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Is Higher Docetaxel Clearance in Prostate Cancer Patients Explained by Higher CYP3A? An In Vivo Phenotyping Study with Midazolam

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Abstract

Patients with prostate cancer (PCa) have a lower docetaxel exposure for both intravenous (1.8-fold) and oral administration (2.4-fold) than patients with other solid cancers, which could influence efficacy and toxicity. An altered metabolism by cytochrome P450 3A (CYP3A) due to castration status might explain the observed difference in docetaxel pharmacokinetics. In this in vivo phenotyping, pharmacokinetic study, CYP3A activity defined by midazolam clearance (CL) was compared between patients with PCa and male patients with other solid tumors. All patients with solid tumors who did not use CYP3A-modulating drugs were eligible for participation. Patients received 2 mg midazolam orally and 1 mg midazolam intravenously on 2 consecutive days. Plasma concentrations were measured with a validated liquid chromatography–tandem mass spectrometry method. Genotyping was performed for CYP3A4 and CYP3A5. Nine patients were included in each group. Oral midazolam CL was 1.26-fold higher in patients with PCa compared to patients with other solid tumors (geometric mean [coefficient of variation], 94.1 [33.5%] L/h vs 74.4 [39.1%] L/h, respectively; $P = .08$). Intravenous midazolam CL did not significantly differ between the 2 groups ($P = .93$). Moreover, the metabolic ratio of midazolam to 1′-hydroxy midazolam did not differ between the 2 groups for both oral administration ($P = .67$) and intravenous administration ($P = .26$). CYP3A4 and CYP3A5 genotypes did not influence midazolam pharmacokinetics. The observed difference in docetaxel pharmacokinetics between both patient groups therefore appears to be explained neither by a difference in midazolam CL nor by a difference in metabolic conversion rate of midazolam.

Keywords

cytochrome P450 3A, in vivo phenotyping, midazolam, pharmacokinetics, prostate cancer

Docetaxel is a well-established anticancer drug for the treatment of patients with multiple solid tumors including metastatic breast cancer, non–small cell lung cancer, prostate cancer, gastric cancer, and squamous cell carcinoma of the head and neck.[1] Docetaxel is commonly administered as a 1-hour intravenous infusion at a dose of 75 or 100 mg/m^2 every 3 weeks.[2,3] To decrease patient burden and toxicity associated with intravenous docetaxel, oral formulations have been designed and investigated in clinical trials.[4-8]

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Recent studies have reported a difference in docetaxel pharmacokinetics between patients with prostate cancer and male patients with other solid tumors. A meta-analysis reported a 1.8-fold lower area under the plasma concentration–time curve (AUC) for intravenous docetaxel in patients with metastatic castration-resistant prostate cancer (mCRPC) compared to male patients with other solid tumors.[9] Furthermore, these mCRPC patients had a 2.2-fold lower odds of developing Grade 3/4 neutropenia,[9] indicating the possible clinical significance of a lower docetaxel AUC. Moreover, oral docetaxel administration resulted in an even more pronounced 2.4-fold decrease in docetaxel AUC in patients with mCRPC as compared to male patients with other solid tumors.[8] The lower docetaxel exposure appears to be independent of disease status since patients with metastatic hormone-sensitive prostate cancer (mHSPC) had a similar pharmacokinetic profile for docetaxel as patients with mCRPC.[10]

Since docetaxel is predominantly metabolized by cytochrome P450 3A (CYP3A),[11,12] altered CYP3A activity in patients with prostate cancer might explain the observed difference in docetaxel pharmacokinetics. The hypothesis behind the altered CYP3A activity is an induction of the CYP3A enzyme caused by the castration status of patients with prostate cancer.[8] However, in vivo phenotyping studies, using the erythromycin breath test, found no significant difference in hepatic CYP3A activity between the above-described patient groups.[13,14] On the other hand, erythromycin is not a specific or validated substrate for CYP3A.[15-17] Therefore, there is a need for a more accurate investigation of hepatic and intestinal CYP3A activity in patients with prostate cancer and male patients with other solid tumors.

The aim of the current study was to quantify in vivo CYP3A activity in patients with prostate cancer and male patients with other solid tumors using the clearance (CL) of the specific CYP3A substrate midazolam as a more accurate metric for enzyme activity. Midazolam is a short-acting benzodiazepine that is, like docetaxel, almost exclusively metabolized by CYP3A into its predominant metabolite 1′-hydroxy midazolam and the lesser metabolite 4′-hydroxy midazolam.[18] Both midazolam AUC and metabolic clearance to 1′-hydroxy midazolam correlate well with hepatic CYP3A content.[19,20] In general, midazolam plasma CL is an accepted accurate metric for CYP3A activity due to its specificity and sensitivity to changes in CYP3A activity.[21] Usually, oral midazolam doses of 2-7.5 mg and intravenous doses of 1-3 mg are used for in vivo phenotyping.[21] Secondary objectives were the comparison of midazolam AUC, and the midazolam metabolic ratio to 1′-hydroxy midazolam between the 2 patient groups with a differentiation between intestinal and hepatic CYP3A activity by administrating oral and intravenous midazolam.

Methods

Study Design and Patients

A prospective, interventional pharmacokinetic study was designed to compare midazolam pharmacokinetics between patients with prostate cancer and male patients with other solid tumors. The study was conducted in Antoni van Leeuwenhoek Hospital and the study protocol was approved by the local accredited medical ethics committee (Netherlands Cancer Institute, Amsterdam). The study was performed in accordance with the Declaration of Helsinki. Written consent was obtained for all participating patients before the start of the study procedures. The study was registered at ClinicalTrials.gov (NCT05518799).

