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Midazolam as a phenotyping probe to predict sunitinib exposure in patients with cancer

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ABSTRACT

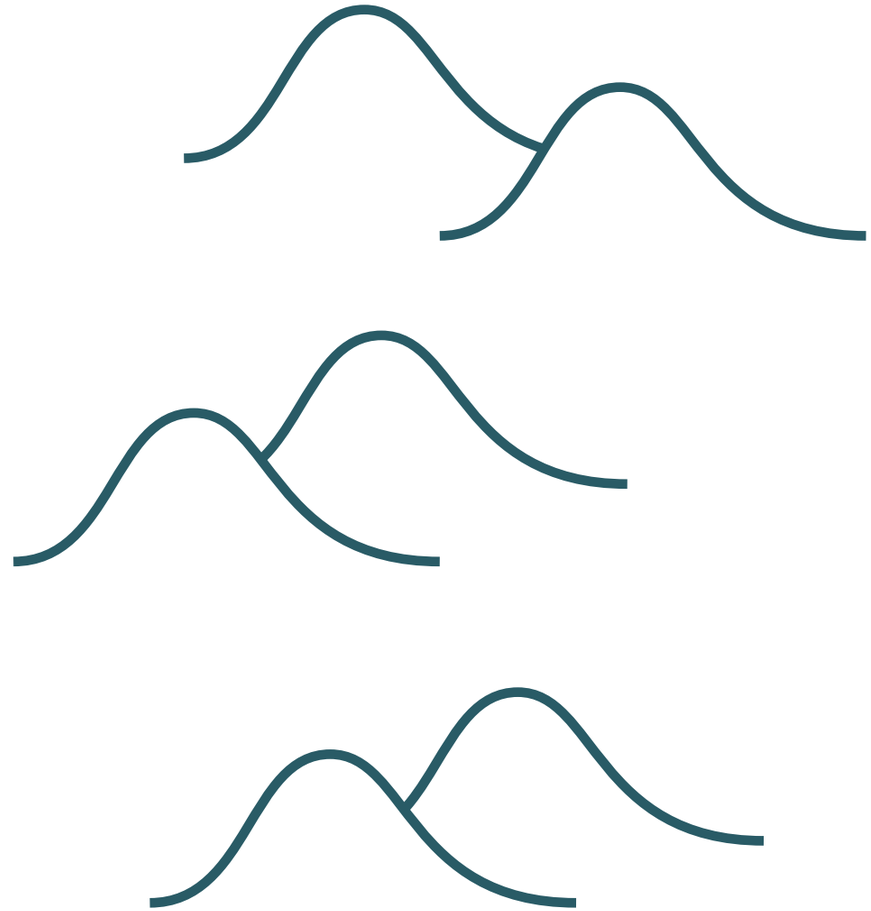
Background Patients treated with sunitinib show substantial inter-patient variability in drug exposure (~30-40%), which is largely unexplained. Since sunitinib is metabolized by cytochrome P450 (CYP) 3A4, variability in the activity of this enzyme may explain a considerable proportion of this inter-patient variability. Midazolam is widely used as a phenotyping probe to assess CYP3A4 activity. The objective of this study was to prospectively evaluate the relationship between midazolam and sunitinib exposure. Additionally, the correlation between sunitinib C_{trough} levels and exposure and the influence of sunitinib on midazolam exposure was determined.

Patients and Methods Thirteen patients treated with sunitinib in a '4 weeks on - 2 weeks off' regimen received twice 7.5 mg midazolam; once with and once without sunitinib. Steady-state sunitinib, its active metabolite SU12662 and midazolam exposures were determined.

Results A significant correlation between midazolam exposure (AUC_{0-7}) and steady-state sunitinib and sunitinib + SU12662 exposure (AUC_{0-24}) was found ($P = 0.006$ and $P = 0.0018$, respectively); midazolam exposure explained 51% and 41% of the inter-patient variability in sunitinib and sunitinib + SU12622 exposure. Furthermore, C_{trough} was highly correlated ($r^2 = 0.94$) with sunitinib AUC_{0-24}. Sunitinib decreased midazolam exposure with 24% ($P = 0.034$).

Conclusion Midazolam exposure is highly correlated with sunitinib exposure and explains a large proportion of the observed inter-patient variability in sunitinib pharmacokinetics. Consequently, midazolam could be used to identify patients that are at risk of under- or overtreatment, respectively, at the start of sunitinib therapy. Moreover, sunitinib and sunitinib + SU12662 C_{trough} levels are highly correlated with drug exposure and can thus be used in clinical practice to individualize sunitinib therapy. The decrease in midazolam exposure by sunitinib needs further investigation.

