Factors Affecting Pharmacokinetic Variability of Docetaxel

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Factors Affecting Pharmacokinetic Variability of Docetaxel

Factoren van Invloed op de Farmacokinetische Variabiliteit van Docetaxel

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Chapter 1

Introduction to the Thesis

Introduction to the thesis

In the early 1960s, the US National Cancer Institute started a program aimed at the discovery of new anticancer drugs by releasing protocols for widespread screening of substances and extracts from various origins for antineoplastic activity. Of the more than 100,000 compounds from 35,000 plant species tested between 1960 and 1981, paclitaxel, a complex diterpene isolated from the Pacific Yew tree (*Taxus brevifolia*), proved one of the most interesting and active agents. Extensive studies on the synthesis of paclitaxel analogues and the development of structure-activity relationships have been carried out over the last few decades, and led in 1986 to the development of docetaxel, a semisynthetic taxane derivative prepared from a noncytotoxic precursor isolated from the European Yew tree (*Taxus baccata*). Following an extensive clinical evaluation program that started in 1990, docetaxel has currently been recognized as one of the most widely active agents available, and it has been approved in most countries for the treatment of advanced breast cancer and non-small cell lung cancer.

Although pharmacokinetic investigations were a principal component of the early clinical development of docetaxel during phase I and phase II trials, it was recognized in the late 1990s that various aspects of docetaxel pharmacology required further investigation in order to improve treatment outcome. Specifically, docetaxel treatment in patients with cancer, despite the use of standard premedication, is often associated with a significant incidence of very severe toxic side effects, including neutropenic fever, that is a direct consequence of unusually high systemic exposure to docetaxel.

Against this background, it was the aim of the work described in this thesis to systematically unravel factors influencing the pharmacokinetic variability of docetaxel in patients with cancer (summarized in Chapter 2), and to lay the foundation for the development of improved future treatment strategies with this agent. Many of the described investigations have been made possible by the development of selective analytical methodologies to simultaneously monitor total concentrations of the parent drug and its formulation vehicle polysorbate 80 in plasma (Chapter 7), as well as to determine the extent of vascular binding of docetaxel (Chapter 8).

Although the prescribed dose of docetaxel is calculated using body-surface area (BSA) as the only independent variable, it has been shown that this approach still

results in a large interpatient variability in drug exposure (Chapters 3 and 4). This suggests that alternate dosing strategies that consider factors other than standard body-size measures are needed. Candidate factors include commonly available characteristics like measures of organ dysfunction, sex, and patient age (Chapter 10), but also the comcomitant use other drugs that might interfere with the elimination pathways of docetaxel, the total amount of drug administered (Chapter 4), as well as the frequency of administration and the rate of drug infusion (Chapter 9).

One of the principal candidates for consideration in future dosing strategies for docetaxel is phenotypic expression of the cytochrome P-450 isozymes of the 3A subfamily (CYP3A). These enzymes are responsible for the metabolic breakdown of docetaxel into several pharmacologically inactive compounds, and the activity of CYP3A is now known to vary up to 14-fold in cancer patients (Chapter 5). Clearly this degree of interindividual variation might have important ramification for treatment of patients with docetaxel, and has instigated the use of various noninvasive *in vivo* probes, such as the erythromycin breath test, for evaluating CYP3A activity, as well as to identify demographic, physiologic, and inheritable factors that influence the activity of this group of enzymes (Chapter 5).

A further complication with the clinical use of docetaxel is the presence of the polyoxyethylated surfactant polysorbate 80 in the currently used pharmaceutical formulation (Taxotere). Over the last few years, evidence has accumulated that the use of polysorbate 80 might be associated with some of the side effects observed with docetaxel treatment. Furthermore, polysorbate 80 has the potential to modulate the pharmacokinetic behavior of docetaxel by altering the availability of the fraction unbound drug in the circulation (Chapter 6), and consequently influence a patient's susceptibility to severe docetaxel-mediated hematologic toxicity (Chapter 11).