Male patients (aged 18 years or older) with histological or cytological proof of a solid tumor were eligible for study participation independent of disease status. Patients with prostate cancer had to have a castration level of testosterone (1.73 nmol/L or less).[22] Adequate hematologic, hepatic, and renal function were required for participation. Patients using concomitant CYP3A-modulating drugs, herbs, or food 14 days before the start of the study or within 5 half-lives of the drug and patients who smoked during or within 7 days before the start of the study were excluded.

All patients received 2 mg of oral midazolam, and 1 mg IV of midazolam on 2 consecutive days. After administration of midazolam, pharmacokinetic exposure was determined. Blood samples (4 mL, dipotassium ethylenediaminetetraacetic acid) were drawn at 7 time points: before dosing and 0.25, 0.5, 1, 2, 4, and 8 hours after administration. Immediately after collection, samples were centrifuged for 10 minutes at 1500 × *g* at 4°C. Plasma was collected and stored at −80°C until analysis.

Bioanalysis

Plasma concentrations of midazolam, 1′-hydroxy midazolam, and 4′-hydroxymidazolam were determined using a liquid chromatography–tandem mass spectrometry method. Sample preparation consisted of liquid–liquid extraction with tert-butylmethylether using 200-µL plasma aliquots. Stable isotopically labeled midazolam and 1′-hydroxy midazolam were used as internal standards. Plasma aliquots were prepared by adding 1000 µL of tert-butylmethylether and 20 µL of internal standard. Samples were mixed with an automatic shaker (1250 rpm, 10 minutes) and centrifuged (18,626 × *g*, 5 minutes). After snap freezing, the organic layer was transferred to a clean tube and evaporated until dryness under a gentile stream of nitrogen (40°C). The residue was reconstituted with a mixture of 100 µL

Table 1. Demographics of the Included Patients

	Prostate cancer group	Other solid-tumor group
Demographics		
Number of patients	9	9
Race		
White	9 (100)	9 (100)
Age (years)	69 (58-79)	64 (38-71)
Body weight (kg)	81.8 (65-100)	91 (62.5-131)
Disease information		
WHO score		
0	9 (100)	8 (89)
1	0 (0)	1 (11)
Primary tumor		
Colorectal	0 (0)	4 (44)
Melanoma	0 (0)	4 (44)
Prostate	9 (100)	0 (0)
SCLC	0 (0)	1 (12)
Disease stage		
Local	0 (0)	2 (22)
Locally advanced	1 (11)	0 (0)
Metastatic	8 (89)	7 (78)
Clinical chemistry[a]		
ALAT (<45 U/L)	22 (17-36)	22 (9-35)
ASAT (<35 U/L)	32 (22-69)	28 (19-42)
eGFR (>60 mL/min)	90 (71-108)	84 (66-96)
Serum creatinine (50-105 µmol/L)	72 (61-90)	84 (66-99)
Testosterone (3.0-33.0 nmol/L)	0.03 (0.02-0.5)	9 (6.3-29)
Total bilirubin (<24 µmol/L)	9 (6-23)	6 (6-18)
Medical history		
Prior therapy		
No	6 (67)	5 (56)
Chemotherapy	3 (33)	1 (11)
Hormone therapy	3 (33)	2 (22)
Immune therapy	0 (0)	4 (44)
Chronic concomitant medication		
Abiraterone	6 (67)	0 (0)
Anti-acids including PPIs	2 (22)	4 (44)
Antibiotics	0 (0)	1 (11)
Antihistamines	1 (11)	0 (0)
Asthma medication	0 (0)	1 (11)
Cardiovascular mediation	8 (89)	4 (44)
Corticosteroids	3 (33)	2 (22)
Encorafenib	0 (0)	1 (11)
Hormone therapy	7 (78)	0 (0)
Immune therapy	0 (0)	6 (67)
Laxatives	1 (11)	4 (44)
Osteoporosis prophylaxis	4 (44)	0 (0)
Paracetamol	2 (22)	2 (22)
Thyreominetics	0 (0)	1 (11)

Data are presented as median (range) or frequency (percentage) unless otherwise specified.

ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; eGFR, estimated glomerular flitration rate; PPI, proton pump inhibitors; SCLC, small cell lung cancer; WHO, World Health Organization.

[a] Normal ranges for laboratory provided.

20 mM ammonium formate in water (pH 3.5) and methanol (7:3 v/v). Samples were centrifuged (18,626 × *g*, 5 minutes) before transferring the supernatant in vials for analysis. Liquid chromatography–tandem mass spectrometry apparatus employed were Nexera X2 Chromatograph LC (Shimadzu) and API4000 triple quadrupole tandem mass spectrometry (Sciex), equipped with a turbo ion spray interface, operated in the positive mode. Separation was accomplished using an Acquity BEH C18 analytical column (50 × 2.1 mm ID, 1.7 µm particles) using gradient elution with 20 mM of ammonium formate (pH 3.5)-methanol (7:3 v/v) and methanol. Detection and quantification were performed using mass/charge transitions m/z 325.9 → 291.0 for midazolam, m/z 342.1 → 203.1 for 1′-hydroxy midazolam, and m/z 342.1 → 234.0 for 4′-hydroxy midazolam. The method was validated according to international guidelines,[23,24] over a concentration range of 0.1-50 ng/mL for all 3 analyses. Accuracy was within 7.9%, and precision was better than 5.6%, at all tested concentration levels during method validation.

