts for >70% of the oral dose administered [4]. Hence,
14 quantification of this pathway by the metabolic clearance of midazolam to 1'-
15 hydroxymidazolam is the most suitable parameter for CYP3A activity. We investigated the
16 correlations between various partial midazolam AUCs and metabolic clearance of midazolam
17 to 1'-hydroxymidazolam in order to find a reliable method to predict midazolam metabolic
18 clearance. When midazolam is administered as an oral solution the best results were obtained
19 using the midazolam partial AUC from 2 to 4 hours comprising of four plasma samples at 2,
20 2.5, 3 and 4 hr after oral administration of midazolam. Resorption and distribution is fast after
21 an oral solution and seems not to be influencing the prediction of CYP3A activity when using
22 plasma samples from 2-4 hours after midazolam administration. The prediction of the
23 metabolic clearance of midazolam to 1'-hydroxymidazolam is only valid for the study
24 conditions described above and for healthy volunteers without any conditions which could
25 modify the absorption, distribution, metabolism or excretion of drugs. However, this method
26 might also be applied to patients with limited metabolism caused by liver diseases in order to
27 identify drug-drug interactions. The alteration of CYP3A activity by the addition of a drug
28 should also be valid in these patients with the limitation that the prediction of the metabolic
29 clearance might not be accurate.
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Using four plasma samples to determine partial AUC instead of one single plasma
4
5 concentration minimises errors in prediction of CYP3A activity due to imprecisions of the
6
7 analytical measurements.
8

9
10 General prediction of CYP3A-activity has to comprise a full range of metabolic activity,
11
12 including induction, inhibition and normal state of enzyme activity. Although this LSS solely
13
14 is derived from induced and normal CYP3A activity, it is clear from Figure 2 that inhibition
15
16 of CYP3A activity will result in high partial AUCs and very low metabolic clearance
17
18 approaching zero when there is no metabolic activity.
19
20
21
22
23

24 **Conclusion**

25
26 We propose a new LSS to predict midazolam metabolic clearance to 1'-hydroxymidazolam
27
28 and thus CYP3A activity using a full pharmacokinetic dataset from an interaction study. The
29
30 determination of the partial AUC from 2-4 hours after oral administration of midazolam
31
32 predicts CYP3A4 activity with high precision and will enable the assessment of the time
33
34 course of drug drug interactions and the clinical application of CYP3A4 phenotyping.
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

REFERENCES

1. Myasoedova, K.N., New findings in studies of cytochromes P450. *Biochemistry (Mosc)*, 2008. 73(9): p. 965-9.
2. Greenblatt, D.J., et al., Inhibition of oral midazolam clearance by boosting doses of ritonavir, and by 4,4-dimethyl-benziso-(2H)-selenazine (ALT-2074), an experimental catalytic mimic of glutathione oxidase. *Br J Clin Pharmacol*, 2009. 68(6): p. 920-7.
3. Streetman, D.S., J.S. Bertino, Jr., and A.N. Nafziger, Phenotyping of drug-metabolizing enzymes in adults: a review of in-vivo cytochrome P450 phenotyping probes. *Pharmacogenetics*, 2000. 10(3): p. 187-216.
4. Thummel, K.E., et al., Oral first-pass elimination of midazolam involves both gastrointestinal and hepatic CYP3A-mediated metabolism. *Clin Pharmacol Ther*, 1996. 59(5): p. 491-502.
5. Quintela, O., et al., A sensitive, rapid and specific determination of midazolam in human plasma and saliva by liquid chromatography/electrospray mass spectrometry. *Rapid Commun Mass Spectrom*, 2004. 18(24): p. 2976-82.
6. Hafner, V., et al., Effect of simultaneous induction and inhibition of CYP3A by St John's Wort and ritonavir on CYP3A activity. *Clin Pharmacol Ther*, 2010. 87(2): p. 191-6.
7. Langguth, P., G. Fricker, and H. Wunderli-Allenspach, *Biopharmazie*. 2004, Weinheim: WILEY-VCH Verlag GmbH & Co.KGaA.
8. Chaobal, H.N. and E.D. Kharasch, Single-point sampling for assessment of constitutive, induced, and inhibited cytochrome P450 3A activity with alfentanil or midazolam. *Clin Pharmacol Ther*, 2005. 78(5): p. 529-39.
9. Egorin, M.J., et al., A limited sampling strategy for cyclophosphamide pharmacokinetics. *Cancer Res*, 1989. 49(11): p. 3129-33.
10. Ratain, M.J. and N.J. Vogelzang, Limited sampling model for vinblastine pharmacokinetics. *Cancer Treat Rep*, 1987. 71(10): p. 935-9.
11. Rogers, J.F., et al., Single plasma concentrations of 1'-hydroxymidazolam or the ratio of 1'-hydroxymidazolam:midazolam do not predict midazolam clearance in healthy subjects. *J Clin Pharmacol*, 2002. 42(10): p. 1079-82.
12. Penzak, S.R., et al., Limitations of using a single postdose midazolam concentration to predict CYP3A-mediated drug interactions. *J Clin Pharmacol*, 2008. 48(6): p. 671-80.