Chapter 2

Clinical Pharmacokinetics of Docetaxel: Recent Developments

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Submitted

Abstract

Docetaxel belongs to the class of taxane antineoplastic agents, and acts through inhibition of tubulin polymerization. The drug has shown a broad spectrum of antitumor activity in preclinical models as well as clinically, with responses observed in various disease types including advanced breast cancer and non-small cell lung cancer. The pharmacokinetics and metabolism of docetaxel are extremely complex, and have been the subject of intensive investigation in recent years. Docetaxel is subject to extensive metabolic conversion by the cytochrome P450 (CYP) 3A isozymes, which forms several pharmacologically inactive oxidation products. Elimination routes of docetaxel are also depending on the presence of drug-transporting proteins, notably P-glycoprotein, present on the bile canalicular membrane. The various processes mediating drug elimination, either through metabolic breakdown or excretion, impact substantially on interindividual variability in drug handling. Strategies to individualize docetaxel administration schedules based on phenotypic or genotype-dependent differences in CYP3A expression are underway, and may ultimately lead to more selective chemotherapeutic use of this agent.

Introduction

The antineoplastic agent docetaxel acts by disrupting the microtubular network, and has activity against numerous cancers including breast, lung, head and neck, ovarian, and prostate. Docetaxel is currently approved to treat patients with locally-advanced or metastatic breast cancer after failure of prior chemotherapy, and patients with unresectable locally-advanced or metastatic non-small cell lung cancer (NSCLC) in combination with cisplatin, who have not received prior chemotherapy. It is also approved for patients with unresectable locally-advanced or metastatic NSCLC after failure of prior platinum-based chemotherapy and for use in combination with prednisone as a treatment for androgen-independent (hormone-refractory) metastatic prostate cancer. An application for the use of docetaxel in treating women with early-stage operable breast cancer with involved axillary lymph nodes is under consideration in the U.S. and Europe. The docetaxel dose used for treating cancer patients ranges from 60 to 100 mg/m^2 as a 1-hour intravenous infusion given once every 3 weeks (3-weekly). In this regimen, neutropenia occurs in virtually all patients, and grade 4 neutropenia occurs in 75% of patients given 100 mg/m^2 (N = 2045; febrile neutropenia incidence, 12%); grade 3/4 neutropenia occurs in 65% of patients given 75 mg/m^2

(n=176; febrile neutropenia incidence, 6.3%), and grade 4 neutropenia occurs in 75% of patients given 60 mg/m^2 (n=174; febrile neutropenia incidence, 0%) (see: http://www.taxotere.com/ - last accessed July 13, 2004). Other side effects with the 3-weekly regimen include alopecia, asthenia, dermatologic reactions, fluid retention, hypersensitivity reactions, stomatitis and diarrhea. There has been a recent trend toward administration of docetaxel at lower doses (25 to 40 mg/m^2) on weekly schedules. Weekly schedules are associated with comparable response rates as 3-weekly schedules, but with reduced myelosuppression [3-5]. However, severe fatigue and asthenia have become dose-limiting with weekly schedules and many physicians are reverting back to administering docetaxel once every 21 days.

Since the last comprehensive review on the clinical pharmacokinetics of docetaxel by Clarke and Rivory [6], numerous studies have further characterized the clinical pharmacokinetics of docetaxel. Recent developments on predictors of docetaxel disposition and pharmacokinetic-pharmacodynamic relationships are herein reviewed.