Pharmacokinetics and Statistical Analysis

The primary aim of the current study was the comparison of midazolam CL between patients with prostate cancer and male patients with other solid tumors. For sample size calculation, a midazolam CL of 81.7 L/h with a standard deviation of 41.5 L/h was used for male patients with other solid tumors.[25] With 9 patients per group, there was 80% power to detect a 2-fold change in midazolam CL, assuming a coefficient of variation on the original scale of 50.8% and alpha 0.05 (2-sided). Therefore, 9 evaluable patients in each patient group were required. Midazolam CL was calculated using noncompartmental analysis. The secondary aim of the study was the determination of AUC from time 0 to 8 hours and AUC extrapolated to infinity (AUC_{inf}), which were calculated using the linear-log trapezoidal method. The metabolic ratio was calculated by dividing AUC_{inf} of 1′-hydroxy midazolam by AUC_{inf} of midazolam. Other pharmacokinetic metrics were derived from the noncompartmental analysis such as the highest measured concentration over 8 hours (C_{max}), time to C_{max}, and volume of distribution were derived from the AUC_{inf}. Oral bioavailability was defined as the dose-corrected ratio between oral midazolam AUC_{inf} and intravenous midazolam AUC_{inf}. Samples below the lower limit of quantification (LLOQ) were imputed with half of LLOQ (0.05 ng/mL) if they were necessary for a reliable estimation of the elimination rate constant.[26] Noncompartmental analysis, statistical analysis, and power calculation were performed using R Version 4.1.2 (R-project). The Wilcoxon signed-rank test was used to determine *P*-values for the comparison of pharmacokinetics between the 2 patient groups. A *P*-value of .05 or less was considered statistically significant. In case of significant difference, post hoc analysis would be performed to differentiate between intestinal and hepatic CYP3A activity.

Table 2. Pharmacokinetic Parameters of Midazolam and 1′-Hydroxy Midazolam

	Oral administration			Intravenous administration		
	Prostate cancer	Other solid tumors	*P*-value	Prostate cancer	Other solid tumors	*P*-value
Midazolam						
Geometric mean (CV%)						
C_{max} (ng/mL)	11.33 (24.9)	8.94 (29.5)	.10	–	–	–
t_{max} (hour)	0.5	0.6	.35	–	–	–
$AUC_{0\text{-}8}$ (ng • h/mL)	19.9 (30.7)	24.0 (32.8)	.09	21.9 (31.8)	21.4 (36.5)	.93
AUC_{inf} (ng • h/mL)	21.3 (33.5)	26.9 (39.1)	.08	23.5 (35.1)	24.6 (40.0)	.93
$t_{1/2}$ (hour)	1.58 (46.6)	2.18 (37.8)	.19	1.63 (52.3)	2.47 (37.9)	.06
CL/F (L/h)	94.1 (33.5)	74.4 (39.1)	.08	–	–	–
CL (L/h)	–	–	–	42.6 (35.1)	40.6 (40.0)	.93
V_d/F (L)	214 (35.7)	234 (20.2)	.34	–	–	–
V_d (L)	–	–	–	100 (44.3)	144 (37.1)	.14
F (%)	45.3 (24.7)	54.5 (28.3)	.22	–	–	–
1′-Hydroxy midazolam						
Geometric mean (CV%)						
$AUC_{0\text{-}8}$ (ng • h/mL)	4.58 (57.0)	4.92 (68.9)	1.00	3.35 (30.7)	2.81 (120)	.39
AUC_{inf} (ng • h/mL)	4.98 (56.3)	5.33 (67.6)	1.00	3.73 (31.5)	3.41 (111)	.67
Metabolic ratio	0.23 (40.8)	0.19 (78.5)	.67	0.16 (18.4)	0.14 (99.2)	.26

$AUC_{0\text{-}8}$, area under the plasma concentration–time curve from time zero to 8 h after administration; AUC_{inf}, area under the plasma concentration–time curve extrapolated to infinity; CL, clearance; CL/F, oral clearance; C_{max}, maximum concentration; CV, coefficient of variation; F, oral bioavailability; metabolic ratio, $AUC_{0\text{-}8}$ of 1′-hydroxy midazolam divided by the $AUC_{0\text{-}8}$ of midazolam; $t_{1/2}$, half-life; t_{max}, time to maximum concentration; V_d, volume of distribution; V_d/F, oral volume of distribution.

Genotyping

For genotyping of CYP3A, 4 mL of blood was collected in dipotassium ethylenediaminetetraacetic acid vials and stored at −20°C until analysis. The following single nucleotide polymorphism (SNPs) were determined: *CYP3A4*2* (*664T>C*), *CYP3A4*17* (*566T>C*), *CYP3A4*22* (*15389C>T*), and *CYP3A5*3* (*6987A>G*). DNA was extracted with QIAmp DNA Mini Kit (Qiagen). DNA concentrations were measured at 260 nm using a nanodrop nd-1000 UV-VIS spectrometer (Thermo Fisher Scientific, Ashville, NC, USA). Genotyping was performed with TaqMan SNP genotyping assays (Applied Biosystems) according to the manufacturer's protocol. Reactions were performed with the Applied Biosystems StepOne. Two negative and 2 positive quality control samples were included on each plate in the TaqMan SNP genotyping assay.

Results

Patient Characteristics

Demographic characteristics of the included patients are summarized in Table 1. All included patients were of White ethnicity. Median age was similar between the 2 patient groups. The patients with solid tumors consisted of colorectal carcinoma (n = 4), melanoma (n = 4), and small cell lung cancer (n = 1). Most patients had metastatic disease; 1 patient with prostate cancer had locally advanced disease, while 2 patients with other solid tumors had localized disease. Patients with prostate cancer used relatively more chronic concomitant medications, especially cardiovascular drugs, abiraterone, and hormone therapy. Furthermore, 5 patients used corticosteroids, which consisted of either prednisolone (max. 10 mg, n = 4) or locally applied budesonide (n = 1) during the study.