Cancer Chemotherapy and Pharmacology 2014, 73(1):87-96



Introduction

Sunitinib malate (Sutent®; SU11248) is an oral multi-targeted tyrosine kinase inhibitor with both antitumor and antiangiogenic activity. It is approved for the treatment of metastatic renal cell carcinoma (mRCC), metastatic gastrointestinal stromal tumors (GIST) and advanced pancreatic neuroendocrine tumors [1-4]. Sunitinib is primarily metabolized by cytochrome P450 3A4 to its equally active *N*-desethyl metabolite SU12662, which is further metabolized to inactive moieties by CYP3A4 as well [5]. Steady-state concentrations of sunitinib and SU12662 are approximately reached after 14 days of daily dosing [6].

Patients treated with sunitinib show substantial inter-patient variability in drug exposure (~30-40%), which is largely unexplained [3,7,8]. Previously, a relationship between systemic sunitinib exposure, efficacy and adverse events has been demonstrated; Houk et al. showed that patients with mRCC, GIST or solid tumors and a sunitinib AUC_{ss} > 800, 600 and 700 µg·hr/L, respectively, had longer time to progression (TTP) and better overall survival (OS) [9]. This study also showed that there was a positive relationship between exposure and fatigue, decreased absolute neutrophil count and change in diastolic blood pressure. Consequently, the inter-patient variability in the pharmacokinetics of sunitinib can result in either sub- or supratherapeutic levels leading to inefficacy or toxicity, respectively. Since sunitinib is predominantly metabolized by CYP3A4, differences in the activity of this enzyme may explain a considerable proportion of the unexplained observed inter-patient variability in pharmacokinetics.

Midazolam is widely used as a noninvasive phenotyping probe to assess CYP3A4 activity. By using this probe, patients potentially at risk of under- or overtreatment, respectively, at the standard dosage regimen of sunitinib can be identified before the start of therapy. Hence, midazolam exposure can be used as a surrogate parameter for sunitinib exposure and could possibly prevent inefficacy or toxicity of sunitinib therapy.

The predictive value of different phenotyping probes for the exposure of various cytotoxic drugs including vinorelbine, docetaxel, irinotecan and tamoxifen, as well as the tyrosine kinase inhibitors gefitinib and imatinib has been investigated before [10-20]. Since, CYP3A4 plays an important role in the metabolism of nearly all tyrosine kinase inhibitors (TKI) and these TKIs also show a large inter-patient variability in pharmacokinetics, phenotyping with midazolam may also be useful for these drugs [21].

This study prospectively evaluated the relationship between midazolam and sunitinib exposure in patients with cancer in order to assess the feasibility of midazolam as a phenotyping probe for sunitinib pharmacokinetics. Total drug exposure is usually determined by collecting multiple samples over the dosing interval. However, for daily clinical practice, less intensive sampling would be a more feasible approach. Therefore, this study also determined the correlation between C_{trough} levels and total sunitinib exposure. This potentially justifies the use of only C_{trough} levels for monitoring and guiding sunitinib therapy in clinical practice. Finally, this study evaluated the effect of sunitinib on the pharmacokinetics of midazolam in patients with cancer.

Patients and Methods

Patients

Patients that used sunitinib for the palliative treatment of various tumors were eligible for study entry. Patients were ≥ 18 years old, had a WHO performance status ≤ 2 and a life expectancy of at least 12 weeks. Cytotoxic chemotherapy or radiation therapy within 4 weeks before study entry and concurrent use of substances known or likely to interfere with CYP3A4 activity within 14 days before study entry were not allowed. All patients had adequate clinical functional reserves as defined by hemoglobin ≥ 6.0 mmol/L, WBC ≥ 3.0×10^9/L, ANC ≥ 1.5×10^9/L, platelets ≥ 100×10^9/L, creatinine clearance ≥ 60 mL/min, bilirubin ≤ 1.75 × the upper limit of institutional normal value. The study was approved by the institutional ethics committee (Leiden University Medical Center, the Netherlands) and all patients gave written informed consent before entering the study.

Study design

All patients were treated in a '4 weeks on - 2 weeks off' dosing schedule, with commercially available sunitinib malate hard capsules (Pfizer, Kent, United Kingdom) at an once daily oral dose of 37.5 or 50 mg. The study was performed during one sunitinib treatment cycle of 6 weeks. Patients were admitted to the hospital for pharmacokinetic (PK) sampling on two separate days. The first PK day was at steady-state sunitinib pharmacokinetics (between days 14-20). The second PK day was on day 42, the final day of the 2 weeks 'off period' after the wash out of sunitinib. On both PK days, patients were given one midazolam 7.5 mg tablet (Roche, Woerden, The Netherlands) which was used either with (first PK day) or without sunitinib (second PK day).

