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ORIGINAL ARTICLE

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Dexamethasone as a probe for docetaxel clearance

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Abstract *Purpose*: A pilot study was conducted in 23 patients in order to assess the correlation between docetaxel clearance (CL) and pharmacokinetics of dexamethasone. Dexamethasone is mainly 6-β hydroxylated by CYP3A4, and is regularly used as standard docetaxel premedication. Genotyping of known functional single nucleotide polymorphism (SNP) of CYP3A5 (G22893A) and mdr-1 (G2677T, G2677A, and C3435T) have been performed in order to tentatively correlate genotype with docetaxel and dexamethasone pharmacokinetics. *Patients and methods*: To be eligible for this study, patients were required to have a solid malignancy for which docetaxel was indicated. A population pharmacokinetic approach was used to determine individual pharmacokinetic parameters of both docetaxel and dexamethasone by Bayesian analysis, and to screen relationships between docetaxel CL and patients' demographic, phenotype and genotype covariates. *Results*: Three different pharmacokinetic parameters of dexamethasone were significantly correlated with docetaxel CL: dexamethasone plasma clearance (DPC) that ranged between 7.7 and 27.2 l/h, urinary amount of 6β-hydroxydexamethasone, and the ratio between urinary amount of 6β-hydroxydexamethasone and unchanged dexamethasone. The best covariate model was $\text{docetaxel CL (l/h)} = 356 \times \text{fu}_{\alpha 1}\text{ - AG} \times (1 - 0.17 \times \text{HPMT})(1 + 0.126 \times \text{DPC})$ where $\text{fu}_{\alpha 1\text{-AG}}$ is the unbound plasma fraction of docetaxel calculated from alpha$_1$-acid glycoprotein plasma level, and HPMT is hepatic metastasis coded as 1 if present or 0 if absent. No significant difference in docetaxel CL was observed between the several genotypes. *Conclusions*: Dexamethasone may be used as a probe to predict docetaxel clearances, hence reducing interindividual variability.

Keywords Cytochrome P450 · P-glycoprotein transporter · Pharmacokinetics · Pharmacogenetics

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Introduction

Interindividual variability in pharmacokinetics is a significant contributor to the variability of both antitumor activity and toxicity with cytotoxic drugs. For drugs eliminated mainly by the renal pathway, the ability to predict the variable clearance led to the method of AUC dosing, which resulted in improvements in both safety and tumor response. For drugs cleared mainly by hepatic metabolism, particularly for those metabolized by CYP3A4, several in vivo probes for CYP3A4 have been proposed. The most widely accepted is the erythromycin breath test [8], but the requirement for an isotopic compound limits the use of this test. Midazolam has also been used as a probe drug to predict docetaxel clearance (CL) [17]. Recently, Yamamoto et al. have observed a close relationship between the total amount of urinary 6-β-hydroxycortisol excreted after cortisol administration and docetaxel CL [21]. Indeed, both cortisol and docetaxel metabolism are catalyzed by CYP3A4. Dexamethasone, a synthetic glucocorticoid, is also mainly hydroxylated in position 6-β by CYP3A4 [5]. Moreover, dexamethasone, in contrast to cortisol, is used as routine standard docetaxel premedication.

The objective of this study was to assess the predictability of the interpatient variability of docetaxel CL estimated by pharmacokinetics of dexamethasone. Since docetaxel undergoes hydroxylation by CYP3A4 and CYP3A5, and is also a substrate for the membrane

transporter P-glycoprotein (P-gp, or mdr-1 protein), genotyping of known functional single nucleotide polymorphism (SNP) of CYP3A5 (G22893A) [10] and mdr-1 (G2677T, G2677A, and C3435T) [4, 9] have been performed in order to correlate genotype with docetaxel and dexamethasone pharmacokinetics. CYP3A5*3 allele (G at nt 22893) leads to an aberrant CYP3A5 mRNA splicing that causes a loss of hepatic expression of CYP3A5. The presence of at least one CYP3A5*1 allele (A at nt 22893) results in expression of functional CYP3A5 that can strikingly enhance the overall CYP3A activity. The two mutations of the mdr-1 gene, T or A at nt 2677 and T at nt 3435, have been correlated with a decrease in P-gp expression and activity, respectively. The population pharmacokinetic approach [2] was used as a tool to determine individual pharmacokinetic parameters of both docetaxel and dexamethasone by Bayesian analysis, and to screen relationships between docetaxel CL and patients' demographic, phenotype and genotype covariates.