Pharmacokinetic Analysis

A summary of the pharmacokinetic metrics is provided in Table 2. The pharmacokinetic profiles of midazolam, 1′-hydroxy midazolam, and 4′-hydroxy midazolam are depicted in Figure 1. Individual pharmacokinetics profiles of midazolam and its metabolites are depicted in Figure S1.

Midazolam. Oral midazolam CL was 1.26-fold higher in patients with prostate cancer compared to male patients with other solid tumors (geometric mean [coefficient of variation], 94.1 [33.5%] L/h vs 74.4 [39.1%] L/h, respectively; $P = .08$), which was not statistically significant (Figure 2). On the other hand, there was no significant difference in intravenous midazolam CL (42.6 [35.1%] L/h vs 40.6 [40.0%] L/h, respectively; $P = .93$). Consistently, oral AUC_{inf} was lower in the prostate cancer group compared to the other solid tumor group (21.3 [33.5%] vs 26.9 [39.1%] ng • h/mL, respectively; $P = .08$), which was statistically insignificant, while intravenous AUC_{inf} was similar between the 2 groups (23.5 [35.1%] vs 24.6 [40.0%], respectively; $P = .93$). Oral bioavailability was lower for patients with prostate cancer compared to patients

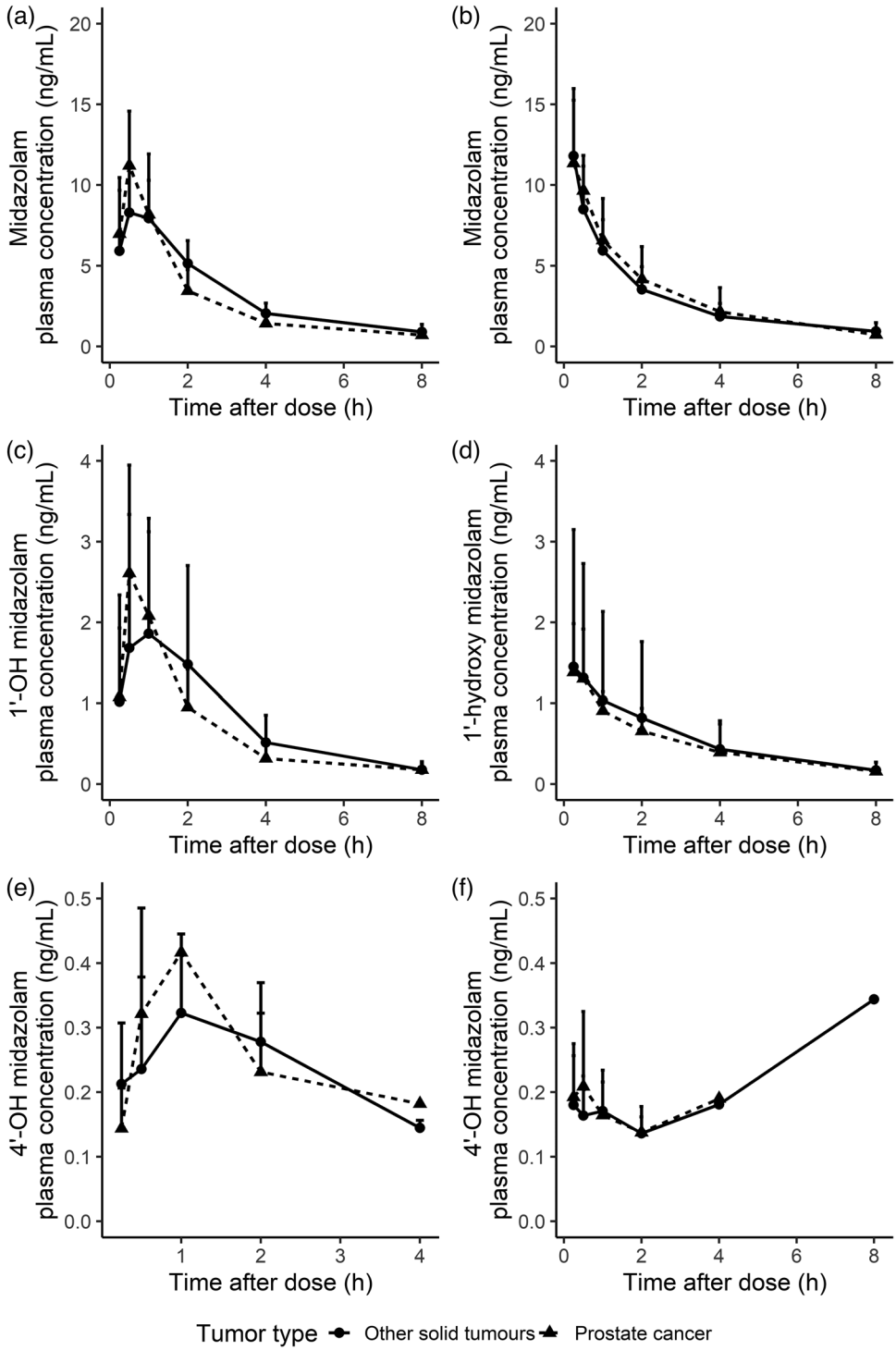

Figure 1. Pharmacokinetic profiles of midazolam, 1′-hydroxy midazolam, and 4′-hydroxy midazolam after oral administration (a, c, e) and intravenous administration (b, d, f). The solid dots and solid line represents the pharmacokinetic profile of patients with other solid tumors and the solid triangles and dashed lines represent the pharmacokinetic profile of patients with prostate cancer. The error bars represent the standard deviation in plasma concentration.

with other solid tumors, but again not statistically significant (45.3% [24.7%] vs 54.5% [28.3%]; $P = .22$). In the current study, no correlation was observed between testosterone levels and midazolam CL (Figure S2).