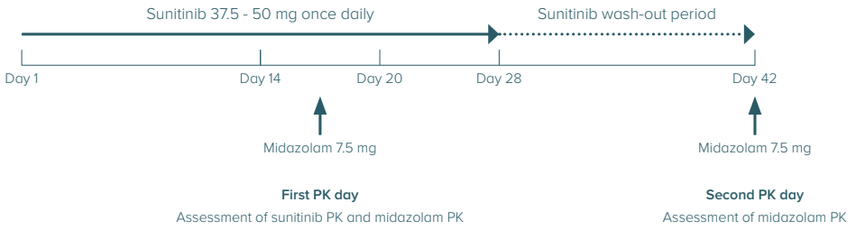
To determine the correlation between midazolam and sunitinib exposure, the $AUC_{0\text{-}7}$ of midazolam without the concomitant use of sunitinib (second PK day) was related to the $AUC_{0\text{-}24}$ and C_{trough} (t = 0) of both sunitinib and sunitinib + SU12662 at steady-state pharmacokinetics (first PK day). Additionally, the steady-state sunitinib data were used to assess the relationship between sunitinib and sunitinib + SU12662 C_{trough} levels (t = 0) and exposure ($AUC_{0\text{-}24}$). To determine the influence of sunitinib on CYP3A4 activity, midazolam exposures ($AUC_{0\text{-}7}$) with

(first PK day) and without (second PK day) the concomitant use of sunitinib were compared (Study Design; Figure 1).

Sunitinib pharmacokinetic analysis

To assess steady-state sunitinib pharmacokinetics, blood samples were collected after 14-20 days of sunitinib therapy (first PK day). The samples were collected into heparin containing tubes at 0, 10, 20, 40 min and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24 hr after the sunitinib dose. Blood samples were centrifuged at 3,000 rpm for 10 min; plasma was divided into two aliquots and stored at -80 °C until the day of analysis. Sunitinib and SU12662 plasma concentrations were determined using a validated Ultra Performance Liquid Chromatography-tandem Mass Spectrometric (UPLC-MS/MS) assay [22].

Figure 1 Study design



Midazolam pharmacokinetic analysis

Blood samples to assess midazolam pharmacokinetics were collected on the first and second PK day after a single oral dose of midazolam. The samples were collected into heparin containing tubes at the following time points: 0, 10, 20, 40 min and 1, 2, 3, 4, 5, 7 hr after the midazolam dose. Blood samples were centrifuged at 3,000 rpm for 10 min and plasma was stored at -80 °C until the day of analysis. Midazolam and its metabolite α-hydroxy midazolam were measured using a validated liquid chromatographic-tandem mass spectrometric (LC-MS/MS) assay. Briefly, 200 µL plasma was extracted by adding 500 µL of acetonitrile containing midazolam D4 (4 µg/L) as the internal standard, followed by vortex mixing and centrifugation at 13,000 rpm for 5 min at ambient temperature. The supernatant was collected and 10 µL was separated on an Atlantis T3 C18 analytical column (50 × 2.1 mm i.d. particle size 3 µm) with a linear gradient. Mobile phase A consisted of 0.1 % v/v formic acid in water and mobile phase B consisted of 0.1 % v/v formic acid in acetonitril. Midazolam was eluted with the following gradient [time scale (min-min): flow rate (mL/min): mobile phase A (%)/mobile phase B (%)] 0-0.5:0.3: 85/15; 0.5-1.5:0.3: 85/15 → 10/90; 1.5-5.8:0.3:10/90; 5.8-6.2:0.5:10/90 → 85/15; 6.2-9.5:0.5:85/15. The effluent was monitored with a Micromass Quattro LC triple-quadrupole mass spectrometric detector (Waters, Milford, MA) using the electrospray positive ionization mode. The retention times for midazolam and α-hydroxy midazolam were 4.82 and 4.83 min, respectively; MRM transitions for midazolam 326.0 > 291.0 and for α-hydroxy midazolam 342.0 > 323.9. The calibration line of midazolam (Bufa, IJsselstein, Netherlands) was linear over the range from 1 to 100 µg/L. The within day and between day imprecision and inaccuracy were less than 5 % within this concentration range.