Analytical Methods

Several reversed-phase high-performance liquid chromatographic (HPLC) methods using solid-phase extraction (SPE) for sample clean-up were developed during early Phase I investigations with docetaxel, permitting the pharmacokinetic behavior of the agent in both brief and prolonged schedules of administration. In general these methods, which were mostly based on the method described by Vergniol et al (7), are not very sensitive (i.e., lower limit of quantitation of 5 to 10 ng/mL) due to the presence of endogenous interferences as well as the limited UV absorption characteristics of the taxane moiety and the low wavelengths required to quantitate the drug. More sensitive and specific reversed-phase HPLC methods capable of detecting docetaxel concentrations in human plasma as low as 0.4 ng/mL have been developed recently by the utilization of liquid chromatography coupled with tandem mass spectrometry (MS/MS) (Table 1). As such, these methods may have potential for clinical monitoring in studies utilizing very low doses that result in plasma concentrations that approach or are below the sensitivity limits for the HPLC-UV assays, and can be used to allow determination of the drug in samples obtained more than 2 days after drug administration. It is of importance to indicate here that the lower limit of quantitation reported in the various publications can be somewhat misleading when it is reported simply in terms of sample concentration rather then the innate sensitivity. This is because it does not give an indication of the sample size required or

the proportion of the analyte in the sample actually injected for analysis. For example, the method reported by Hou et al. has a lower limit of quantitation of only ~20 ng/mL but it requires only 40 microliters of plasma [8], which might be advantageous when serial analyses are being planned on the same material collected from laboratory animals or patients.

In addition to analytical methods available for the determination of total drug concentrations in human plasma, assays have been developed for measuring the unbound fraction of docetaxel in patient plasma samples. These procedures are based on either ultrafiltration [9] or equilibrium dialysis [10,11]. The latter procedure uses only 200-microliters of sample in a 96-well dialysis plate, which makes the assay more cost-efficient and less time-consuming than the other currently available assays, and likely introduces less bias and random error.

Table 1. Analytical methods for quantitation of docetaxel in human plasma

Sample Pretreatment	I.S.	Sample Volume	LLOQ (ng/mL)	ULOQ (ng/mL)	Detection	Reference
LLE	paclitaxel	50 µL	5	1000	MS/MS	[112]
SPE	paclitaxel	1 mL	0.21	800	MS/MS	[113]
LLE	paclitaxel	1 mL	25	1000	227 nm	[114]
LLE	paclitaxel	1 mL	10	20,000	230 nm	[115]
LLE	paclitaxel	1 mL	0.40	80	MS/MS	[116]
SPE	Paclitaxel	1 mL	0.25	800	MS	[117]
SPE	paclitaxel	1 mL	5	1000	227 nm	[118]
SPE	2'-methyl paclitaxel	1 mL	10	1000	227 nm	[119]
SPE	paclitaxel	1 mL	5	1000	227 nm	[120]
SPE	paclitaxel	1 mL	10	2500	227 nm	[7]
SPE + column switching	paclitaxel	900 µL	2.5	2000	227 nm	[121]
PP	paclitaxel	40 µL	20	2000	MS/MS	[8]

Abbreviations: LLE, liquid-liquid extraction; SPE, solid phase extraction; PP, protein precipitation; MS, mass spectrometry; MS/MS, tandem mass spectrometry; LLOQ, lower limit of quantitation; ULOQ, upper limit of quantitation.

Protein Binding

In vitro studies have demonstrated that docetaxel is extensively bound to albumin and alpha$_1$-acid glycoprotein (orosomucoid; ORM; AAG), and that the latter is the main determinant of variability in docetaxel serum binding [9]. In cancer patients, AAG concentrations vary approximately 7-fold between patients [12], which may contribute to differences in protein binding and systemic drug clearance. From a population pharmacokinetic model involving 547 patients, AAG was identified as a significant predictor of docetaxel total clearance, where an AAG concentration of 260 mg/dL (95% quantile) was associated with a 19% decrease in docetaxel clearance [13]. However, low concentrations of AAG have been shown to be an independent predictor of the severity of neutropenia [14] and improved survival in patients with non-small cell lung cancer [15]. Regarding the latter observation, high AAG concentrations are associated with inflammation in cancer patients and may be a marker for advanced disease and worse prognosis [16,17].

