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

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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

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The determination of the partial AUC using four plasma samples from 2-4 hours after oral administration of midazolam as a solution is proposed to be an easy and reliable CYP3A phenotyping measure which of course needs to be validated in prospective clinical trials.

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Introduction

Cytochromes P (CYPs) 450 represent a multifaceted family of heme-containing enzymes belonging to the group of monooxygenases accountable for phase-1-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

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drug to determine the time course of CYP3A activity is very limited. A useful strategy for the clinical practice of CYP3A4 phenotyping might be the reduction of the number of plasma samples with still accurate determination of CYP3A activity. It will then be possible to obtain CYP3A activity over many days and assess the time course of drug drug interactions.

We here propose a new limited sampling strategy to predict the midazolam metabolic clearance to 1'-hydroxymidazolam and subsequently CYP3A-activity in an easy and reliable way using much less plasma samples and no urine sampling. Limited sampling is a useful strategy to simplify proceedings and reduce costs and labour. This is relevant for the clinical application of CYP3A phenotyping.

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Methods

The development of this LSS is based on data of an in vivo drug-drug interaction study carried out at the clinical research unit of the Department of Clinical Pharmacology and Pharmacoepidemiology, University of Heidelberg. In this study twelve participants were included. The protocol was approved by the competent authority (BfArM, Germany) and the Ethics Committee of the University of Heidelberg, written informed consent was obtained from each subject prior to study-related procedures. On the first occasion they received midazolam in order to determine the baseline metabolic clearance of midazolam to 1'-hydroxymidazolam. Thereafter efavirenz (400 mg p.o. once daily) was administered over 14 days to study the inducing effect of efavirenz on CYP3A. Midazolam was given orally on day 1 and 14 of this period. After an overnight fast 3 mg midazolam (Dormicum® Roche 5mg/5ml, solution for injection or infusion) were administered in 100 ml tap water. 4 hours thereafter a standard meal was provided for each subject. Blood samples were taken after 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 completely over 24 hours. Concentrations of midazolam and 1'-hydroxymidazolam in plasma

and urine were quantified by high performance liquid chromatography coupled to tandem mass spectrometry after modification of a published method [5] as described elsewhere [6]. The LC/MS/MS system (Thermo Fisher, Dreieich, Germany) consisted of a Surveyor Plus HPLC System and a triple stage quadrupole mass spectrometer (TSQ 7000 with API-2 ion source and performance kit). For quantification of the drugs a Phenomenex Max RP column (150 x 2 mm, 4 µm, Phenomenex, Aschaffenburg, Germany) with integrated guard column was used at 40°C for chromatographic separation. The bioanalytical method was validated according to the recommendations of the FDA in the range of 0.61-460 nmol/L for midazolam and 0.59-439 nmol/L for 1'-hydroxymidazolam in plasma and 1.53-153 nmol/L for midazolam and 73.2-7315 nmol/L for 1'-hydroxymidazolam in urine. Lower limits of quantification for midazolam and 1'-hydroxymidazolam in plasma were 0.61 and 0.59 nmol/l, respectively, (with accuracy/precision of –4.2%/5.0% and +11.6%/4.6%, respectively); lower limit of quantification in urine was 1.53 nmol/l for midazolam and 72.2 nmol/l for 1'-dydroxymidazolam (accuracy/precision of +8.0%/7.9% and +3.8%/7.1%, respectively). Metabolic clearance of midazolam to 1'-hydroxymidazolam was calculated as the amount of total (conjugated + unconjugated) 1'-hydroxymidazolam excreted in urine (0-24 h) divided by midazolam $AUC_{0-\infty}$.

In order to predict metabolic clearance of midazolam to 1'-hydroxymidazolam various partial midazolam AUCs were calculated and correlated with the metabolic clearance of midazolam to 1'-hydroxymidazolam. Stepwise multiple regressions were used to identify optimal time points and intervals of midazolam plasma concentrations, respectively. Standard pharmacokinetic parameter and partial AUCs (1-2 hours, 1-3 hours, 1-4 hours, 2-4 hours, and 1-6 hours) after oral administration of midazolam were calculated by noncompartmental analysis using WinNonlin 5.2 (Pharsight Corp., Mountain View, CA, USA). Midazolam concentrations were expressed per mg dose of midazolam. Non-linear regressions were performed using Prism 5.03 (GraphPad Software Inc., La Jolla, CA, USA).