Pharmacokinetic analysis

Sunitinib and midazolam plasma concentrations were analyzed using a non-compartmental trapezoidal approach (Phoenix® Winnonlin® v6.3). For sunitinib and SU12662 the following pharmacokinetic parameters were assessed: AUC over the dosing interval (0-24); C_{trough} = trough plasma concentration; T_{max} = time to reach peak plasma concentration and C_{max} = peak plasma concentration. Since sunitinib and SU12662 pharmacokinetics are known to be dose-proportional over at least the dose range of 25-100 mg, the AUC_{0-24}, C_{trough} and C_{max} were dose normalized to a sunitinib dose of 50 mg in order to account for sunitinib dose differences (37.5 and 50 mg) between patients [6]. For midazolam and its metabolite

Table 1 Patient characteristics

Characteristic	Value
Number of patients	15
Sex	
Male (n)	12
Female (n)	3
Age (years)	61 (41 - 78)
Creatinine (µmol/L)	76 (56 - 122)
Total bilirubine (µmol/L)	9 (6 - 27)
ALT (µmol/L)	24 (9 - 68)
AST(µmol/L)	38 (23 - 203)
Hb (mmol/L)	8.8 (7.0 - 10.3)
WBC (x109)	5.0 (3.2 - 38.2)
Thrombocytes (x109/L)	158 (82 - 318)
Neutrophils (%)	53.3 (31.3 - 96.6)
WHO performance score	
0 (n)	3
1 (n)	12

Data are presented as median values with lower and upper limit. Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; Hb, hemoglobin; WBC, white blood count.

α-hydroxy midazolam the following pharmacokinetic parameters were assessed: AUC over the sampling period (0-7), T_{max} and C_{max}.

Statistical analysis

The relationship between midazolam exposure and both sunitinib and sunitinib + SU12662 exposure, as well as the relation between both sunitinib and sunitinib + SU12662 C_{trough} and AUC_{0-24} were examined by correlation analysis. The Pearson square correlation coefficient (r^2) was used to assess the percentage of variability in sunitinib exposure that could be explained by midazolam exposure. To determine the influence of sunitinib on midazolam exposure, midazolam AUC_{0-7} on the first and second PK day were compared using a two-tailed paired student's t-test. *P* values < 0.05 were considered statistically significant. Statistical calculations were performed using SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Patients and treatment

Fifteen patients using sunitinib were included in this pharmacokinetic study. Of these patients, 12 received 50 mg and 3 received 37.5 mg sunitinib once daily. Patient characteristics are summarized in Table 1. No unexpected side effects were observed during sunitinib treatment or on the day of midazolam co-administration. Two patients used or recently stopped using mitotane therapy during the study. At the time of inclusion it was unknown that mitotane is a very potent inducer of CYP3A4 and that the combination of both drugs would thus result in markedly decreased sunitinib and midazolam concentrations. The observed interaction between sunitinib and mitotane in this present study is reported separately [23] and thereafter also described elsewhere [24-26].

Table 2 Summary of sunitinib and midazolam pharmacokinetic parameters

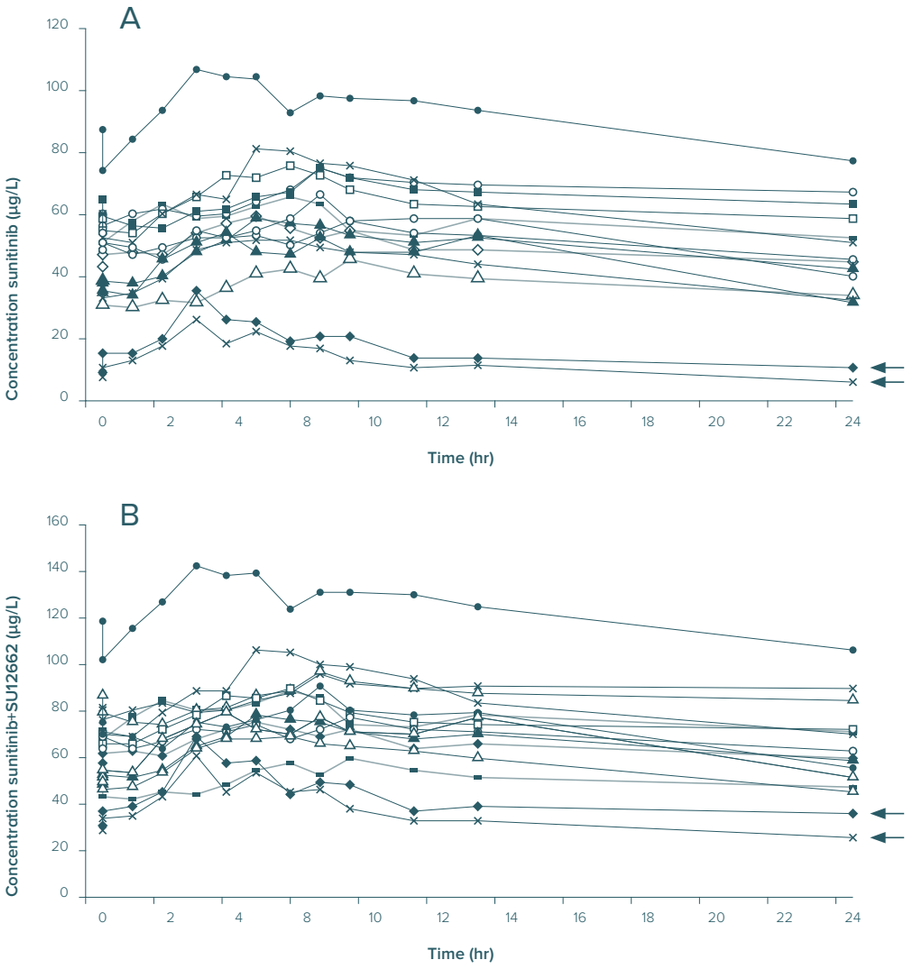
	Sunitinib (n = 13)	Sunitinib + SU12662 (n = 13)		Midazolam[a] (n = 13)	α-OH-midazolam[a] (n = 13)
AUC_{0-24} (μg·hr/L)	1442 (329)	1929 (423)	AUC_{0-7}[a] (μg·hr/L)	152 (54)	40 (13)
C_{trough} (μg/L)	55.2 (14.6)	74.4 (19.2)	C_{trough} (μg/L)	N/A	N/A
T_{max} (hr)	6.0 (1.5)	6.0 (1.5)	T_{max} (hr)	1.0 (1.0)	1.0 (0.9)
C_{max} (μg/L)	71.6 (15.4)	94.1 (19.7)	C_{max} (μg/L)	69.4 (28.9)	20.2 (10.8)