Unbound docetaxel pharmacokinetic parameters are summarized in Table 2. In several studies, mean unbound clearance values were 315 and 565 mL/min and varied up to 8.5-fold [11,18]. The pre-treatment unbound fraction expressed as a percentage was 4.7% (range, 1.2 – 8.65); however, docetaxel unbound fraction calculated as the ratio of $AUC_{unbound}/AUC_{total}$ (expressed as a percentage) was 5.7%, representing a 20% increase in fraction unbound compared to that measured in pre-treatment samples [18]. Consistent with this observation, complete concordance was not observed between pre-treatment fu and $AUC_{unbound}/AUC_{total}$ by linear regression (R^2 = 0.4593) [18], indicating that accurate assessment of unbound docetaxel exposure cannot be determined from pre-treatment unbound fraction only.

The discordance between docetaxel pre-treatment unbound fraction and $AUC_{unbound}/AUC_{total}$ suggests a time-dependent change in unbound fraction after the start of the infusion, which may be due to the formulation vehicle, polysorbate 80. *In vitro*, increasing concentrations of polysorbate 80 from 0 to 0.5 µL/mL resulted in a 13% increase in docetaxel unbound fraction [11]. When assessed in cancer patients treated with docetaxel, the unbound fraction of docetaxel increased during the docetaxel infusion and declined post-infusion to values close to baseline unbound fraction at 3 to 7 hours post-infusion [18]. The mechanistic basis for the influence of polysorbate 80 on docetaxel fraction unbound during the infusion is unknown, but it is possible that polysorbate 80 or its metabolites (e.g., oleic acid) interfere with the

binding of docetaxel to albumin and AAG and lead to temporary increase in the unbound fraction.

Similar to docetaxel unbound fraction, polysorbate 80 concentrations increased during the docetaxel infusion, and declined post-infusion with concentrations below the assay limit of detection at 3 to 7 hours post-infusion (Figure 1). The parallel time profile for docetaxel unbound fraction and polysorbate 80 concentrations suggests a causative relationship similar to that observed from *in vitro* studies [11]. Polysorbate 80 pharmacokinetic parameters are summarized in Table 3. Measures of exposure to polysorbate 80 increased in near proportion with an increase in dose over the range of 650 to 1950 mg/m^2. Polysorbate 80 clearance was independent of administered dose and infusion duration (1-h versus 30-min), although most subjects received a 1-h infusion providing limited data to assess the effect of infusion duration on polysorbate 80 clearance. The disappearance of polysorbate 80 from plasma is characterized by a short terminal half-life of approximately 30 min. The volume of distribution at steady state for polysorbate 80 is approximately 5 to 6 L, indicating limited distribution of this excipient outside the blood volume.