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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.

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

Ae (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

$$Ae_{\infty} = CL\int_{0}^{\infty} C \cdot dt = CL \cdot AUC \qquad (2)$$

Ae_{∞} (total eliminated drug until time point of infinity), AUC (area under the curve) which is also equal to systemically available dose (F x D)

$$Ae_{\infty} = CL \cdot AUC = F \cdot D \qquad (3)$$

F (bioavailability), D (dose)

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

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:

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

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:

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

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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:

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

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

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

Discussion

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

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

Midazolam pharmacokinetics is well known und understood. Primary metabolic pathway is the formation of 1'-hydroxymidazolam via CYP3A which is subsequently conjugated and excreted in urine and accounts for >70% of the oral dose administered [4]. Hence, quantification of this pathway by the metabolic clearance of midazolam to 1'-hydroxymidazolam is the most suitable parameter for CYP3A activity. We investigated the correlations between various partial midazolam AUCs and metabolic clearance of midazolam to 1'-hydroxymidazolam in order to find a reliable method to predict midazolam metabolic clearance. When midazolam is administered as an oral solution the best results were obtained using the midazolam partial AUC from 2 to 4 hours comprising of four plasma samples at 2, 2.5, 3 and 4 hr after oral administration of midazolam. Resorption and distribution is fast after an oral solution and seems not to be influencing the prediction of CYP3A activity when using plasma samples from 2-4 hours after midazomal administration. The prediction of the metabolic clearance of midazolam to 1'-hydroxymidazolam is only valid for the study conditions described above and for healthy volunteers without any conditions which could modify the absorption, distribution, metabolism or excretion of drugs. However, this method might also be applied to patients with limited metabolism caused by liver diseases in order to identify drug-drug interactions. The alteration of CYP3A activity by the addition of a drug should also be valid in these patients with the limitation that the prediction of the metabolic clearance might not be accurate.

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Using four plasma samples to determine partial AUC instead of one single plasma concentration minimises errors in prediction of CYP3A activity due to imprecisions of the analytical measurements.

General prediction of CYP3A-activity has to comprise a full range of metabolic activity, including induction, inhibition and normal state of enzyme activity. Although this LSS solely is derived from induced and normal CYP3A activity, it is clear from Figure 2 that inhibition of CYP3A activity will result in high partial AUCs and very low metabolic clearance approaching zero when there is no metabolic activity.

Conclusion

We propose a new LSS to predict midazolam metabolic clearance to 1'-hydroxymidazolam and thus CYP3A activity using a full pharmacokinetic dataset from an interaction study. The determination of the partial AUC from 2-4 hours after oral administration of midazolam predicts CYP3A4 activity with high precision and will enable the assessment of the time course of drug drug interactions and the clinical application of CYP3A4 phenotyping.

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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.

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13. Link, B., et al., Pharmacokinetics of intravenous and oral midazolam in plasma and saliva in humans: usefulness of saliva as matrix for CYP3A phenotyping. Br J Clin Pharmacol, 2008. 66(4): p. 473-84.

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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

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Figure legends:

Figure 1: Mean ± 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 $AUC_{2\text{-}4}$ 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

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

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

Solid line = mean; Dashed line = 1.96*SD

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Figure 1:

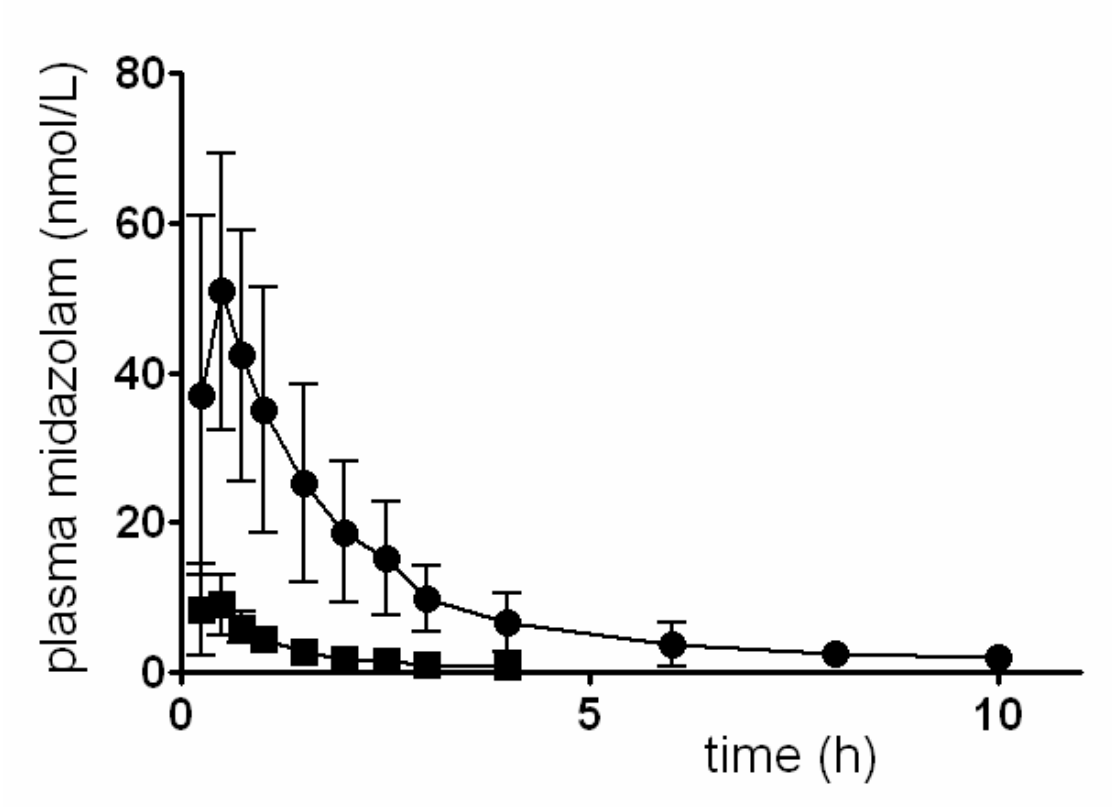

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Figure 2:

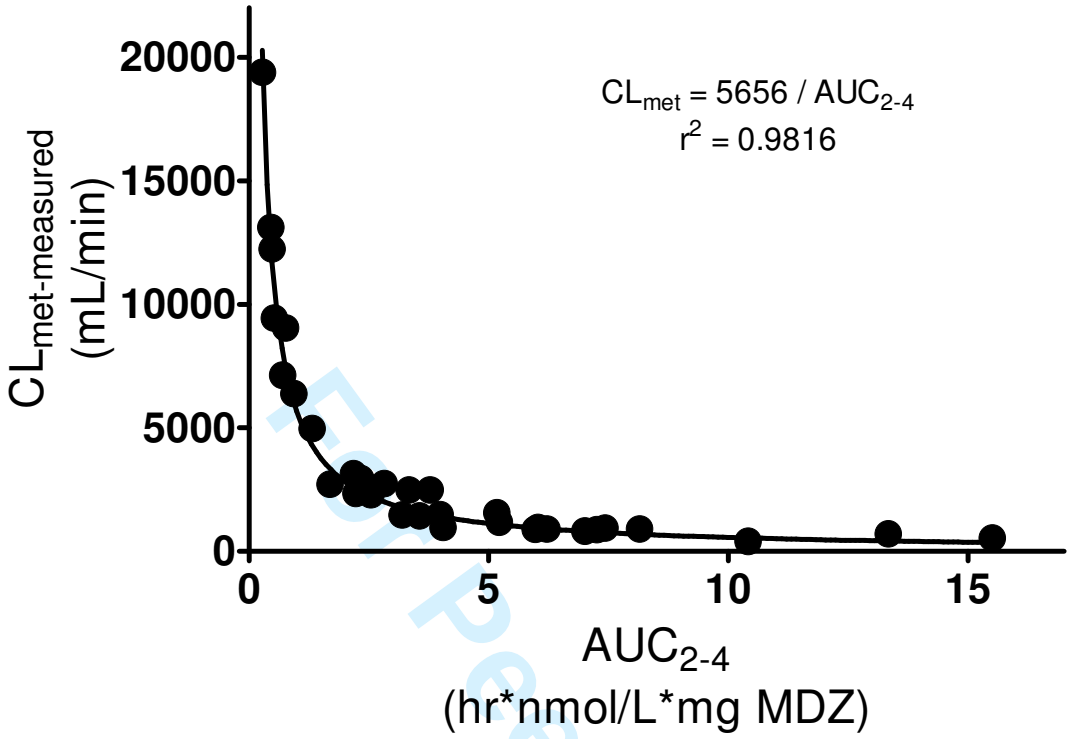

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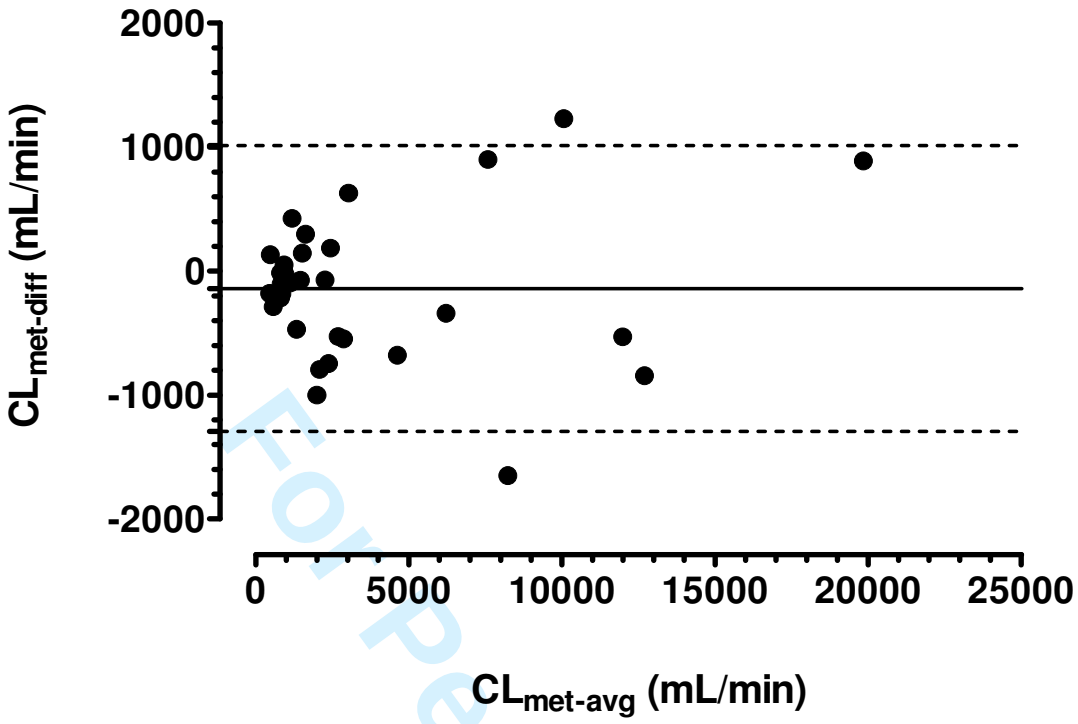
Figure 3:



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The development of this LSS is based on data of an in vivo drug-drug interaction study carried out at the clinical research unit of the Department of Clinical Pharmacology and Pharmacoepidemiology, University of Heidelberg. In this study twelve participants were included. The protocol was approved by the competent authority (BfArM, Germany) and the Ethics Committee of the University of Heidelberg, written informed consent was obtained from each subject prior to study-related procedures. On the first occasion they received midazolam in order to determine the baseline metabolic clearance of midazolam to 1'-hydroxymidazolam. Thereafter efavirenz (400 mg p.o. once daily) was administered over 14 days to study the inducing effect of efavirenz on CYP3A. Midazolam was given orally on day 1 and 14 of this period. After an overnight fast 3 mg midazolam (Dormicum® Roche 5mg/5ml, solution for injection or infusion) were administered in 100 ml tap water. 4 hours thereafter a standard meal was provided for each subject. Blood samples were taken after 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 completely over 24 hours. Concentrations of midazolam and 1'-hydroxymidazolam in plasma