1′-Hydroxy midazolam. Pharmacokinetics of 1′-hydroxy midazolam demonstrated high interpatient variability (Figure 1c and d) with higher variability for patients with other solid tumors than prostate cancer. There was no significant difference in

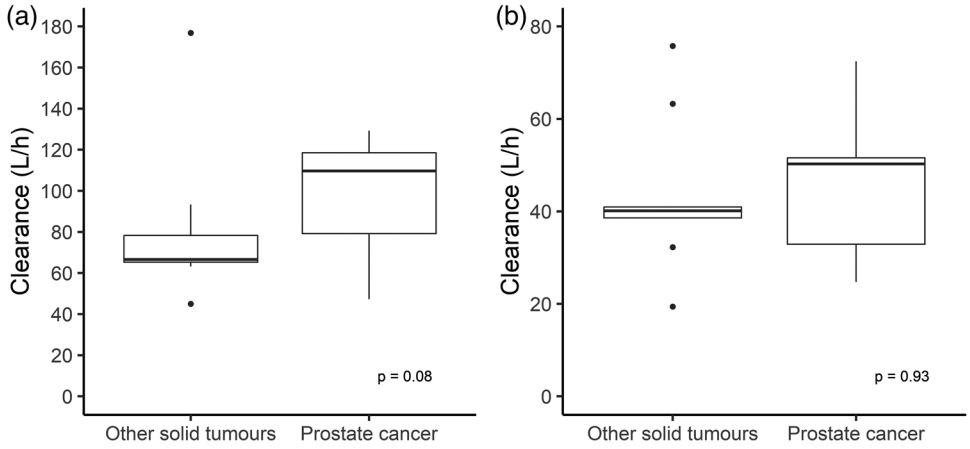

Figure 2. Oral midazolam clearance (a) and intravenous midazolam clearance (b) for patients with other solid tumors and patients with prostate cancer.

1′-hydroxy midazolam AUC_{inf} for oral administration (4.98 [56.3%] ng • h/mL vs 5.33 [67.6%] ng • h/mL, respectively; $P = 1.00$) and for intravenous administration (3.73 [31.5%] ng • h/mL vs 3.41 [111%] ng • h/mL, respectively; $P = .67$). Correspondingly, there was no significant difference in metabolic ratio of 1-hydroxy midazolam to midazolam for both oral administration (0.23 [40.8%] and 0.19 [78.5%]; $P = .67$) and intravenous administration (0.16 [18.4%] vs 0.14 [99.2%]; $P = .26$).

4′-Hydroxy midazolam. The pharmacokinetic profile of 4′-hydroxy midazolam also demonstrated high interpatient variability (Figure 1e and f). The plasma concentrations of 4′-hydroxy midazolam were lower than anticipated. The frequency of less than LLOQ samples did not differ between patients with prostate cancer and patients with other solid tumors (46.3% [50 samples, 10 patients] vs 47.2% [51 samples, 8 patients], respectively). Moreover, the interquartile range of the measured plasma concentrations did not differ between patients with prostate cancer (0.134-0.284 ng/mL) and patients with other solid tumors (0.138-0.273 ng/mL). However, 4′-hydroxy midazolam plasma concentrations for male patients with other solid tumors exhibited a prolonged time above the LLOQ (Figure S1), suggesting a trend toward lower 4′-hydroxy midazolam exposure in prostate cancer patients. The outlier at 4 hours after administration of midazolam depicted in Figure 1f could not be explained by errors in sampling time or a bioanalytical error.

Genotyping

Pharmacogenetic analysis revealed 3 SNPs in the CYP3A gene. One patient with prostate cancer was heterozygous for *CYP3A4*2* and homozygous for *CYP3A5*3* (nonexpressor phenotype). *CYP3A4*2* is in vitro associated with decreased activity (−83% decrease in the predictor of in vivo intrinsic midazolam CL; the ratio of maximum reaction rate and the Michaelis–Menten rate constant) of CYP3A4.[27] The influence of *CYP3A4*2* on the in vivo pharmacokinetics of midazolam is unclear due to the low prevalence of the SNP.[28] Two patients (one in each patient group) were homozygous for *CYP3A4*1* and heterozygous for *CYP3A5*3* (expressor phenotype). This genotype is associated with the phenotype of an extensive metabolizer.[29] The influence of *CYP3A5*3* on the pharmacokinetics of midazolam seems to be inconsistent. Previous studies reported either no significant difference[28,30] or a significant difference[25,31] in midazolam pharmacokinetics. The presence of SNPs did not significantly affect the results from the pharmacokinetic analysis. Furthermore, both patients who were heterozygous for *CYP3A5*3* (expressor phenotype) had midazolam CL values within the established range of CL for both oral and intravenous administration. The patient who was heterozygous for *CYP3A*2* had the highest oral midazolam CL. This is inconsistent with the expected decreased CYP3A4 activity. Therefore, it was concluded that there was limited effect of the detected SNPs on the pharmacokinetics of midazolam in our study.

Discussion

The primary aim of the current study was to quantify CYP3A activity, defined as midazolam CL, in patients with prostate cancer compared to patients with other solid tumors. Oral midazolam CL was 1.26-fold higher in patients with prostate cancer compared to patients with other solid tumors. This nonsignificant increase in oral clearance only partially explains the observed 2.4-fold difference in oral docetaxel exposure.[8]

Moreover, the secondary objectives, midazolam AUC and metabolic ratio, were also not significantly different between patients with prostate cancer and patients with other solid tumors. Because no significant differences between the groups were established, no post hoc analysis was performed to differentiate between intestinal and hepatic CYP3A activity. However, there was a trend toward a higher CL for oral administration, suggesting a possible higher intestinal CYP3A activity for patients with prostate cancer.

The current study was able to reject the hypothesis of an increased CYP3A activity as the sole physiological mechanism behind the 1.8-2.4-fold lower docetaxel exposure observed in patients with prostate cancer.[8,9] Two other studies have also investigated CYP3A activity in patients with prostate cancer. One study in male castrated and noncastrated patients reported no significant difference in hepatic CYP3A activity determined with the erythromycin breath test.[13] Another study observed no significant change in hepatic CYP3A activity, determined with the erythromycin breath test, in 11 men with prostate cancer before the start of luteinizing hormone–releasing hormone agonists and 2 months after the start of therapy.[14] These studies have 2 important limitations. First, erythromycin is not fully specific for CYP3A activity since it is also a substrate of several drug transporters including P-glycoprotein and it is not a validated CYP3A probe.[15] Second, the erythromycin breath test quantifies only hepatic CYP3A activity and not intestinal CYP3A activity. The current study used midazolam CL as a measure for CYP3A activity, which is a generally accepted and validated metric for CYP3A activity.[21] Moreover, midazolam was administered both orally and intravenously to enable the quantification of both intestinal and hepatic CYP3A activity. While the current study found a 1.26-fold higher oral midazolam CL for patients with prostate cancer, it cannot fully explain the observed difference in docetaxel pharmacokinetics.[8]

An alternative explanation for the observed difference in docetaxel pharmacokinetics could be increased hepatic uptake due to increased expression of hepatic drug transporters. Preclinical studies in rats reported a significantly higher docetaxel exposure in the liver of castrated rats compared to noncastrated rats (37.0 vs 18.0 μg • h/mL; $P = .01$).[13] The expression of solute carrier genes encoding for organic cation transporters *rOct1* (*Slc22a1*), organic anion transporter *rOat2* (*Slc22a7*), and organic anion transporter polypeptide *rOatp1a1* (*Slco1a1*) were increased in rat hepatic biopsies.[13] We suspect *rOat2* to mainly contribute to the altered docetaxel pharmacokinetics because docetaxel is both a substrate for rOat2 and rOatp1a1, and erythromycin is only a substrate for rOatp1a1.[13] Furthermore, midazolam is neither a substrate for OATP1a1 and a2, while it is still unknown whether it is a substrate for OAT2.[32,33] Cells ex vivo overexpressing *rOat2* demonstrated a 3.7-fold increase in docetaxel-mediated cytotoxicity compared to control cells and an approximately 2-fold increase in docetaxel uptake.[13] *rOat2* expression seems to be regulated by liver receptor homolog 1 (Lrh-1).[34] Overexpression of Lrh-1 resulted in a 2.2-fold increase in *rOat2* mRNA, while Lrh-1 knockout mice demonstrated a decrease in *rOat2* mRNA.[34] Accordingly, Lrh-1 knockout mice demonstrated increased docetaxel C_{max} and AUC in plasma with lowered hepatic docetaxel concentrations.[34] Finally, several studies have investigated the relationship between androgens, such as testosterone, and Lrh-1; however, the exact relationship remains to be elucidated.[35-39] In the current study, no correlation was observed between testosterone levels and midazolam CL. Moreover, a comparison in oral docetaxel pharmacokinetics between patients with mCRPC and newly diagnosed hormone-sensitive prostate cancer found no differences between the 2 groups.[10]

The current study has several limitations. First, the majority of the patients had metastatic disease. Disease state could be associated with CYP3A enzyme activity.[40] Prostate cancer tumors are reported to express CYP3A proteins.[41] Furthermore, SNPs in CYP3A are associated with prostate cancer risk and aggressiveness.[41] With such a small sample size, 1 patient with local disease and 2 patients with locally advanced disease could influence the current results. However, a comparison of intravenous docetaxel pharmacokinetics between patients with mCRPC and newly diagnosed hormone-sensitive prostate cancer found no differences between the 2 groups.[10] Finally, all included patients were White. This could limit the extrapolation of the current study to different patient populations. However, the effect of the CYP3A genotype, which can differentiate between different ethnicities, appears to be limited to *CYP3A4*22*,[42,43] which was not present in patients enrolled in the current study. Additionally, the effect of *CYP3A5*3* on midazolam pharmacokinetics is inconsistent.[25,28,30,31] Our study also has several strengths. We used midazolam, which, as a more specific and sensitive probe for CYP3A activity,[21] is recommended by both the European Medicines Agency and the US Food and Drug Administration.[44,45] Second, hepatic and intestinal CYP3A activity could be investigated by administration of midazolam both intravenously and orally, while previous studies measured only hepatic CYP3A enzyme activity.[13,14] Finally, short-time-interval pharmacokinetic sampling in the absorption phase ensured the capture of both the

absorption phase of midazolam and the early formation of both 1′-hydroxy midazolam and 4′-hydroxy midazolam.

Conclusions

Oral midazolam CL was 1.26-fold higher in patients with prostate cancer compared to patients with solid tumors, while intravenous midazolam CL was similar between the 2 patient groups. Although not statistically significant, these results suggests a trend toward an increased intestinal CYP3A activity in patients with prostate cancer. However, the observed difference in oral midazolam CL could not explain the observed 1.8- to 2.4-fold difference in docetaxel exposure between patients with prostate cancer and patients with other solid tumors as observed in other studies. An alternative (but currently hypothetical) explanation for the difference in docetaxel pharmacokinetics could be the upregulation of hepatic OAT2, increasing hepatic uptake and CL of docetaxel.

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Conflicts of Interest

J.B. is a part-time employee, co-founder, and indirect stockholder of Modra Pharmaceuticals BV, a small spin-off company of the Netherlands Cancer Institute developing oral taxane treatments a.o. in patients with prostate cancer. J.B. is one of the inventors on a patent of oral taxane formulations, licensed to Modra Pharmaceuticals BV. The remaining authors declare no conflicts of interest.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Sanofi-Aventis (2005) Taxotere, INN-docetaxel – European Medicines Agency. *Summary of product characteristics*. Accessed January 25, 2023. https://www.ema.europa.eu/en/medicines/human/EPAR/taxotere
2. Di Maio M, Perrone F, Chiodini P, et al. Individual patient data meta-analysis of docetaxel administered once every 3 weeks compared with once every week second-line treatment of advanced non-small-cell lung cancer. *J Clin Oncol*. 2007;25(11):1377-1382.
3. Camps C, Massuti B, Jiménez A, et al. Randomized phase III study of 3-weekly versus weekly docetaxel in pretreated advanced non-small-cell lung cancer: a Spanish Lung Cancer Group trial. *Ann Oncol*. 2006;17(3):467-472.
4. de Weger VA, Stuurman FE, Hendrikx J, et al. A dose-escalation study of bi-daily once weekly oral docetaxel either as ModraDoc001 or ModraDoc006 combined with ritonavir. *Eur J Cancer*. 2017;86:217-225.
5. de Weger VA, Stuurman FE, Koolen SLW, et al. A phase I dose escalation study of once-weekly oral administration of docetaxel as ModraDoc001 capsule or ModraDoc006 tablet in combination with ritonavir. *Clin Cancer Res*. 2019;25(18):5466-5474.
6. Moes JJ, Koolen SL, Huitema AD, Schellens JH, Beijnen JH, Nuijen B. Pharmaceutical development and preliminary clinical testing of an oral solid dispersion formulation of docetaxel (ModraDoc001). *Int J Pharm*. 2011;420(2):244-250.
7. Vermunt MAC, Robbrecht DGJ, Devriese LA, et al. ModraDoc006, an oral docetaxel formulation in combination with ritonavir (ModraDoc006/r), in metastatic castration-resistant prostate cancer patients: a phase Ib study. *Cancer Rep (Hoboken)*. 2021;4(4):e1367.
8. Vermunt MAC, van der Heijden LT, Hendrikx J, et al. Pharmacokinetics of docetaxel and ritonavir after oral administration of ModraDoc006/r in patients with prostate cancer versus patients with other advanced solid tumours. *Cancer Chemother Pharmacol*. 2021;87(6):855-869.
9. de Vries Schultink AHM, Crombag MBS, van Werkhoven E, et al. Neutropenia and docetaxel exposure in metastatic castration-resistant prostate cancer patients: a meta-analysis and evaluation of a clinical cohort. *Cancer Med*. 2019;8(4):1406-1415.
10. Vermunt MAC, van Nuland M, van der Heijden LT, Rosing H, Beijnen JH, Bergman AM. Comparison of docetaxel pharmacokinetics between castration-resistant and hormone-sensitive metastatic prostate cancer patients. *Cancer Chemother Pharmacol*. 2022;89(6):785-793.
11. Royer I, Monsarrat B, Sonnier M, Wright M, Cresteil T. Metabolism of docetaxel by human cytochromes P450: interactions with paclitaxel and other antineoplastic drugs. *Cancer Res*. 1996;56(1):58-65.
12. de Weger VA, Beijnen JH, Schellens JH. Cellular and clinical pharmacology of the taxanes docetaxel and paclitaxel-a review. *Anticancer Drugs*. 2014;25(5):488-494.
13. Franke RM, Carducci MA, Rudek MA, Baker SD, Sparreboom A. Castration-dependent pharmacokinetics of docetaxel in patients with prostate cancer. *J Clin Oncol*. 2010;28(30):4562-4567.
14. Hutson PR, Oettel K, Douglas J, et al. Effect of medical castration on CYP3A4 enzyme activity using the erythromycin breath test. *Cancer Chemother Pharmacol*. 2008;62(3):373-377.
15. Franke RM, Baker SD, Mathijssen RH, Schuetz EG, Sparreboom A. Influence of solute carriers on the pharmacokinetics of CYP3A4 probes. *Clin Pharmacol Ther*. 2008;84(6):704-709.
16. Frassetto LA, Poon S, Tsourounis C, Valera C, Benet LZ. Effects of uptake and efflux transporter inhibition on erythromycin breath test results. *Clin Pharmacol Ther*. 2007;81(6):828-832.
17. Kurnik D, Wood AJ, Wilkinson GR. The erythromycin breath test reflects P-glycoprotein function independently of cytochrome P450 3A activity. *Clin Pharmacol Ther*. 2006;80(3):228-234.
18. Nguyen HQ, Kimoto E, Callegari E, Obach RS. Mechanistic modeling to predict midazolam metabolite exposure from in vitro data. *Drug Metab Dispos*. 2016;44(5):781-791.
19. Thummel KE, Shen DD, Podoll TD, et al. Use of midazolam as a human cytochrome P450 3A probe: I. In vitro-in vivo correlations in liver transplant patients. *J Pharmacol Exp Ther*. 1994;271(1):549-556.

20. Thummel KE, Shen DD, Podoll TD, et al. Use of midazolam as a human cytochrome P450 3A probe: II. Characterization of inter- and intraindividual hepatic CYP3A variability after liver transplantation. *J Pharmacol Exp Ther*. 1994;271(1):557-566.
21. Hohmann N, Haefeli WE, Mikus G. CYP3A activity: towards dose adaptation to the individual. *Expert Opin Drug Metab Toxicol*. 2016;12(5):479-497.
22. Lee TY, Pierrillas PB, Lin YW, et al. Population PK and semimechanistic PK/PD modeling and simulation of relugolix effects on testosterone suppression in men with prostate cancer. *Clin Pharmacol Ther*. 2023;113(1):124-134.
23. Agency EEM. Guideline on Bioanalytical Method Validation. Committee for Medicinal Products for Human Use and European Medicines Agency. Accessed May 8, 2022.
24. Administration FFaD. Bioanalytical Method Validation. Silver Springs, Maryland: US Food and Drug Administration. Accessed May 9, 2022.
25. Wong M, Balleine RL, Collins M, Liddle C, Clarke CL, Gurney H. CYP3A5 genotype and midazolam clearance in Australian patients receiving chemotherapy. *Clin Pharmacol Ther*. 2004;75(6):529-538.
26. Irby DJ, Ibrahim ME, Dauki AM, et al. Approaches to handling missing or "problematic" pharmacology data: pharmacokinetics. *CPT Pharmacometrics Syst Pharmacol*. 2021;10(4):291-308.
27. Miyazaki M, Nakamura K, Fujita Y, Guengerich FP, Horiuchi R, Yamamoto K. Defective activity of recombinant cytochromes P450 3A4.2 and 3A4.16 in oxidation of midazolam, nifedipine, and testosterone. *Drug Metab Dispos*. 2008;36(11):2287-2291.
28. Eap CB, Buclin T, Hustert E, et al. Pharmacokinetics of midazolam in CYP3A4- and CYP3A5-genotyped subjects. *Eur J Clin Pharmacol*. 2004;60(4):231-236.
29. Sanchez Spitman AB, Moes D, Gelderblom H, Dezentje VO, Swen JJ, Guchelaar HJ. Effect of CYP3A4*22, CYP3A5*3, and CYP3A combined genotypes on tamoxifen metabolism. *Eur J Clin Pharmacol*. 2017;73(12):1589-1598.
30. de Jonge H, de Loor H, Verbeke K, Vanrenterghem Y, Kuypers DR. Impact of CYP3A5 genotype on tacrolimus versus midazolam clearance in renal transplant recipients: new insights in CYP3A5-mediated drug metabolism. *Pharmacogenomics*. 2013;14(12):1467-1480.
31. Seng KY, Hee KH, Soon GH, et al. CYP3A5*3 and bilirubin predict midazolam population pharmacokinetics in Asian cancer patients. *J Clin Pharmacol*. 2014;54(2):215-224.
32. Ziesenitz VC, Weiss J, Haefeli WE, Mikus G. Cytochrome P450-3A phenotyping using midazolam is not altered by OATP1B1 polymorphisms. *Clin Pharmacol Ther*. 2013;93(5):388.
33. Imanaga J, Kotegawa T, Imai H, et al. The effects of the SLCO2B1 c.1457C >T polymorphism and apple juice on the pharmacokinetics of fexofenadine and midazolam in humans. *Pharmacogenet Genomics*. 2011;21(2):84-93.
34. Yu F, Zhang T, Guo L, Wu B. Liver receptor homolog-1 regulates organic anion transporter 2 and docetaxel pharmacokinetics. *Drug Metab Dispos*. 2018;46(7):980-988.
35. Wu YG, Bennett J, Talla D, Stocco C. Testosterone, not 5α-dihydrotestosterone, stimulates LRH-1 leading to FSH-independent expression of Cyp19 and P450scc in granulosa cells. *Mol Endocrinol*. 2011;25(4):656-668.
36. Wu Y, Baumgarten SC, Zhou P, Stocco C. Testosterone-dependent interaction between androgen receptor and aryl hydrocarbon receptor induces liver receptor homolog 1 expression in rat granulosa cells. *Mol Cell Biol*. 2013;33(15):2817-2828.
37. Yang X, Wang Q, Wang Y, et al. LRH-1 high expression in the ovarian granulosa cells of PCOS patients. *Endocrine*. 2021;74(2):413-420.
38. Xiao L, Wang Y, Xu K, et al. Nuclear receptor LRH-1 functions to promote castration-resistant growth of prostate cancer via its promotion of intratumoral androgen biosynthesis. *Cancer Res*. 2018;78(9):2205-2218.
39. Zhou J, Wang Y, Wu D, et al. Orphan nuclear receptors as regulators of intratumoral androgen biosynthesis in castration-resistant prostate cancer. *Oncogene*. 2021;40(15):2625-2634.
40. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther*. 2013;138(1):103-141.
41. van Eijk M, Boosman RJ, Schinkel AH, Huitema ADR, Beijnen JH. Cytochrome P450 3A4, 3A5, and 2C8 expression in breast, prostate, lung, endometrial, and ovarian tumors: relevance for resistance to taxanes. *Cancer Chemother Pharmacol*. 2019;84(3):487-499.
42. Vanhove T, de Jonge H, de Loor H, et al. Relationship between in vivo CYP3A4 activity, CYP3A5 genotype, and systemic tacrolimus metabolite/parent drug ratio in renal transplant recipients and healthy volunteers. *Drug Metab Dispos*. 2018;46(11):1507-1513.
43. Matthaei J, Bonat WH, Kerb R, et al. Inherited and acquired determinants of hepatic CYP3A activity in humans. *Front Genet*. 2020;11:944.
44. European Medicines Agency. Guideline on the investigation of drug interactions. European Medicines Agency (EMA). Accessed February 27, 2023.
45. Guidance for industry drug interaction studies – study design, data analysis, implications for dosing, and labeling recommendations. US Food and Drug Administration (FDA). Accessed February 27, 2023.

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