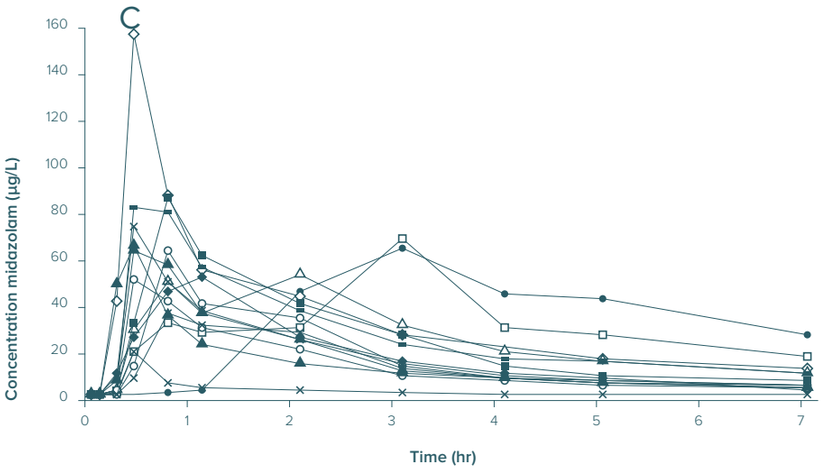
Data are presented as mean values (standard deviation). Parameters were dose normalized to sunitinib 50 mg.
Abbreviations: AUC, area under the concentration time curve; C_{max}, peak plasma concentration and T_{max}, time to reach peak plasma concentration.
[a]Midazolam data from PK day 2 were used; midazolam was then given without the co-administration of sunitinib.

Sunitinib and midazolam pharmacokinetics

Individual sunitinib, sunitinib + SU12662 and midazolam concentration versus time profiles are shown in Figure 2. A wide overlap is seen in the curves of patients that received 37.5 mg sunitinib and those that received 50 mg. The two patients that (recently) used mitotane are represented by the two lowest curves (indicated with an arrow). Since one of the

Figure 2 Individual observed concentration versus time profiles for A) sunitinib B) sunitinib +SU12662 C) midazolam. The *gray lines* represent the patients that received 37.5 mg sunitinib, and the *black lines* patients that received 50 mg sunitinib. The *arrows* indicate the two patients that used or recently stopped using mitotane





exclusion criteria was the use of CYP3A4 inducers, these two patients were excluded from further analysis after discovering that mitotane is a potent CYP3A4 inducer. Summaries of the pharmacokinetics of sunitinib and midazolam are presented in Table 2. The mean dose-normalized AUC_{0-24} (standard deviation) for sunitinib and sunitinib + SU12662 were 1442 µg·hr/L (330 µg·hr/L) and 1929 µg·hr/L (423 µg·hr/L), respectively. C_{trough} levels were 55.2 µg/L (14.6 µg/L) and 74.4 µg/L (19.2 µg/L) for, respectively, sunitinib and sunitinib + SU12662. The two patients that used mitotane had a sunitinib + SU12662 AUC_{0-24} of 1014 and 855 µg·hr/L. Their C_{trough} sunitinib + SU12662 levels were, respectively, 36.7 and 31.6 µg/L.

Correlation between sunitinib and midazolam pharmacokinetic parameters

Both sunitinib C_{trough} and sunitinib exposure (AUC_{0-24}), as well as sunitinib + SU12662 C_{trough} and sunitinib + SU12662 AUC_{0-24} were highly correlated ($r^2 = 0.94$, $P < 0.001$ and $r^2 = 0.93$, $P < 0.001$, respectively) as shown in Figure 3.

Significant correlations were observed between midazolam and sunitinib pharmacokinetics: midazolam exposure (AUC_{0-7}) could explain 51% of the variability in sunitinib AUC_{0-24} ($P = 0.006$), 47% of the variability in sunitinib C_{trough} ($P = 0.010$), 41% of the variability in sunitinib + SU12622 AUC_{0-24} ($P = 0.0018$) and 39% of the variability in sunitinib + SU12622 C_{trough} ($P = 0.023$) (Figure 4).

Influence of sunitinib on midazolam exposure

The mean midazolam exposures (AUC_{0-7}) without and with concomitant sunitinib use were 151.8 µg·hr/L and 115.3 µg·hr/L respectively. This indi-

Figure 3 Correlations between sunitinib pharmacokinetics for A) sunitinib C_{trough} levels and AUC_{0-24} B) sunitinib + SU12662 C_{trough} levels and AUC_{0-24} and C) sunitinib AUC_{0-24} and sunitinib + SU12662 AUC_{0-24}

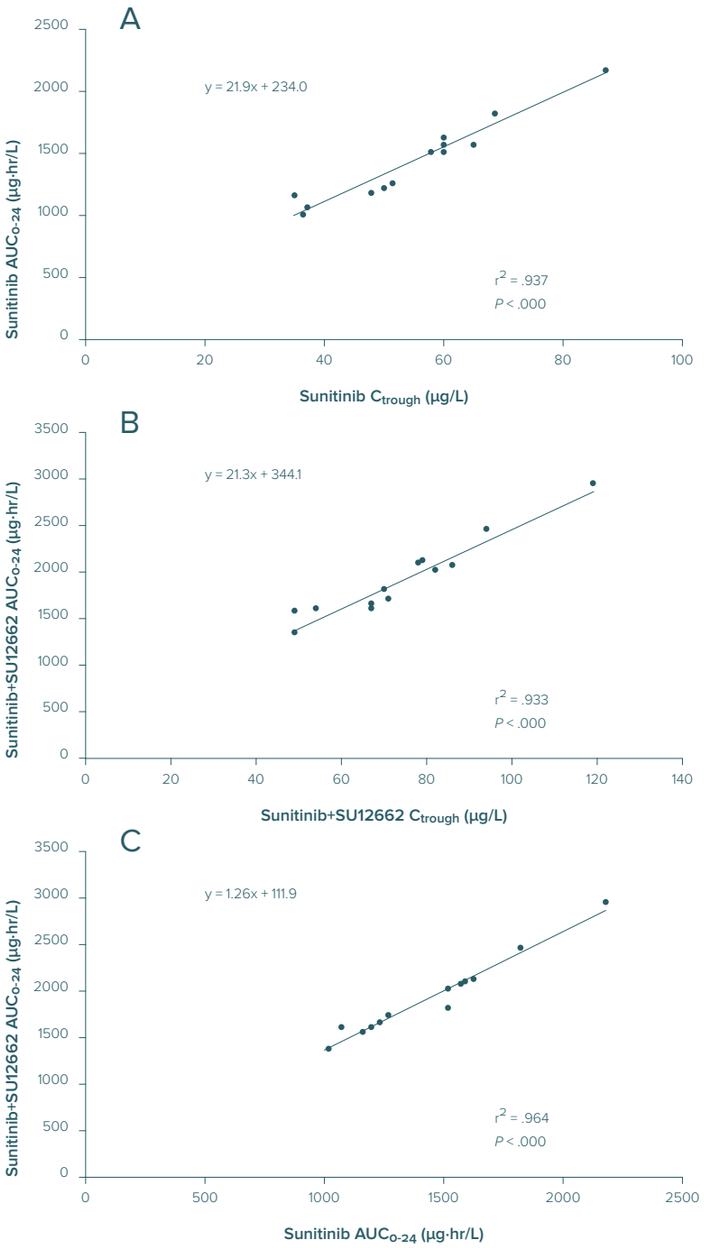
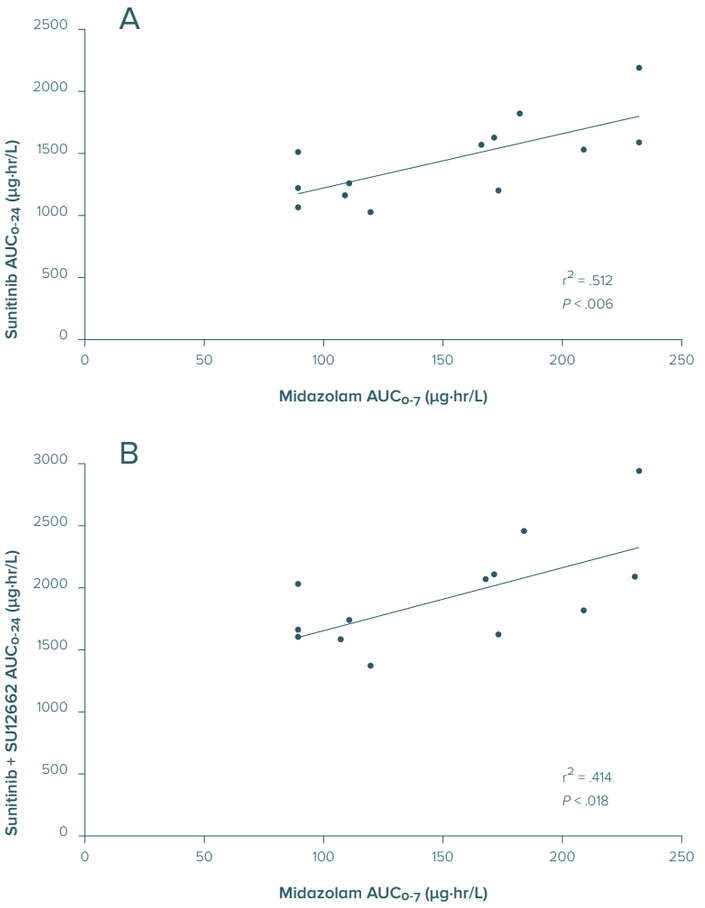


Figure 4 Correlations between midazolam and sunitinib pharmacokinetics A) midazolam AUC_{0-7} and sunitinib AUC_{0-24} B) midazolam AUC_{0-7} and sunitinib + SU12662 AUC_{0-24}



cates a decrease of 24% in midazolam exposure due to the co-administration of sunitinib ($P = 0.034$).

Discussion

This study shows that midazolam exposure is highly correlated with sunitinib exposure and explains a large proportion of the observed inter-patient variability in sunitinib pharmacokinetics. In addition, sunitinib and sunitinib + SU12662 C_{trough} levels were highly correlated with, respectively, sunitinib and sunitinib + SU12662 AUC_{0-24}. Finally, this study indicates that sunitinib significantly affects midazolam exposure in vivo.

This study not only demonstrates the excellent correlation of midazolam exposure with sunitinib pharmacokinetics, but also that up to 51% of the previously unexplained inter-patient variability in sunitinib pharmacokinetics can actually be elucidated by differences in midazolam exposure. By using midazolam as a phenotypic probe, patients at risk for either under- or overtreatment with sunitinib at the standard dosage regimen can be identified before the start of therapy. In fact, the two patients that used mitotane had very low sunitinib exposures with almost unmeasurable midazolam exposure. Since mitotane turned out to be a very potent CYP3A4 inducer which was one of the a priori exclusion criteria of this study, these two patients were excluded from statistical analyses. However, this interaction was unknown at the time of inclusion and actually became apparent by phenotyping these two patients, which shows us the clinical value of phenotyping [23]. By starting sunitinib therapy at an individualized dose, inefficacy due to under treatment as well as dose modifications (~50%) and discontinuations (~19%) due to toxicity can be prevented [1]. Moreover, for patients whose correct starting dose is debatable (e.g., due to comorbidities), midazolam could be useful to establish a safe and effective individualized sunitinib dose. However, before midazolam phenotyping can be used in clinical practice, the suitability of an individualized dosing strategy for sunitinib based on midazolam exposure would require prospective validation in a clinical trial.

Another approach to individualize sunitinib therapy could be therapeutic drug monitoring (TDM). Whereas TDM can be used to identify those patients that are over-/under dosed while on sunitinib therapy, phenotyping could be used to identify these patients beforehand.

Earlier identified covariates including tumor type, race, gender, body weight and elevated ECOG score could only explain 2-17 % of the observed changes in AUC and/or C_{max} [7,27]. Additionally, one study correlated pharmacogenetics with sunitinib exposure and treatment outcome; a significant higher exposure and more toxicity were found in patients harboring a polymorphism in the efflux transporter gene ABCG2 [28,29]. Phenotyping with midazolam might explain such a large percentage of the variability in sunitinib pharmacokinetics because it represents the influence of both genetic differences as well as environmental covariates (comorbidities, medication, life style, etc.) that might affect sunitinib exposure [7].

Although midazolam is officially recommended by the FDA for CYP3A4 activity phenotyping, variability in the oral exposure to midazolam can also be caused by co-influencing factors that affect both sunitinib and midazolam exposure (e.g., body composition, age, gender). Therefore, the percentage of sunitinib variability that can be truly explained by

CYP3A4-activity will be somewhat lower than the 51 % found in this present study.

The time frame of 0-7 hours over which midazolam samples were collected is based on the pharmacokinetic characteristics of midazolam. This frame represents 2-3 times the half-life of midazolam and therefore adequately describes the exposure to midazolam. Accordingly, the difference between the calculated midazolam AUC_{0-7} and extrapolated AUC_{inf} is < 15 % (not shown). Therefore, a longer midazolam sampling schedule would not have substantially improved the correlation between midazolam and sunitinib exposure. Midazolam is, besides CYP3A4, also metabolized by CYP3A5. Since sunitinib is only metabolized by CYP3A4, this difference in metabolism could have influenced the primary outcomes of this study [6, 8]. However, CYP3A5 is only active in 20% of the Caucasian population and in Dutch Caucasians even less [30]. Since all included patients were Dutch Caucasians, we do not expect that the correlation between midazolam and sunitinib exposure is considerably affected by the influence of CYP3A5 activity on midazolam metabolism.

According to the secondary objectives, this study shows the excellent correlation between sunitinib C_{trough} and sunitinib AUC_{0-24}, as well as sunitinib + SU12662 C_{trough} levels and sunitinib + SU12662 exposure. Taking into account the long half-life of sunitinib, it was expected that steady-state C_{trough} levels would well represent total drug exposure. However, the exact correlation between both parameters has, to our knowledge, not been reported before. The results of this present study legitimate the use of only C_{trough} levels to individualize sunitinib therapy in clinical practice. Additionally, the results of this study could be used to translate the threshold sunitinib AUCs associated with a beneficial clinical response into corresponding sunitinib C_{trough} levels [9]. These sunitinib AUCs would correspond with sunitinib + SU12662 AUC_{ss} greater than 1120, 868 and 994 µg·h/L and C_{trough} levels of sunitinib + SU12662 greater than 36.4, 24.6 and 30.5 µg/L for patients with mRCC, GIST and solid tumors, respectively, to achieve longer TTP and OS. Preclinical in vivo research identified 50 - 100 µg/L as the minimum concentration of sunitinib + SU12662 required to show anti-tumor activity which is in the same range [31].

In the current study, we found that treatment with sunitinib resulted in a 24% decrease in midazolam exposure. The mechanism by which this interaction occurs is not entirely clear. The prescribing information states that sunitinib does not induce CYP3A4 [6]. This is consistent with previous studies indicating that sunitinib does not influence the metabolism of the CYP3A4 substrates paclitaxel, docetaxel, or irinotecan [32-35]. A possible mechanistic explanation for the change in midazolam pharmacokinetics observed in this present study is heterotrophic cooperativity, whereby reversible binding of sunitinib causes a three-dimensional change in enzyme structure that ultimately affects a distant active site for some,

but not all substrates. This mechanism was previously proposed in an in vitro study where sunitinib was found to enhance CYP3A5-mediated hydroxylation of midazolam [36]. Since sunitinib and midazolam were administered simultaneously, a decreased uptake of midazolam due to sunitinib cannot be ruled out. Additional investigation is warranted to confirm the exact mechanism of the identified interaction observed in our current study.

In conclusion, this study demonstrates that midazolam exposure is highly correlated with sunitinib exposure and explains a large proportion of the observed inter-patient variability in sunitinib pharmacokinetics. Midazolam could be useful in clinical practice to identify those patients that are at risk of under- or overtreatment, respectively, at start of the standard sunitinib dosage regimen. Moreover, using C_{trough} levels as a surrogate parameter of total sunitinib exposure is a good and feasible approach for monitoring and guiding sunitinib therapy in clinical practice. Finally, the exposure to midazolam is decreased by the co-administration of sunitinib therapy, but this finding needs further investigation.

Acknowledgement

We thank Dr. Ron Wolterbeek for his statistical support. This work was supported in part by the American Lebanese Syrian Associated Charities (ALSAC)

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