and urine were quantified by high performance liquid chromatography coupled to tandem mass spectrometry after modification of a published method [5] as described elsewhere [6]. The LC/MS/MS system (Thermo Fisher, Dreieich, Germany) consisted of a Surveyor Plus HPLC System and a triple stage quadrupole mass spectrometer (TSQ 7000 with API-2 ion source and performance kit). For quantification of the drugs a Phenomenex Max RP column (150 x 2 mm, 4 µm, Phenomenex, Aschaffenburg, Germany) with integrated guard column was used at 40°C for chromatographic separation. The bioanalytical method was validated according to the recommendations of the FDA in the range of 0.61-460 nmol/L for midazolam and 0.59-439 nmol/L for 1'-hydroxymidazolam in plasma and 1.53-153 nmol/L for midazolam and 73.2-7315 nmol/L for 1'-hydroxymidazolam in urine. Lower limits of quantification for midazolam and 1'-hydroxymidazolam in plasma were 0.61 and 0.59 nmol/l, respectively, (with accuracy/precision of –4.2%/5.0% and +11.6%/4.6%, respectively); lower limit of quantification in urine was 1.53 nmol/l for midazolam and 72.2 nmol/l for 1'-dydroxymidazolam (accuracy/precision of +8.0%/7.9% and +3.8%/7.1%, respectively). Metabolic clearance of midazolam to 1'-hydroxymidazolam was calculated as the amount of total (conjugated + unconjugated) 1'-hydroxymidazolam excreted in urine (0-24 h) divided by midazolam $AUC_{0-\infty}$.

In order to predict metabolic clearance of midazolam to 1'-hydroxymidazolam various partial midazolam AUCs were calculated and correlated with the metabolic clearance of midazolam to 1'-hydroxymidazolam. Stepwise multiple regressions were used to identify optimal time points and intervals of midazolam plasma concentrations, respectively. Standard pharmacokinetic parameter and partial AUCs (1-2 hours, 1-3 hours, 1-4 hours, 2-4 hours, and 1-6 hours) after oral administration of midazolam were calculated by noncompartmental analysis using WinNonlin 5.2 (Pharsight Corp., Mountain View, CA, USA). Midazolam concentrations were expressed per mg dose of midazolam. Non-linear regressions were performed using Prism 5.03 (GraphPad Software Inc., La Jolla, CA, USA).

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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.

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

Ae (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

$$Ae_{\infty} = CL\int_{0}^{\infty} C \cdot dt = CL \cdot AUC \qquad (2)$$

Ae_{∞} (total eliminated drug until time point of infinity), AUC (area under the curve) which is also equal to systemically available dose (F x D)

$$Ae_{\infty} = CL \cdot AUC = F \cdot D \qquad (3)$$

F (bioavailability), D (dose)

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

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:

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

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:

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

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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:

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

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

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

Discussion

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

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

Midazolam pharmacokinetics is well known und understood. Primary metabolic pathway is the formation of 1'-hydroxymidazolam via CYP3A which is subsequently conjugated and excreted in urine and accounts for >70% of the oral dose administered [4]. Hence, quantification of this pathway by the metabolic clearance of midazolam to 1'-hydroxymidazolam is the most suitable parameter for CYP3A activity. We investigated the correlations between various partial midazolam AUCs and metabolic clearance of midazolam to 1'-hydroxymidazolam in order to find a reliable method to predict midazolam metabolic clearance. When midazolam is administered as an oral solution the best results were obtained using the midazolam partial AUC from 2 to 4 hours comprising of four plasma samples at 2, 2.5, 3 and 4 hr after oral administration of midazolam. Resorption and distribution is fast after an oral solution and seems not to be influencing the prediction of CYP3A activity when using plasma samples from 2-4 hours after midazomal administration. The prediction of the metabolic clearance of midazolam to 1'-hydroxymidazolam is only valid for the study conditions described above and for healthy volunteers without any conditions which could modify the absorption, distribution, metabolism or excretion of drugs. However, this method might also be applied to patients with limited metabolism caused by liver diseases in order to identify drug-drug interactions. The alteration of CYP3A activity by the addition of a drug should also be valid in these patients with the limitation that the prediction of the metabolic clearance might not be accurate.

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Using four plasma samples to determine partial AUC instead of one single plasma concentration minimises errors in prediction of CYP3A activity due to imprecisions of the analytical measurements.

General prediction of CYP3A-activity has to comprise a full range of metabolic activity, including induction, inhibition and normal state of enzyme activity. Although this LSS solely is derived from induced and normal CYP3A activity, it is clear from Figure 2 that inhibition of CYP3A activity will result in high partial AUCs and very low metabolic clearance approaching zero when there is no metabolic activity.