- 1
2
3 13. Link, B., et al., Pharmacokinetics of intravenous and oral midazolam in plasma and
4 saliva in humans: usefulness of saliva as matrix for CYP3A phenotyping. Br J Clin
5 Pharmacol, 2008. 66(4): p. 473-84.
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

Table 1: Non linear regression of different midazolam partial AUCs / mg midazolam and measured metabolic clearance of midazolam to 1'-hydroxymidazolam (r^2 – coefficient of correlation).

partial AUC / mg MDZ	r^2
1 – 2 hr	0.8471
1 – 3 hr	0.8427
1 – 4 hr	0.8558
1 – 6 hr	0.8657
2 – 4 hr (2, 4 hr)	0.8795
2 – 4 hr (2, 3, 4 hr)	0.9062
2 – 4 hr (2, 2.5, 3, 4 hr)	0.9816

Figure legends:

Figure 1: Mean \pm SD (n=12) plasma concentration– time course of midazolam after oral administration of 3 mg midazolam alone (●) and during efavirenz treatment (■)

Figure 2: Non-linear regression of midazolam AUC_{2-4} and measured metabolic clearance of midazolam to 1'-hydroxymidazolam

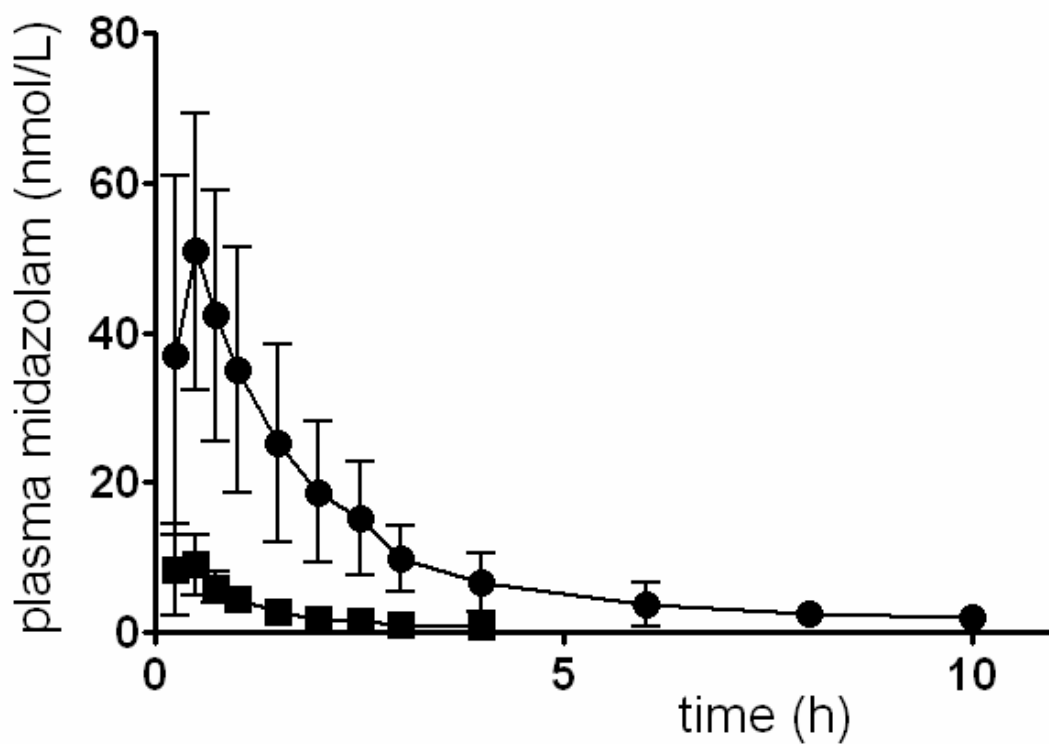
Figure 3: Bland-Altman analysis of predicted metabolic clearance and measured metabolic clearance of midazolam to 1'-hydroxymidazolam