Patients and methods

Patients

To be eligible for this study, patients were required to have histologically or cytologically proven solid malignancy for which docetaxel was indicated, and to be older than 18 years. Exclusion criteria included a known history of hypersensitivity reactions to glucocorticoids, and blood sampling impossibility. The study was approved by the regional ethics committee. Written informed consent was obtained from all patients before they were entered onto the study.

Drug administration

Docetaxel (Taxotere, Aventis Pharma, Paris, France) was administered intravenously (i.v.) over 1 h at doses ranging from 75 to 100 mg/m^2 depending on the standard regimen. All patients were treated by docetaxel as monotherapy. Each patient received the following premedications: (a) dexamethasone (20 mg) diluted in 10 ml 0.9% saline and administered i.v. for 5 min 24 h before the docetaxel infusion; (b) methylprednisolone (8 mg) administered orally twice daily for 3 days (first tablet administered the evening before the docetaxel infusion); and (c) granisetron (3 mg), or ondansetron (8 mg) diluted in 100 ml 0.9% saline and administered i.v. 30 min before the docetaxel infusion.

Blood and urine sampling

Samples for pharmacokinetic studies were obtained from all patients during the first cycle therapy. The blood samples were collected into heparinized tubes through an indwelling cannula in the arm opposite that used for drug administration: (a) before infusion, 30 min, 2 h and 4 h after the beginning of the dexamethasone administration; and (b) before infusion, 25 min, 1.5 h, 3 h and 7 h after the beginning of the docetaxel infusion. All blood samples were centrifuged immediately at 1760 *g* for 10 min, and the plasma was removed and kept at −20°C until analysis. After dexamethasone administration, urine was collected over 24 h, the collection being divided into three periods: 0–4, 4–8, and 8–24 h.

Measurements

Analytical grade dexamethasone was obtained from Sigma (Saint-Quentin Fallavier, France). 6-β-Hydroxydexamethasone (6β-OHD) was obtained from dexamethasone 21-acetate, provided by Steraloids, by oxidation with selenium dioxide [18]. This oxidation is not stereospecific since both 6β-OHD and 6α-OHD are obtained in a ratio of 85/15. Tedious purifications are required to obtain pure 6β-OHD in a low 5% yield. Optimization of the synthesis and the isolation of 6β-OHD will be published elsewhere. Dexamethasone and 6β-OHD were measured by reverse-phase high-performance liquid chromatography (HPLC) using ultraviolet (UV) absorbance detection. Drugs were extracted from both urine and plasma samples (1.0 ml) by solid–liquid extraction using Waters Oasis HLB 1 ml extraction cartridges (Waters, Milford, Mass.) eluted by 2.0 ml ethyl acetate after washing successively with methanol/2% acetic acid (20/80) and methanol/2% ammonium hydroxide (20/80). The solution obtained after elution was washed successively with 1% aqueous acetic acid and 0.3% aqueous sodium hydroxide, and then evaporated. The residue was dissolved in 130 µl methanol/water (50/50), and 100 µl was injected into a Prontosil 120-5-C18-H 5.0 µm column (Bischoff, Leonberg, Germany). The mobile phase (flow rate of 1 ml/min) was methanol/water in sequential proportions: 30/70 for the first 12 min, followed by 50/50 for 38 min. Flumethasone (purchased from Sigma) was added before extraction as internal standard. The detection wavelength was 244 nm. The limits of quantification were 50 ng/ml for plasma and urinary concentrations for both dexamethasone and 6β-OHD. The between-day coefficients of variations (CV) were lower than 7.0%, 17.0%, 16.1% and 6.9% for plasma dexamethasone, urinary dexamethasone, plasma 6β-OHD and urinary 6β-OHD, respectively.

Docetaxel plasma concentrations were measured by reverse-phase HPLC using UV absorbance detection according to a previously described method [20], with some modifications. Drug was extracted from plasma samples (1.0 ml) by solid/liquid extraction using Waters Oasis HLB 1 ml extraction cartridges (Waters, Milford, Mass.) without sample pretreatment, washed with 46% methanol, then eluted with 1.0 ml 90% methanol. The

residue obtained by evaporation of the elution solution was dissolved in 200 µl methanol/water (50/50), and 100 µl was injected onto the column. The mobile phase was methanol/0.3% orthophosphoric acid (65/35). The column was a Polarity 150-4.6 C18-H 5.0 µm (Waters). The flow rate was 0.9 ml/min, and the detection wavelength 235 nm. The limit of quantification was 10 ng/ml. The between-day CV varied between 2.0% and 15.7%.

Genotyping

CYP3A5. Genotyping of CYP3A5 A22893G SNP was performed using sequencing as described previously by Kuehl et al. [10].

MDR1. The MDR1 C3435T, G2677T and G2677A polymorphisms were genotyped using PCR-based restriction enzyme digestion by *Sau*3AI, *Ban*I, and *Bsr*I respectively, as described by Cascorbi et al. [4].

Pharmacokinetic analyses

Concentration versus time profiles of both docetaxel and dexamethasone were analyzed using NONMEM (version V, level 1.1) and the PREDPP package [2] running on a personal computer.

Dexamethasone. Plasma and urinary concentrations of dexamethasone were analyzed simultaneously using the first-order conditional estimation method. Due to analytical interference of methylprednisolone (or one of its metabolite) administered from 12 h after dexamethasone administration, only the urinary concentrations observed between time 0 and 8 h were considered. The two POSTHOC individual pharmacokinetic parameters of interest generated by this analysis were the DPC and the total amount of dexamethasone eliminated unchanged in the urine. The 6β-OHD data, restricted to urinary 6β-OHD data since plasma concentrations were not detectable, were not integrated into the model. The ratio between the amounts of 6β-OHD and unchanged dexamethasone (MR) in urine was calculated from measured total amount of 6β-OHD between 0 and 24 h (T6β-OHD) and the model-dependent value of total unchanged dexamethasone in the urine.

Docetaxel. First, individual docetaxel plasma CL was determined by Bayesian estimation using the POSTHOC option of NONMEM program according to the method of Baille et al. [1]. A three-compartment structural model with first-order elimination was used. Second, covariate screening was performed by considering the typical value of the docetaxel CL as a dependent of patients' covariates: DPC, MR, T6β-OHD (expressed as percentage of administered dexamethasone), MDR1 genotype (coded as 1 for patients who were both homozygous for thymine at 2677 and 3435, and 0 for other genotypes), CYP3A5 genotype (coded as 1 in the presence of at least one CYP3A5*1 allele, 0 in the other case), sex, age, alanine aminotransferase plasma level (ALT), aspartate aminotransferase (AST) plasma level, body weight, height, body surface area (BSA), bilirubin plasma level, albumin plasma level (ALB), presence of hepatic metastasis (HPMT, coded as 1 if present, 0 if absent), and unbound plasma docetaxel fraction ($fu_{\alpha 1\text{-AG}}$, fu_{ALB} or $fu_{\alpha 1\text{-AG;ALB}}$). The covariates $fu_{\alpha 1\text{-AG}}$, fu_{ALB}, and $fu_{\alpha 1\text{-AG;ALB}}$ corresponded to the ratio of unbound and total plasma docetaxel estimated according to the alpha$_1$-acid glycoprotein (AAG), ALB, and both AAG and ALB, respectively, and the equations proposed by Urien et al. [19]: $fu_{\alpha 1\text{-AG}} = 1/(11.20045 + 0.14478 \times AAG)$; $fu_{ALB} = 1/(11.54348 + 0.0072734 \times ALB)$; and $fu_{\alpha 1\text{-AG;ALB}} = 1/(7.20008 + 0.0072734 \times ALB + 0.14478 \times AAG)$ with AAG and ALB in micromoles. The covariates were first tested individually. Those having a potential impact on docetaxel CL were entered into the model sequentially using forward addition. Covariates that significantly ($P < 0.05$) reduced the minimum value of the objective function were retained in the covariate model.

Results

Of 23 patients entered into the study, 21 were assessable. For the other two patients, urine collection was not complete. Patient characteristics are shown in Table 1. Docetaxel doses ranged between 110 and 200 mg.

Genotyping

CYP3A5. Three patients were heterozygous for the CYP3A5*1 and CYP3A5*3 alleles; all others were homozygous for the CYP3A5*3 allele.

MDR1. No G2677A SNP was observed. Genotypes for G23677T and C3435T SNP are shown in Table 2. The two mutations were closely linked.

Pharmacokinetics of dexamethasone

A one-compartment model with first-order elimination well-described both plasma and urinary dexamethasone concentrations. Dexamethasone plasma levels declined according to a mean half-life of 3.1 h (range 1.9–4.8 h). DPC ranged between 7.7 and 27.2 l/h. Mean total amount of 6β-OHD (T6β-OHD, expressed as the percentage of administered dexamethasone) and ratio urinary 6β-OHD/dexamethasone (MR) were 11.6% (4.3–19.7%) and 5.2 (0.6–10.2), respectively. A slight correlation between DPC and MR was observed ($P < 0.05$, Fig. 1). No significant difference in either DPC or MR was observed between the several genotypes.

Table 1 Patients characteristics ($n = 21$)

Characteristics	Mean	Range	Abbreviation
Demographics			
Age (years)	55	19–71	
Weight (kg)	67.1	49–106	
Height (m)	1.67	1.51–1.84	
Body surface area (m^2)[a]	1.75	1.46–2.25	BSA
Sex (female/male)	11/10		
Laboratory measurements			
Alanine aminotransferase (IU/l)	37.8	11–102	
Aspartate aminotransferase (IU/l)	37.6	11–108	
Alkaline phosphatase (IU/l)	231	61–739	
Bilirubinemia (μM)	7.3	3.6–13.9	
Serum α_1-acid glycoprotein (μM)	37.1	18.6–70.9	AAG
Albuminemia (μM)	548	348–773	ALB
Proteinemia (g/l)	67	52–76	
Serum creatinine (μM)	77	55–121	
Disease and treatment			
WHO performance status (0/1/2/3)	4/13/3/1		
Docetaxel dosage: 75/100 mg/m^2	9/12		
Liver metastasis: yes/no	8/13		HPMT
Tumor types: breast/prostate/others	9/4/8		

[a]Calculated according to the Dubois formula

Table 2 Distribution of the 21 patients according to their MDR-1 genotype and the two single nucleotide polymorphism G2677T and C3435T

		C3435T		
		CC	CT	TT
G2677T	GG	4	0	0
	GT	2	8	1
	TT	0	0	6

Pharmacokinetics of docetaxel

Docetaxel CL ranged between 28.9 and 73.4 l/h. During individual testing of the covariates, $fu_{\alpha 1\text{-AG}}$, corresponding to the estimated unbound fraction of plasma docetaxel estimated from $alpha_1$-acid glycoprotein plasma levels, was significantly correlated with docetaxel CL. Since docetaxel binds mainly to $alpha_1$-acid glycoprotein, high plasma levels of this protein limit the free fraction available for CL in the liver. Then, $fu_{\alpha 1\text{-AG}}$ was considered for individual testing of the other covariates. Seven covariates (i.e., sex, DPC, MR, T6β-OHD,

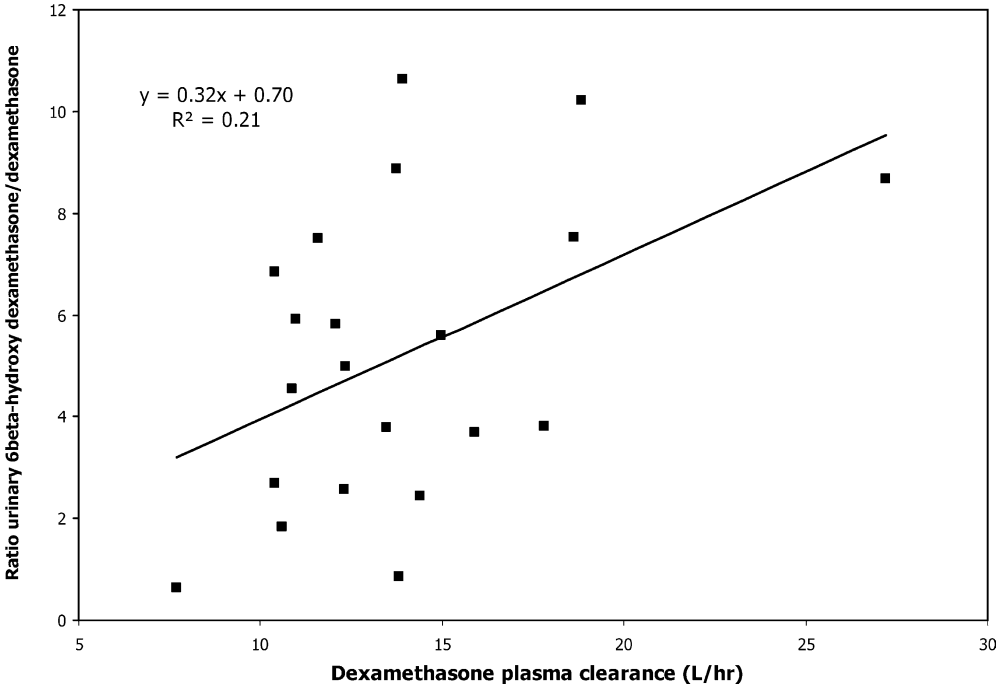

Fig. 1 DPC vs urinary 6β-OHD/dexamethasone ratio (MR). The linear regression line (*solid line*) is shown

Table 3 Mean, interindividual variability and covariate models of docetaxel CL (l/h)

	Coefficient values	ΔOBJ[a]	*P* value	%CV[b]
Final covariate model[c]				
$CL = \theta_1 \times fu_{\alpha 1}\text{ - AG} \times (1 - \theta_2 \times HPMT)(1 + \theta_3 \times DPC)$	$\theta_1 = 356$; $\theta_2 = 0.170$; $\theta_3 = 0.126$			15
Alternative covariate models				
$CL = \theta_1 \times fu_{\alpha 1}\text{ - AG} \times (1 - \theta_2 \times HPMT)(1 + \theta_3 \times MR)$	$\theta_1 = 441$; $\theta_2 = 0.201$; $\theta_3 = 0.066$	+5.2	–	19
$CL = \theta_1 \times fu_{\alpha 1}\text{ - AG} \times (1 - \theta_2 \times HPMT)(1 + \theta_3 \times T6\beta\text{ - OHD})$	$\theta_1 = 705$; $\theta_2 = 0.271$; $\theta_3 = 3.82$	+9.5	–	17
$CL = \theta_1 \times (1 - \theta_2 \times HPMT)(1 + \theta_3 \times DPC)$	$\theta_1 = 34.9$; $\theta_2 = 0.267$; $\theta_3 = 0.060$	+7.2	<0.01	24
$CL = \theta_1 \times fu_{\alpha 1}\text{ - AG} \times (1 - \theta_2 \times HPMT)$	$\theta_1 = 996$; $\theta_2 = 0.206$	+19.7	<0.001	22
$CL = \theta_1 \times fu_{\alpha 1}\text{ - AG} \times (1 + \theta_2 \times DPC)$	$\theta_1 = 214$; $\theta_2 = 0.247$	+4.7	<0.05	23
$CL = \theta_1$	$\theta_1 = 55.8$	+33.1	<0.001	38
$CL = \theta_1 \times BSA$	$\theta_1 = 32.9$	+28.2	–	32

[a]Change in objective function by comparison with the final covariate model

[b]Coefficient of variation for interindividual variability (not explained by the covariate, if any)

[c]Where $fu_{\alpha 1\text{-AG}}$ is the ratio of unbound and total plasma docetaxel calculated according to the equation proposed by Urien et al. [19], HPMT is hepatic metastasis (1 if present, 0 if absent), DPC is plasma dexamethasone clearance, MR is the ratio between the amount of 6β-OHD and unchanged dexamethasone in the urine, and T6β-OHD is the total amount of 6β-hydroxydexamethasone in the urine expressed as percentage of dexamethasone dose administered

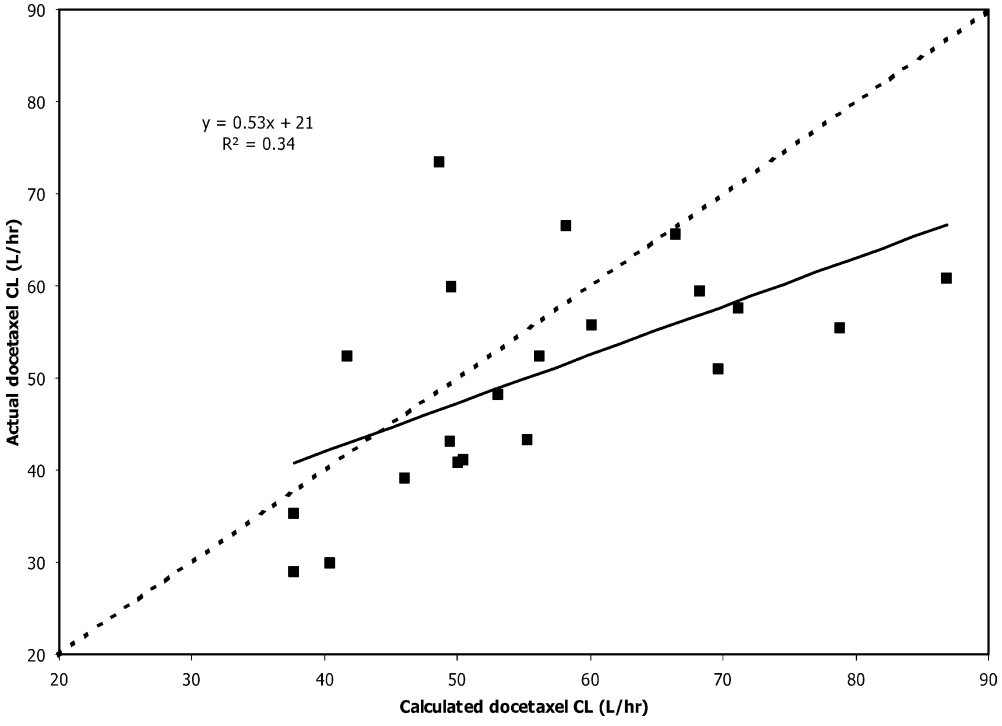

Fig. 2 Observed docetaxel CL values vs CL according to predicted final model: $CL = 356 \times fu_{\alpha 1}\text{ - AG} \times (1 - 0.17 \times HPMT)(1 + 0.126 \times DPC)$ where $fu_{\alpha 1\text{-AG}}$ is the plasma unbound fraction of docetaxel, HPMT is hepatic metastasis (1 if present, 0 if absent), and DPC is plasma dexamethasone clearance. The line of identity (*dashed line*) and the linear regression line (*solid line*) are shown

HPMT, height, and CYP3A5 genotype) were significantly correlated with docetaxel CL. Intermediate covariate models were built using either dexamethasone urinary data (i.e., MR or T6β-OHD) or plasmatic data (i.e., DPC). Significant covariates were forced into multivariate models including one of these dexamethasone data. Each covariate was eliminated in a backward stepwise approach to determine if its exclusion was statistically significant. The best covariate model (model associated with the lowest value of the objective function) was $CL = \theta_1 \times fu_{\alpha 1}\text{ - AG} \times (1 - \theta_2 \times HPMT)(1 + \theta_3 \times DPC)$. Coefficients corresponding to each covariate are presented in Table 3. Independent deletion of each of covariate from the final model was associated with a significant increase in the value of the objective function. The correlation between calculated (according to this covariate model) and actual docetaxel CL values is shown in Fig. 2. Alternative models were obtained by substitution of DPC by MR or T6β-OHD [i.e., $CL = \theta_1 \times fu_{\alpha 1}\text{ - AG} \times (1 - \theta_2 \times HPMT)(1 + \theta_3 \times MR)$ or $CL = \theta_1 \times fu_{\alpha 1}\text{ - AG} \times (1 - \theta_2 \times HPMT)(1 + \theta_3 \times T6\beta\text{ - OHD})$] that were also superior to the model including only $fu_{\alpha 1\text{-AG}}$ and HPMT (Table 3).

Docetaxel CL values were compared between patients according to CYP3A5 and MDR-1 genotype and are presented in Table 4. No significant difference in docetaxel CL was observed between the several genotypes.

Table 4 Mean docetaxel CL according to genotype

SNP	Genotype	Number of patients	Mean CL (l/h) (range)	*P* value[a]
CYP3A5 (A22893G)	CYP3A5*1/3	3	55.1 (43.2–66.5)	0.37
	CYP3A5*3/3	18	49.6 (28.9–66.5)	
MDR-1 G2677T	Homozygous for thymine	6	55.2 (35.2–73.4)	0.28
	Others	15	48.5 (28.9–66.5)	
MDR-1 C3435T	Homozygous for thymine	7	55.9 (35.2–73.4)	0.14
	Others	14	47.7 (28.9–66.5)	
MDR-1 G2677T and C3435T	Homozygous for thymine	6	55.2 (35.2–73.4)	0.27
	Others	15	48.5 (28.9–66.5)	

[a]Calculated according using the Mann-Whitney *U*-test

Relationship between docetaxel pharmacokinetics and toxicity

Although assessment of toxicity was not the primary objective of this study, serious adverse events occurring within the 21 days after docetaxel infusion were reported. Seven patients experienced severe neutropenia (i.e., neutrophil count less than 500 mm^{-3}, and/or febrile neutropenia). Both total AUC and unbound AUC (calculated according to $AUC_u = fu_{\alpha 1\text{ - }AG} \times Dose/CL$) of docetaxel were compared between patients with or without severe neutropenia. Mean total AUC was not significantly larger in patients who experienced severe neutropenia: 3.9 mg h/l (range 2.4–5.8 mg h/l) vs 2.9 mg h/l (1.7–5.3 mg h/l), respectively (NS, Mann-Whitney *U*-test). The difference was significant for AUC_u in patients who experienced severe neutropenia: 0.24 mg h/l (0.15–0.31 mg h/l) vs 0.17 mg h/l (0.09–0.29 mg h/l; $P = 0.02$).

Discussion

We found a large interindividual variability in both DPC and the ratio 6β-OHD/dexamethasone ratio (MR) in the urine in vivo. Moreover, the two parameters (i.e., DPC and MR) were slightly correlated, confirming the in vitro observations of Gentile et al. [5] who studied the metabolism of dexamethasone by human liver microsomes. They showed that 6β-OHD is the major metabolite and that dexamethasone 6-hydroxylation is catalyzed by CYP3A4. The pharmacokinetic parameters we observed (mean DPC 3.5 ± 0.9 ml/min/kg and mean half-life 3.1 h) were consistent with those previously reported (i.e., clearance of 3.7 ± 0.9 ml/min/kg and half-life of 3.0 ± 0.8 h) [7].

Since dexamethasone is one of the synthetic glucocorticoids used as standard docetaxel premedication, we studied the possibility of decreasing interpatient variability in docetaxel systemic exposure by considering dexamethasone pharmacokinetic parameters for docetaxel dosing.

Yamamoto et al. retained the total amount of 6β-hydroxycortisol in the urine to estimate docetaxel CL [21]. However, for other drugs used to reflect in vivo hepatic CYP3A4 activity such as midazolam or CYP2D6 activity with dextromethorphan, the metabolic ratio is a better probe than the amount of metabolite in the urine [17]. In the present study, T6β-OHD and MR had similar performances, but the best covariate model was obtained when considering the dexamethasone plasma CL (DPC). Although determination of this parameter requires blood sampling, it presents some practical advantages over the determination of urinary parameters. In our study, the last blood sample was obtained 4 h after administration, while determination of the total amount of metabolite in the urine required a collection period of more than 8 h. Moreover, urinary data may not be suitable.

We observed a large interindividual pharmacokinetic variability of docetaxel CL with a mean value (28.9 $l/h/m^2$) larger than that reported by Bruno et al. from clinical development (20.6 $l/h/m^2$) [3], but similar to that observed lately by Rosing et al. (34.8 $l/h/m^2$) [15]. The final covariate model we obtained illustrates that drug metabolism is a multifactorial process. Although seven covariates were significantly correlated with docetaxel CL during the individual testing, only three remained independent after testing of the intermediate models. As found by Bruno et al. [3] using population pharmacokinetic analysis of data collected during phase I and phase II trials, we also observed a significant impact of AAG on docetaxel CL. CL tends to decrease with increasing AAG as a consequence of the binding of docetaxel to this protein. This relationship has recently been confirmed by Loos et al. [13]. By determination of the actual unbound fraction of plasma docetaxel, they demonstrated that AAG is the main determinant of variability in docetaxel plasma binding. Moreover, the range of actual values they observed were similar to that for $fu_{\alpha 1\text{-AG}}$ we estimated from AAG (3.9–7.9% [13] vs 4.7–7.2%, respectively). However, these consistent values do not imply that unbound fractions for individual patients were equal using the model or measurement.

Further studies should include measurement of unbound fraction of docetaxel. In previous work [3], the hepatic factor has been shown to correspond to elevated hepatic enzymes, while we observed only metastasis involvement. As shown by the major increase in both the value of the objective function and the unexplained interindividual variability in docetaxel CL (Table 3), the covariate DPC significantly improved the model. However, no pharmacogenetic covariate was retained in the final covariate model. Only consideration of the CYP3A5 SNP was associated with a slight decrease in

the objective function value during the individual testing of the covariates on docetaxel CL. However, DPC and CYP3A5 were redundant covariates in the multivariate NONMEM analysis, which indicates that dexamethasone may be a substrate of CYP3A5. In vivo the contribution of CYP3A5 to overall docetaxel biotransformation is probably limited, as suggested by the tenfold lower affinity of CYP3A5 for docetaxel compared to CYP3A4 [16]. Moreover, Goh et al. [6] have previously investigated the relationship between docetaxel CL and CYP3A5 genotype corresponding to the same SNP: they did not observe a higher docetaxel CL in patients with at least one CYP3A5*1 allele. Genotyping of CYP3A4 would be more appropriate than that of CYP3A5, but so far, out of 38 described allelic variants of CYP3A4, no SNP have been proved to be relevant in explaining a low CYP3A activity [12].

Active efflux by P-gp in intestine and the biliary system represents an alternative pathway of elimination of docetaxel [22]. Thus, the MDR1 genotype was investigated in the patients. Two alleles were well documented as being responsible for the altered P-gp in vivo activity which results in variation of the absorption and disposition profile of some drugs. Indeed, it has been shown that both variant genotypes T3435T and T2677T are related to enhanced oral bioavailability of digoxin [11]. We did not observe any difference in docetaxel CL between various MDR-1 genotypes. Again, the results are consistent with those of Goh et al. [6] who investigated an Asian population. The discrepancy between digoxin and docetaxel may be explained by the fact that the percentage of the dose of digoxin that is metabolized is limited so that the contribution of P-gp is larger than that for docetaxel. Furthermore, van Zuylen et al. did not observe any change in docetaxel CL when combined with a potent inhibitor of P-gp [23].

Overall, it seems more realistic to predict the docetaxel CL by phenotype strategies than from genotype information. This is the first clinical study showing that dexamethasone may be used as a probe for drug metabolism. Previous determination of dexamethasone CL may be clinically useful in tailoring doses of CYP3A4 substrates, such as docetaxel, in certain individuals, particularly in those with elevated hepatic enzymes. Furthermore, actual and model-predicted docetaxel CL were more consistent for low values (Fig. 2) corresponding to low dexamethasone CL. The use of dexamethasone as a probe would allow the docetaxel dose to be decreased more rationally than we do according to the actual guideline (i.e., dose decrease of 25% if AST or ALT over 1.5-fold, or alkaline phosphatase over 2.5-fold the normal upper limit, and contraindicated if over 3.5-fold and 6-fold, respectively). First, the expected unbound docetaxel AUC (AUC_u) corresponding to the "regular" docetaxel dose (e.g., 100 mg/m^2), and individual covariates (i.e., $fu_{\alpha 1\text{-AG}}$, HPMT, and DPC) would be calculated. Second, comparison between this calculated value and target AUC_u would allow an adapted docetaxel dose to be proposed.

Ideally, pharmacokinetics of dexamethasone would be performed after the administration of dexamethasone 24 h before docetaxel as a therapeutic requirement. However, for technical reasons (time for chemical analysis and interpretation of the results), an extra dose of dexamethasone administered more than 24 h before docetaxel would be necessary. Alternatively, a test dose of docetaxel may be considered rather than using a probe, but that would be associated with the analytical difficulty of quantifying very low plasma concentrations or would require a technique such as LC-MS. Moreover, a potential benefit of a CYP3A probe over a test dose of specific drug would be in predicting individual capacity of elimination of several cytotoxic drugs which may be combined or sequentially administered in a particular patient.

A prospective study is now planed to confirm dexamethasone as a pertinent covariate as observed in this pilot study. Patients treated with either docetaxel or vinorelbine will be included in order to evaluate its non-specificity characteristic (which would be a positive property in this circumstance). Moreover, further refinements are still needed—in particular, a more powerful comparison of the respective predictive values of the urinary data (i.e., MR and T6β-OHD) vs plasma dexamethasone clearance. Indeed, the other in vivo probes of CYP3A4, such as the erythromycin breath test, continued to be optimized several years after their first description [14]. Lastly, the larger values of docetaxel AUC (particularly unbound AUC estimated from AAG) observed in patients with severe neutropenia confirm the potential of individual dosing of docetaxel. These results also suggest that any pharmacokinetic-pharmacodynamic study of docetaxel should include measurement, or at least calculation, of the unbound plasma fraction.

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