Conclusion

We propose a new LSS to predict midazolam metabolic clearance to 1'-hydroxymidazolam and thus CYP3A activity using a full pharmacokinetic dataset from an interaction study. The determination of the partial AUC from 2-4 hours after oral administration of midazolam predicts CYP3A4 activity with high precision and will enable the assessment of the time course of drug drug interactions and the clinical application of CYP3A4 phenotyping.

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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.

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13. Link, B., et al., Pharmacokinetics of intravenous and oral midazolam in plasma and saliva in humans: usefulness of saliva as matrix for CYP3A phenotyping. Br J Clin Pharmacol, 2008. 66(4): p. 473-84.

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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

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Figure legends:

Figure 1: Mean ± 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 $AUC_{2\text{-}4}$ 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

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

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

Solid line = mean; Dashed line = 1.96*SD

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Figure 1:

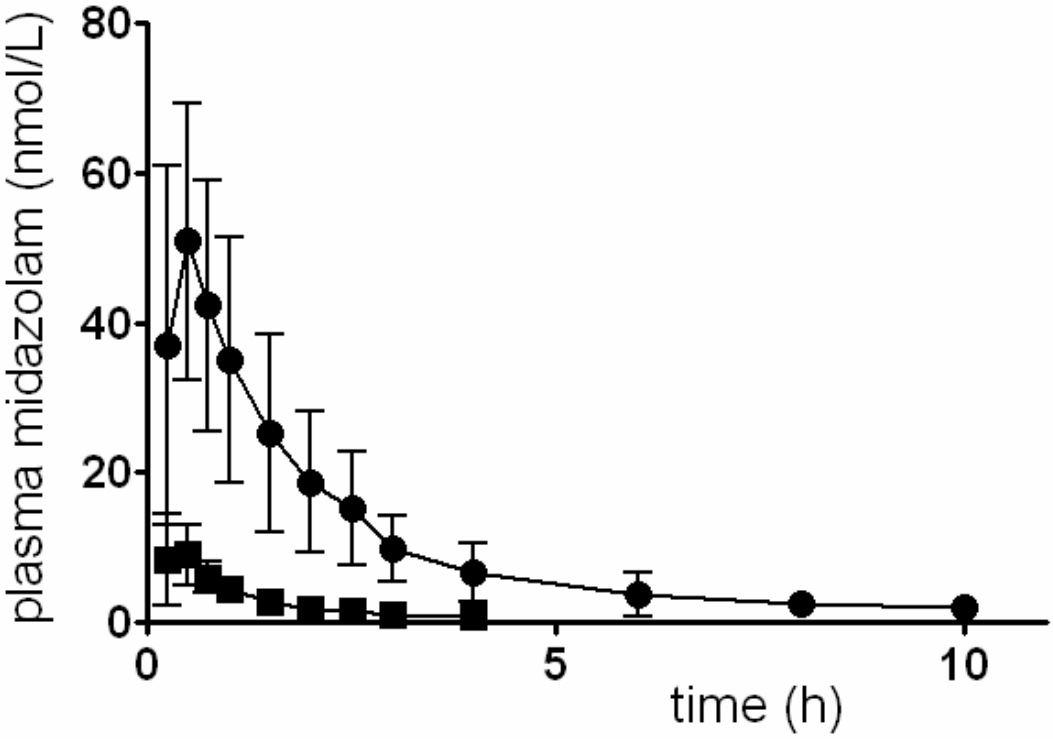

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Figure 2:

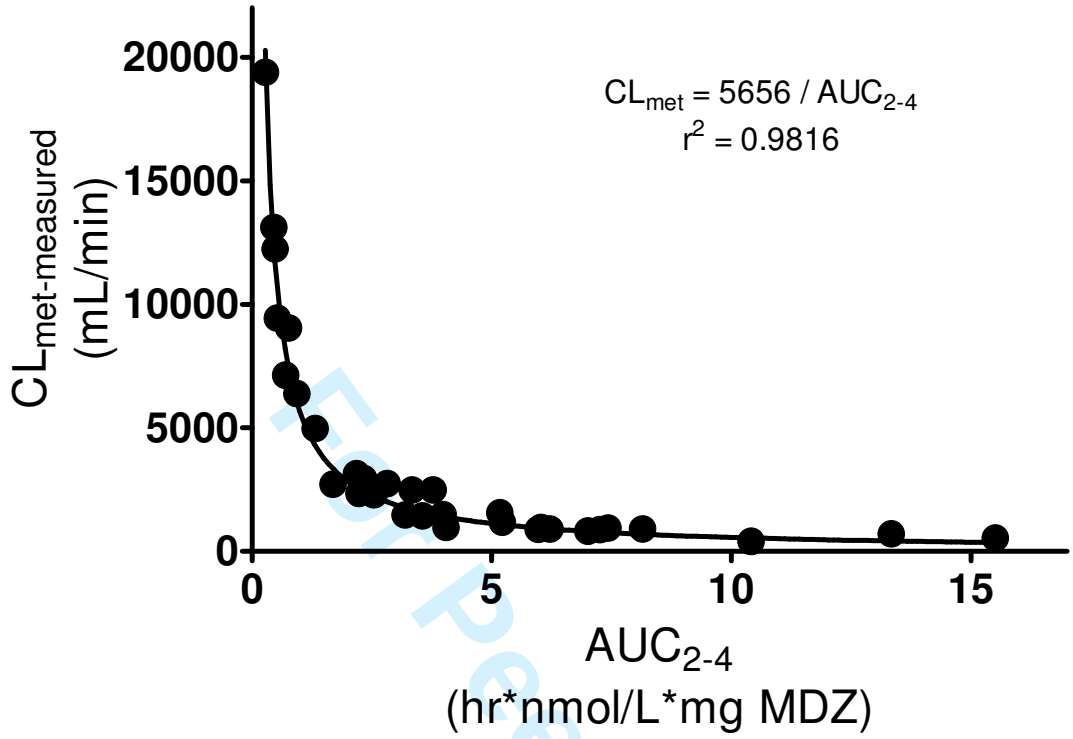

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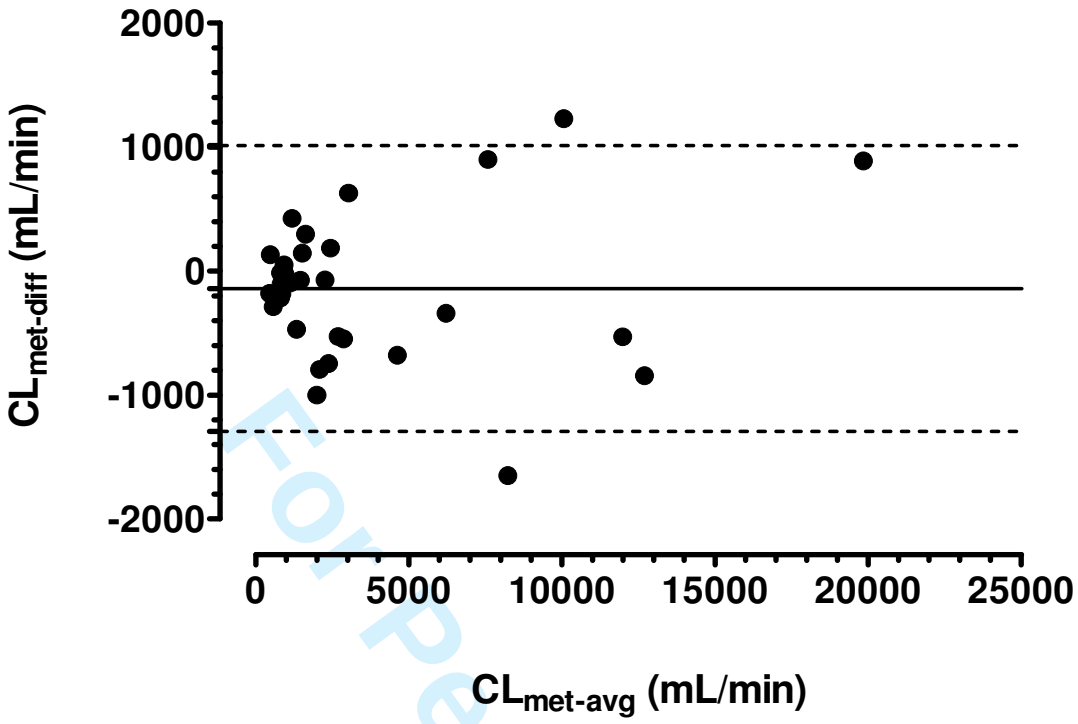
Figure 3:



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