$CL_{met-diff}$ = difference of $CL_{met-predicted}$ and $CL_{met-measured}$

$CL_{met-avg}$ = average of $CL_{met-predicted}$ and $CL_{met-measured}$

Solid line = mean; Dashed line = $1.96*SD$

Figure 1:



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 2:

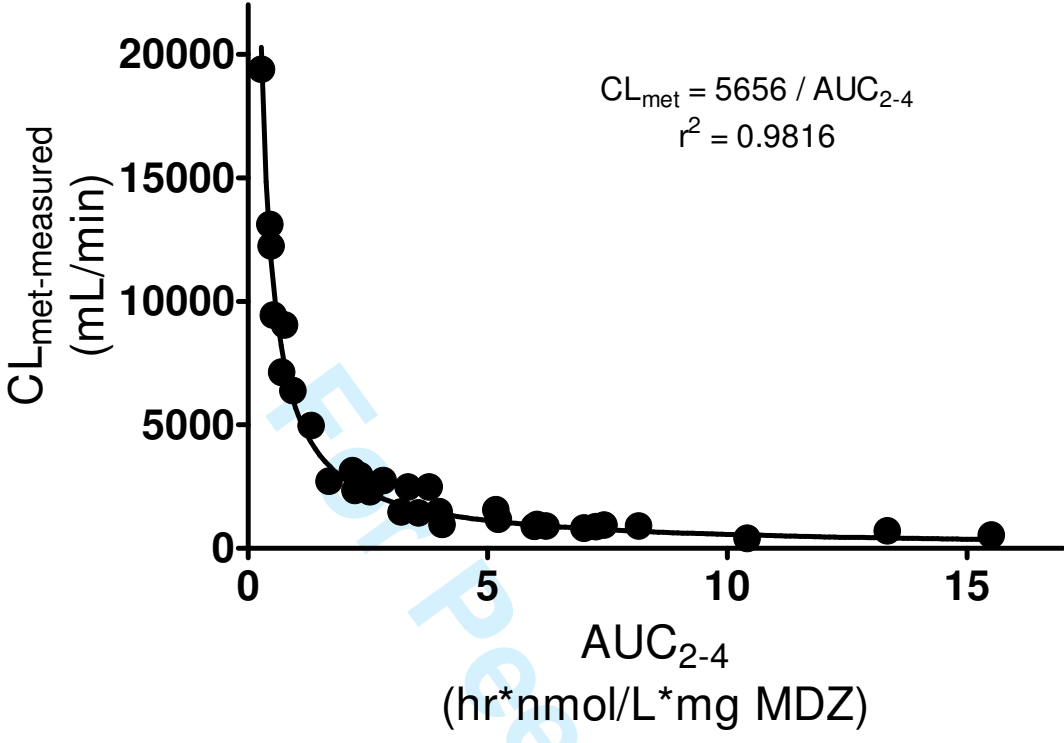


Figure 3:

