Clinical pharmacology of the tyrosine kinase inhibitors imatinib and sunitinib
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Relationship between CYP3A4 phenotype and sunitinib exposure in cancer patients

Abstract

Purpose: The objective of this study was to explore the feasibility of CYP3A4 phenotyping for dose individualization of sunitinib. The relationship between CYP3A4 activity and sunitinib exposure was assessed. Moreover, the impact of sunitinib exposure on CYP3A4 activity was evaluated.

Patients and Methods: Sunitinib and midazolam pharmacokinetics were evaluated in cancer patients receiving sunitinib monotherapy in a “four weeks on - 2 weeks off” regimen. Serial blood samples for pharmacokinetic analysis of midazolam and sunitinib were collected on two separate days. On both PK days the patients received a single oral dose of 7.5 mg midazolam as a CYP3A4 phenotyping probe. The first PK day was at steady-state sunitinib pharmacokinetics (between day 14-20), the second PK day was after two weeks wash out of sunitinib (day 42). The influence of sunitinib on midazolam exposure was assessed by comparing midazolam exposure on the both PK days. For the phenotyping study, midazolam exposure at the second PK day, after the wash out of sunitinib, and sunitinib exposure at the first PK day were associated.

Results: A linear correlation between midazolam exposure and 1] steady-state sunitinib exposure ($R^2= 0.56$, $P = 0.02$) and 2] steady state sunitinib plasma trough levels ($R^2 = 0.51$, $P = 0.03$) were found. Additionally a strong linear relation was found between sunitinib plasma trough levels and sunitinib exposure ($R^2 = 0.90$, $P < .0001$). Co-administration of sunitinib reduced the exposure to midazolam. However this reduction was not significant ($P = .113$).

Conclusion: Steady-state sunitinib exposure and sunitinib trough levels are strongly related to CYP3A4 activity. Therefore, CYP3A4 phenotyping could be useful for individualization of the sunitinib starting dose. In addition, sunitinib exposure relates well to sunitinib plasma trough levels and sunitinib appears to be a mild inducer of CYP3A4.

Introduction

Sunitinib malate (Sutent®; SU11248) is an oral, multi-targeted tyrosine kinase inhibitor that specifically inhibits vascular endothelial growth factor receptors 1, 2 and 3 (VEGFR1, -2 and -3, respectively), platelet derived growth factor receptor alpha and beta (PDGFR-α and -β), KIT, Fms-like tyrosine kinase-3 receptor (FLT3), colony stimulating factor receptor type 1 (CSF-1R) and the receptor encoded by the ret proto-oncogene (RET)1-2. Sunitinib is approved for first line treatment of metastatic renal cell carcinoma (mRCC) and imatinib-resistant metastatic gastrointestinal stromal tumors (GIST)3-5. Sunitinib is metabolized by cytochrome P450 (CYP) 3A4 to an equally active metabolite SU12662, which is further metabolized to inactive moieties by CYP3A46.

Clinical pharmacokinetics of sunitinib show high interpatient variability (~ 40%) which is mainly unexplained7. This could result in supra- or sub-therapeutic sunitinib levels leading to toxicity or inefficacy, respectively. Since sunitinib is predominantly metabolized by CYP3A4, variability in the activity of this enzyme may explain a considerable proportion of the observed interpatient variability in sunitinib pharmacokinetics. A noninvasive and inexpensive phenotypic probe to measure the CYP3A4 activity might be useful for therapeutic optimization of the dosage of sunitinib. The probe could also be used to evaluate the potential impact of sunitinib on CYP3A4 activity. In this study, midazolam was used for CYP3A4 phenotyping. The design of the study was not only suitable to assess the activity of CYP3A4, but also to evaluate the potential impact of sunitinib on the pharmacokinetics of midazolam in cancer patients. It is thought that sunitinib is neither an inhibitor nor an inducer of CYP-enzymes and therefore the drug is considered not prone to drug-drug and drug-food interactions, while other tyrosine kinase inhibitors (e.g. imatinib, erlotinib, gefitinib) appear to be substrates and/or inhibitors of several CYP-enzymes in vivo and in vitro7-12. For sunitinib, in vivo confirmatory studies to define an effect of sunitinib on CYP-enzymes are lacking. Moreover, recently it was shown in an in vitro study that sunitinib is a substrate for and an inhibitor of the transporter proteins ABCG2 and to some extent ABCB1, which may also lead to drug-drug interactions13. Therefore, in this study the relationship is determined between CYP3A4 activity and sunitinib exposure in cancer patients, using the phenotypic probe midazolam. In addition, the relationship between sunitinib plasma trough levels and sunitinib exposure is investigated and the effect of sunitinib on CYP3A4 activity in vivo is assessed.
Methods

Patients
Eligibility for study entry included adult cancer patients that were on sunitinib treatment for palliative treatment of various tumors, mainly mRCC and GIST, at a dose level of 25 – 50 mg once daily in a “four weeks on – two weeks off” schedule. 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 four weeks before entering the study was not allowed. Concurrent use of substances known or likely to interfere with the pharmacokinetics of sunitinib or with CYP3A4 activity, such as ketoconazole, fluconazole, rifampicin, St. John’s wort etc., were not allowed within 14 days before study entry. All patients had adequate clinical functional reserves as defined by hemoglobin ≥ 6.0 mmol/L, WBC ≥ 3.0 x 10⁹/L, ANC ≥ 1.5 x 10⁹/L, platelets ≥ 100 x 10⁹/L, creatinine clearance ≥ 60 mL/min, bilirubin ≤ 1.75 x 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
In this study, midazolam was used as a probe to assess CYP3A4 activity. The study was designed to determine the relationship between CYP3A4 activity and sunitinib exposure in cancer patients and additionally to evaluate the effect of sunitinib on CYP3A4 activity by studying its effect on midazolam pharmacokinetics. All patients were treated in a “four weeks on – two weeks off” dosing schedule, with commercially available sunitinib malate hard capsules (Pfizer, Kent, United Kingdom) at an oral once daily dose ranging between 25 and 50 mg. The study was performed during one sunitinib treatment cycle of six weeks. Patients were admitted to the hospital on two separate days. The first PK day was on day 14 - 20, the second PK day was on day 42, the last day of the two weeks “off period” after the sunitinib treatment cycle of six weeks. 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 four weeks before entering the study was not allowed. Concurrent use of substances known or likely to interfere with the pharmacokinetics of sunitinib or with CYP3A4 activity, such as ketoconazole, fluconazole, rifampicin, St. John’s wort etc., were not allowed within 14 days before study entry. All patients had adequate clinical functional reserves as defined by hemoglobin ≥ 6.0 mmol/L, WBC ≥ 3.0 x 10⁹/L, ANC ≥ 1.5 x 10⁹/L, platelets ≥ 100 x 10⁹/L, creatinine clearance ≥ 60 mL/min, bilirubin ≤ 1.75 x 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.

Sunitinib Pharmacokinetics
Blood samples were collected on the first PK day of the study for assessing sunitinib pharmacokinetics at steady-state. The samples were collected into heparin-containing tubes at 0, 10, 20, 40 minutes, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24 hours after the sunitinib dose. Blood samples were centrifuged at 3,000 rpm for 10 minutes and plasma was divided into two aliquots and stored at −80°C until the day of analysis. Total sunitinib plasma concentrations were determined in plasma using a validated liquid chromatographic tandem mass spectrometric (LC/MS/MS) assay, as described previously14.

Midazolam Pharmacokinetics
Blood samples to assess midazolam pharmacokinetics were collected on the first and second PK day after a single dose of midazolam. The samples were collected into heparin-containing tubes at the following time points: 0, 10, 20, 40 minutes, 1, 2, 3, 4, 5, 7 hours after the midazolam dose. Blood samples were centrifuged at 3,000 rpm for 10 minutes and plasma was stored at −80°C until the day of analysis. Midazolam was 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 ng/L) and eluted with the following mobile phase: water and acetonitrile 90:10 (v:v) plasma was extracted by adding 500 μL of acetonitrile containing midazolam D4 (4 ng/L) and eluted with the following mobile phase: water and acetonitrile 90:10 (v:v). The supernatant was collected and 10 μL plasma was stored at −80°C until the day of analysis. Total midazolam plasma concentrations were determined in plasma using a validated liquid chromatographic tandem mass spectrometric (LC/MS/MS) assay, as described previously14.
Sunitinib pharmacokinetics

Dose normalized sunitinib exposure (AUC 0–24hr) and trough levels (C_{ss,min}) at steady-state pharmacokinetics varied 8 to 9.5-fold (geometric mean = 1,105 ng·hr/mL, range 267 – 2,119 ng·hr/mL and geometric mean = 43.6 ng/mL, range 7.2 – 68.7 ng/mL, respectively) (Table 2). The interpatient variabilities (defined as the coefficient of variation (CV%)) in sunitinib exposure and sunitinib trough levels were large: 51% and 56%, respectively (Figure 2). Pharmacokinetic parameters of sunitinib are listed in Table 2. Sunitinib trough levels (C_{ss,min}) and sunitinib exposure (AUC 0–24hr) were found highly related (R^2 = .90, \(P < 0.0001\)) (Figure 3A).

Midazolam pharmacokinetics related to sunitinib exposure

Dose normalized midazolam exposure (AUC_{0–7hr}) and trough levels (C_{ss,min}) at steady-state pharmacokinetics varied 8 to 9.5-fold (geometric mean = 1,105 ng·hr/mL, range 267 – 2,119 ng·hr/mL and geometric mean = 43.6 ng·hr/mL, range 7.2 – 68.7 ng·hr/mL, respectively) (Table 2). The interpatient variabilities (defined as the coefficient of variation (CV%)) in sunitinib exposure and sunitinib trough levels were large: 51% and 56%, respectively (Figure 2). Pharmacokinetic parameters of sunitinib are listed in Table 2. Sunitinib trough levels (C_{ss,min}) and sunitinib exposure (AUC 0–24hr) were found highly related (R^2 = .90, \(P < 0.0001\)) (Figure 3A).

Midazolam pharmacokinetics related to sunitinib exposure

Midazolam exposure (AUC_{0–7hr}) and C_{max} and T_{max} are listed in Table 2. Midazolam exposure (AUC_{0–7hr}) was highly correlated to sunitinib trough levels (C_{ss,min}) (R^2 = .51, \(P = .030\)) and sunitinib exposure (AUC_{0–24hr}) (R^2 = .56, \(P = .021\)) could thereby reduce both the interpatient variability in sunitinib trough levels and sunitinib exposure to 29% (Figure 3B, 3C).

Midazolam pharmacokinetics to evaluate the effect of sunitinib on CYP3A4

The mean midazolam exposure (AUC_{0–7hr}) with and without sunitinib were 91.0 \(\mu\)g·hr/L and 130.4 \(\mu\)g·hr/L, respectively (\(P = .113\), Table 3).

Table 1  Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>9</td>
</tr>
<tr>
<td>Sex (female / male)</td>
<td>2/7</td>
</tr>
<tr>
<td>Age, years*</td>
<td>56 (41 - 78)</td>
</tr>
<tr>
<td>Baseline renal and liver function parameters</td>
<td></td>
</tr>
<tr>
<td>Creatinine, µM*</td>
<td>76 (56 - 122)</td>
</tr>
<tr>
<td>Total bilirubin, µM*</td>
<td>8 (6 - 15)</td>
</tr>
<tr>
<td>ALT, units/L*</td>
<td>33 (14 - 68)</td>
</tr>
<tr>
<td>Baseline bone marrow function parameters</td>
<td></td>
</tr>
<tr>
<td>Hb, g/dL</td>
<td>8.7 (7 - 9.4)</td>
</tr>
<tr>
<td>WBC, x 10^9/L*</td>
<td>5.5 (3.5 - 38.2)</td>
</tr>
<tr>
<td>Thrombocytes, x 10^9/L*</td>
<td>193 (122 - 318)</td>
</tr>
</tbody>
</table>

* median values (range)
Table 2  Pharmacokinetic parameters for evaluation of the relation between sunitinib and midazolam

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sunitinib at steady-state</th>
<th>Midazolam after a single dose without sunitinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{0-24hr} (ng·hr/mL)</td>
<td>1105 ± 189</td>
<td>130.4 ± 22.9</td>
</tr>
<tr>
<td>Cl/F (L/hr)</td>
<td>71.8 ± 21.8</td>
<td>162.1 ± 34.7</td>
</tr>
<tr>
<td>C_{ss,min} (ng/mL)</td>
<td>43.6 ± 8.1</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>T_{max} (hr)</td>
<td>6.1 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>C_{max} (ng/mL)</td>
<td>576 ± 8.6</td>
<td>51.9 ± 5.8</td>
</tr>
</tbody>
</table>

Results are presented as mean values ± the standard error of the mean (SEM). *Sunitinib C_{ss,min}, C_{max} and AUC_{0-24hr} values are normalized to a sunitinib dose of 50 mg.

Abbreviations: AUC_{0-24hr} = area under the plasma concentration-time curve at steady-state over a dose interval of sunitinib; AUC_{0-7hr} = area under the plasma concentration-time curve over the observed interval after a single midazolam dose; AUC_{0-} = area under the plasma concentration-time curve over a time interval 0 - infinity; Cl/F = apparent oral clearance; C_{ss,min} = average trough plasma concentration; T_{max} = time to reach peak plasma concentration; C_{max} = peak plasma concentration.

Figure 2  Individual observed sunitinib plasma concentrations versus time profiles

Seven solid lines represent patients with sunitinib 50 mg/day. The two dotted lines represent the patients with sunitinib 37.5 mg/day.

Figure 3  Correlation between A: sunitinib trough levels (C_{ss,min}) and sunitinib exposure (AUC_{0-24hr}), B: Midazolam exposure (AUC_{0-7hr}) and sunitinib trough levels (C_{ss,min}), C: Midazolam exposure (AUC_{0-7hr}) and sunitinib exposure (AUC_{0-24hr})

y = 22,055x + 143.26  \( R^2 = 0.9003 \)

y = 0.2549x + 10.367  \( R^2 = 0.5142 \)

y = 6,1577x = 301.89  \( R^2 = 0.5356 \)
Table 3  Midazolam pharmacokinetics with and without sunitinib

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Midazolam alone</th>
<th>Midazolam with sunitinib</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0-7hr (ng·hr/mL)</td>
<td>130.4 ± 22.9</td>
<td>91.0 ± 21.3</td>
<td>.113</td>
</tr>
<tr>
<td>AUC0-∞ (ng·hr/mL)</td>
<td>162.1 ± 34.7</td>
<td>118.8 ± 28.4</td>
<td>.092</td>
</tr>
<tr>
<td>Tl,max (hr)</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.5</td>
<td>.895</td>
</tr>
<tr>
<td>C1,max (ng/mL)</td>
<td>51.9 ± 5.8</td>
<td>50.6 ± 12.2</td>
<td>.926</td>
</tr>
</tbody>
</table>

Results are presented as mean values ± the standard error of the mean (SEM).

Abbreviations: AUC0-7hr = area under the plasma concentration-time curve over the observed interval after a single midazolam dose; AUC0-∞ = area under the plasma concentration-time curve over a time interval 0 - infinity; Tl,max = time to reach peak plasma concentration; C1,max = peak plasma concentration.

Discussion

This study shows that sunitinib exposure is highly related to CYP3A4 activity. Also a strong relationship between sunitinib trough levels and sunitinib exposure is observed. Moreover, sunitinib appears to show a trend towards CYP3A4 induction, however this was not found significant.

Sunitinib is metabolized by cytochrome P450 3A4 (CYP3A4). No other enzymes are known to be involved in sunitinib metabolism5, 6. In addition, sunitinib appears to be an in vitro substrate and inhibitor for the ATP-binding cassette transporters ABCG2 and ABCB1 and these transporters may, therefore, also contribute to sunitinib disposition in vivo11. Similarly, midazolam is extensively metabolized by CYP3A4 with less affinity for CYP3A515. It, however, appears to be a poor substrate of ABCB1 (P-glycoprotein; MDR1) and ABCG2 (BCRP; MXR)16, 17.

Oral midazolam is widely accepted and used as a probe for evaluating (hepatic and intestinal) CYP3A4 activity, without influencing the activity of this enzyme18, 19. Former studies have shown that sunitinib pharmacokinetics comprises high interpatient variability (~ 40%) with respect to drug exposure. Until now, this high interpatient variability was only marginally explained by the studied variables; tumor type, race, sex, body weight, and Eastern Cooperative Oncology Group score22, 23. The relationship between sunitinib exposure and clinical efficacy or toxicity has not yet been elucidated but substantial pharmacokinetic variability is likely to impact treatment outcome. Phenotyping patients for CYP3A4 activity may not only help to understand variability in sunitinib pharmacokinetics but it may also be a future clinical tool to individualize and optimize sunitinib treatment.

In the presented study the interindividual variability in the sunitinib exposure was large (51%) and partially explained by midazolam exposure. Therefore, CYP3A4 activity as assessed by oral midazolam phenotyping adds to the variables identified in explaining the variability in sunitinib exposure23. In fact, CYP3A4 activity explained a large part (51%) of the total interpatient variability in sunitinib exposure and might therefore help to identify patients predisposed to relatively high sunitinib exposure or those that are potentially underdosed. When plasma drug concentrations are monitored to guide individual therapy, drug exposure is typically assessed by estimating the area under the plasma concentration time curve after taking blood samples at different time points. However, this strategy is difficult and time consuming in clinical practice and the use of surrogate parameters, such as drug trough levels, are favorable to determine drug exposure. To our knowledge, this is the first study that shows that sunitinib trough levels are highly correlated to sunitinib exposure. Therefore, measuring sunitinib trough levels instead of sunitinib exposure, which is a more feasible approach than monitoring total exposure curves in treated patients. Before therapeutic drug monitoring is considered in clinical practice additional information on concentration-effect relationship of sunitinib for the different tumor subtypes is warranted26, 27. Sunitinib shows a trend towards induction of CYP3A4 metabolism resulting in a reduced midazolam exposure while co-administered. Only gefitinib showed a similar interaction with midazolam in an in vivo study; however this effect has not been confirmed in vivo yet10. All other tyrosine kinase inhibitors are either CYP3A4 inhibitors (imatinib, dasatinib, and nilotinib) or show no influence on CYP3A4 metabolism, however caution with concomitant administered CYP3A4 substrates is still warranted20, 21.

The relatively small number of patients in this study may be considered as a limitation especially for regarding accurate estimation of interpatient variability in sunitinib PK and assessment of relationships. However, the dense sampling results in a reliable determination of the exposure to sunitinib and midazolam. Moreover, the interpatient variability in our study is consistent with the interpatient variability of ~ 40% reported so far23, 31. In conclusion, variability in sunitinib PK is strongly related to CYP3A4 activity and therefore CYP3A4 phenotyping could be useful for individualizing the sunitinib starting dose. Sunitinib trough levels relate well to sunitinib exposure, making this more assessable approach suitable for studying the exposure-efﬁcacy and exposure-toxicity relations.

Chapter 6

Relationship between CYP3A4 pharmacokinetics and sunitinib exposure in cancer patients
References