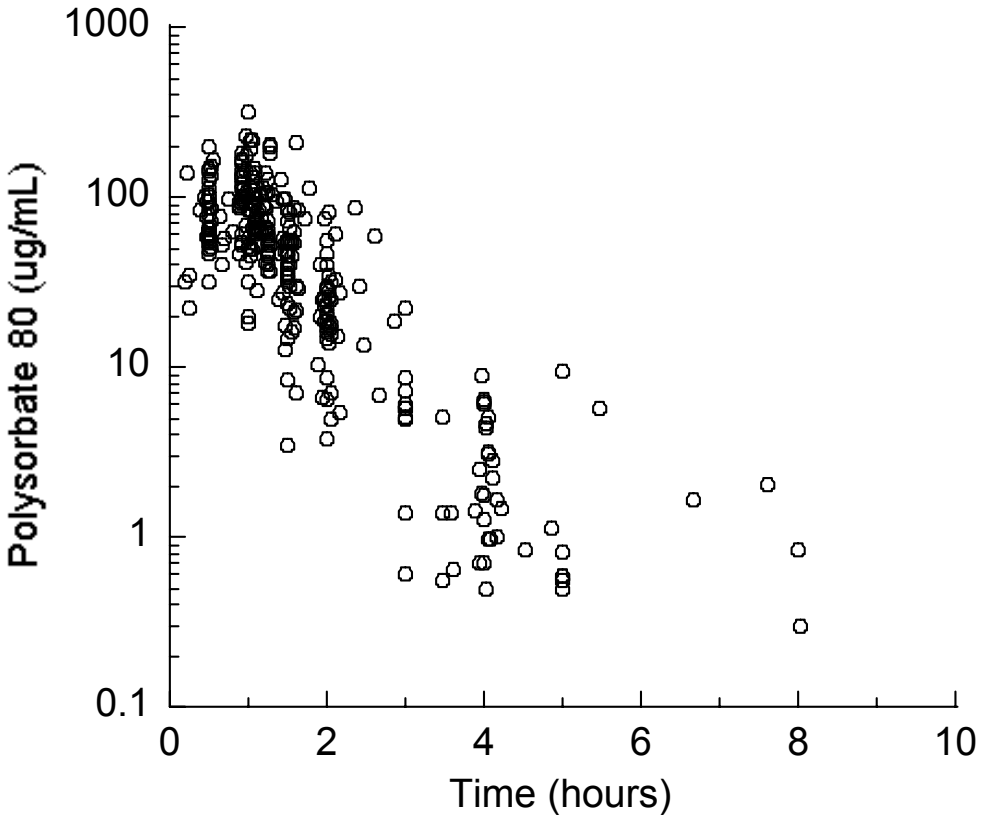

Figure 1. Concentration-time profiles of polysorbate 80 after dose adjustments to 1000 mg absolute dose. Data are from reference [1].

Table 2. Unbound docetaxel pharmacokinetic parameters[a]

Dose (mg/m^2)	n	C_{max} (ng/mL)	AUC (ng/mL×h)	CL (L/h)	Pre-treatment fu (%)	$AUC_{unbound}$/ AUC_{total} (%)	Reference
75 - 85	23	NA	NA	315 ± 71.4 (188 – 446)	5.49 ± 1.01 (3.93 – 7.93)	6.00 ± 1.03 (4.12 – 8.06)	[11]
75	40	233 ± 101 (57.4 – 489)	321 ± 143 (96.4 – 584)	565 ± 329 (207 – 1763)	4.72 ± 1.79 (1.19 – 8.63)	5.66 ± 1.40 (3.41 – 8.59)	[18]

[a]Data represent mean ± standard. Abbreviations: Cmax, maximum plasma concentration; AUC, area under the plasma concentration-time curve from time zero to infinity; CL, systemic clearance; fu, unbound fraction; n, number of patients studied; NA, not available.

Table 3. Polysorbate 80 pharmacokinetic parameters[a]

Docetaxel dose mg/m^2)[b]	Polysorbate 80 dose (mg/m^2)	n	C_{max} (mg/mL)	AUC (mg/mL×h)	CL (L/h)	V_{ss} (L)	$t_{1/2,z}$ (h)	Reference
25	650	3	0.139 ± 0.0174	0.200 ± 0.0799	7.63 ± 2.72	8.30 ± 3.72	0.640 ± 0.071	[1]
30	780	1	0.160	0.213	8.34	4.56	0.421	[1]
35	910	4	0.181 ± 0.0645	0.180 ± 0.0417	8.58 ± 1.63	7.63 ± 2.44	0.568 ± 0.114	[1]
50	1300	7	0.302 ± 0.0516	0.385 ± 0.133	6.53 ± 2.03	6.25 ± 1.93	0.739 ± 0.328	[1]
75	1560	40	0.451 ± 0.221	0.528 ± 0.217	8.18 ± 3.66	NA	NA	[18]
100	1950	19	0.457 ± 0.233	0.576 ± 0.252	7.27 ± 3.22	2.87 ± 1.47	0.629 ± 0.246	[1]

[a] Data represent mean ± standard deviation. [b]Docetaxel administered as a 1-h intravenous infusion, except at the 35 mg/m^2 dose level, which was infused over 30 min. Abbreviations: Mean values ± standard deviation; C_{max}, maximum plasma concentration; AUC, area under the plasma concentration-time curve from time zero to infinity; CL, systemic clearance; V_{ss}, volume of distribution at steady state; $t_{1/2,z}$, half-life of the terminal disposition phase; n, number of patients studied.

AAG concentration has been shown to be significantly correlated with pre-treatment docetaxel unbound (free) fraction both *in vitro* [9] and *in vivo* [11,18], with higher free fraction values in the presence of lower AAG concentrations. Based on these findings, and the observation of an association between low concentrations of AAG and the incidence of the severity of neutropenia [14], it has been postulated that low AAG concentrations will result in higher exposure to unbound docetaxel. However, in a recent analysis of 40 cancer patients receiving docetaxel 75 mg/m^2, where AAG concentrations ranged from 60 to 257 mg/dL (mean, 141 mg/dL) and unbound docetaxel AUC ranged from 96.4 to 584 μg/mL×h (mean, 321 μg/mL×h), no relationship was observed between AAG and unbound docetaxel AUC by linear regression analysis (R^2 = .028, P = .2994) (data from reference [18]). This is not unexpected because unbound drug clearance is used to examine factors other than protein-binding that influence clearance (e.g., drug metabolizing enzyme activity) by eliminating the confounding affects of binding to proteins or other macromolecules in the systemic circulation. In addition, because total docetaxel clearance decreases with increasing AAG concentrations, but free fraction also decreases with increasing AAG concentrations, clearance of unbound drug and AUC of free drug, may be unaffected by varying AAG concentrations, as was shown [18]. A multiple linear regression analysis was performed to determine the influence of other factors including age, liver function, and polysorbate 80 exposure on unbound docetaxel clearance. Only polysorbate 80 AUC and liver impairment, defined as concurrent elevations in liver transaminases ≥ 1.5 times the upper limit of institutional normal (× ULN) and alkaline phosphatase ≥ 2.5 × ULN or elevated total bilirubin ≥ 1.5 × ULN, were retained in the final model and explained 32% of the variation in unbound clearance among patients. In this analysis, higher polysorbate 80 AUC values were associated with lower unbound clearance values. One possible explanation for this observation is that polysorbate 80 or its metabolites (e.g., oleic acid) inhibit cytochrome P-450 3A [19,20] and/or P-glycoprotein [21] mediated elimination of docetaxel.

Elimination pathways

Pharmacogenetics of metabolism and transport

Based on catalytic potential, CYP3A4 and CYP3A5 are the most important among the four members of the CYP3A subfamily (CYP3A4, CYP3A5, CYP3A7, CYP3A43) involved in docetaxel metabolism and elimination [22]. The major docetaxel metabolites and less than 10% of the parent drug are excreted into the feces, whereas

total urinary excretion is less than 10% [6]. The metabolites demonstrate substantially reduced cytotoxic activity as compared to the parent drug, making biotransformation by CYP3A a major route of inactivation [23].

CYP3A4 is considered the major isoform expressed in adult livers and intestine in all racial/ethnic populations studied [24]. Over 30 single nucleotide polymorphisms (SNPs) in CYP3A4 have been published, most of which are very rare and unlikely to impact on CYP3A activity *in vivo* [25]. The best-characterized polymorphism, a promoter variant with an A to G transition at nucleotide -392 (CYP3A4*1B), was initially shown *in vitro* to have increased transcriptional activity [26]. This variant was not detected in 25 cancer patients of Asian descent receiving docetaxel therapy, precluding genotype-pharmacokinetic correlation analysis [27]. However, a recent report indicates no association between the CYP3A4*1B variant and CYP3A activity using the phenotypic probes midazolam clearance and the erythromycin breath test, under constitutive and induced (by pretreatment with rifampin) conditions in 57 healthy subjects including 23 Caucasian- and 34 African-Americans [28]. In line with this data, no association was observed between this variant and the erythromycin breath test in 118 patients with advanced cancer (n=106, wild type; n=12, heterozygous variant), which were predominantly of Caucasian descent [12].

In contrast to CYP3A4, CYP3A5 is polymorphically expressed in some adults, which varies by race/ethnicity. To date, 13 allelic variants involving both coding and intronic sequences have been identified [24]. A splice variant in intron 3 of CYP3A5 at nucleotide position 6986 (6986A>G, CYP3A5*3C) is the most common variant across all racial/ethnic populations, which results in the absence of functional CP3A5 protein [24,29,30]. Only individuals with at least one wild type allele at nucleotide position 6986 (CYP3A5*1) express large amounts of CYP3A5 protein [30]. The allele frequency of the CYP3A5*3C variant is 71 - 75% in East Asians, 55-65% in South Asians, 29-35% in Blacks, 89 - 94% in Caucasians, and 60 - 66% in Hispanics [24]. Goh et al. examined the association between the CYP3A5*3C variant and docetaxel and midazolam clearance in cancer patients of Asian descent [27]. No relationship was observed in this population, where 3, 13, and 9 patients had the wildtype (*1/*1), heterozygous variant (*1/*3), or homozygous variant (*3/*3) genotype, respectively. One recent study involving a predominantly Caucasian population of 67 cancer patients observed 1.7 folder higher midazolam clearance in 9 patients with the *1/*3 genotype compared to 58 patients with the *3/*3 genotype [31]. This data is not consistent with another recent study in a larger population of 121 patients with advanced cancer (n=2, *1/*1; n=19, *1/*3; and n=100, *3/*3 genotype), where no

association was observed between CYP3A5*3C genotype and the erythromycin breath test. The discordance in results from these studies assessing genotype-phenotype relationships could be explained when the CYP3A probe drug is considered: docetaxel and erythromycin are deemed CYP3A4 substrates but poor substrates for CYP3A5, whereas midazolam is a substrate for both CYP3A4 and CYP3A5 (32). However, studies in healthy subjects involving populations of similar sample size and diverse race/ethnicity including African Americans [28], Asians [33] and Caucasians [28,34], showed no association between CYP3A5*3C genotype and midazolam clearance. In addition, the one study showing an association between CYP3A5*3C genotype and midazolam clearance used a limited sampling scheme involving one plasma sample at 4-h, whereas the other studies utilized intensive sampling schemes for midazolam [28,33,34]. Furthermore, CYP3A5 genotype-phenotype relationships are more likely to be observed in healthy volunteers compared to cancer patients because interpatient variation in CYP3A activity is much lower in the former (4- versus 14-fold) [12,28], most likely due to the absence of comorbidities and environmental influences (e.g., drug-drug and drug-herb interactions) that would confound genotype-phenotype relationships. Another explanation for the lack of CYP3A5 genotype-phenotype associations may be due to the administration of drug doses that produce plasma concentrations well below the threshold for CYP3A4 enzyme saturation. A recent study showed a dose-dependent association between CYP3A5*3C genotype and plasma concentrations of the investigational antibiotic ABT-773, where drug exposure was higher in CYP3A5 negative individuals (those that were homozygous variant [*3/*3 genotype]) only at the highest dose administered (450 mg versus 150 or 300 mg) [35]. When CYP3A4 drug metabolizing capability becomes saturated, individuals that express CYP3A5 (those that are wild type or heterozygous variant with at least one *1 allele), may metabolize the compound more quickly because of the additional activity of a second major CYP3A enzyme.

In addition to CYP3A-mediated elimination, docetaxel is also a substrate for the efflux membrane-localized transporter P-glycoprotein, encoded by the ABCB1 gene [36]. This protein is expressed on the apical surface of the small-intestinal epithelium and the biliary surface of hepatocytes [37]. Preclinical observations in mice lacking mouse Abcb1 have indicated that docetaxel AUC following intravenous administration was not different in knockout and wildtype mice, indicating that metabolism rather than transport is the prominent elimination pathway for docetaxel [38]. However, ABCB1-mediated intestinal secretion has been demonstrated to contribute significantly to fecal elimination of taxane drugs both in mice lacking Abcb1 [39] and

humans [40], without changing plasma concentrations.

Recent re-sequencing of the ABCB1 gene has revealed a number of allelic variants that affect activity and/or expression of the encoded protein in vivo [37]. In total, 50 SNPs and 3 insertion/deletion polymorphisms in ABCB1 have been reported [41]. The most common ABCB1 variants are a silent mutation at the 3435C>T transition in exon 26 (ABCB1*6), a nonsynonymous ABCB1 2677G>T/A variant (ABCB1*7) resulting in an amino acid change in exon 21 (Ala893Ser/Thr), and a silent ABCB1 1236 C>T variant (ABCB1*8). The homozygous T allele of ABCB1*6 has been associated with reduced protein expression in different human tissues, including duodenum biopsies and CD56+ natural killer cells, although an association with higher protein expression has been observed and this relationship is somewhat controversial [37]. The frequency of this allele varies extensively with race/ethnicity as follows: 37 - 66% in Asians, 10 - 27% in Blacks, and 46 - 59% in Caucasians [37]. No association was observed between ABCB1*6 genotype and docetaxel and midazolam clearance in 25 cancer patients of Asian descent [27], consistent with similar findings in healthy subjects receiving intravenous midazolam [28,34] and the erythromycin breath test [28]. Close linkage of the ABCB1*6, ABCB1*7, and ABCB1*8 polymorphisms has been found, which makes a variant haplotype termed ABCB1*2 [42]. Recent work indicates that the use of the ABCB1*2 haplotype is superior to single SNP analysis to predict substrate drug pharmacokinetics [43-45] and this remains to be assessed for docetaxel. It is noteworthy that ABCB1 variants may be associated with toxicity and response unrelated to the potential influence on docetaxel plasma pharmacokinetics.

CYP3A phenotype-pharmacokinetic relations

Several studies have assessed the ability of CYP3A phenotyping probes to predict total docetaxel clearance. One study involved the administration of docetaxel 100 mg/m^2 to 21 patients with sarcoma and a pre-treatment CYP3A phenotypic test using the erythromycin breath test [46]. A strong linear correlation was observed between CYP3A activity, assessed as the log transformation of % ^{14}C dose exhaled during the first hour calculated from a 20-min sample and the equation from Wagner [47], and docetaxel clearance, expressed in units of $L/h/m^2$ (R^2 = .67, P = .0001). However, one patient had greatly reduced CYP3A activity and docetaxel clearance. Inclusion of this patient increased the range of docetaxel clearance values from 3- to 6-fold in the studied population and most likely improved the correlation between CYP3A activity and clearance. In another study, urinary 6-beta- hydroxycortisol excretion was used to determine the association between CYP3A activity and docetaxel clearance in 29

cancer patients with advanced NSCLC from Japan receiving docetaxel 60 mg/m^2 [48]. In the population studied, a strong linear correlation was observed between CYP3A activity and docetaxel clearance (R^2 = .73) and values for these parameters varied approximately 4.5- and 3.5-fold, respectively. In a third study, CYP3A activity, determined by assessment of midazolam clearance after intravenous injection, was correlated with docetaxel clearance (R^2 = .36, P = .0005) in 31 cancer patients receiving docetaxel 75 mg/m^2, where CYP3A activity and drug clearance varied approximately 4.5- and 3.5-fold, respectively [27]. Finally, a pilot study was recently reported suggesting potential usefulness to using dexamethasone as a CYP3A4 phenotyping probe for docetaxel clearance in a group of 23 cancer patients [49]. Regression equations for prediction of docetaxel clearance derived from these studies involving a small number of patients may limit the application of these phenotypic tests for prediction of docetaxel clearance in larger populations, where docetaxel clearance may vary up to 7- to 10-fold [50,51].

A population pharmacokinetic approach was recently utilized to identify patient covariates that significantly influence the clearance of docetaxel when administered weekly at a dose of 40 mg/m^2 to 54 patients with advanced cancer [52]. Pharmacokinetic studies included sparse sampling (2 to 3 samples) and CYP3A activity was phenotyped pre-treatment using the erythromycin breath test. Significant relationships were found between docetaxel clearance and the erythromycin breath test parameter, $1/T_{max}$ (R2 = .15, P = .0003), and liver function enzymes (alkaline phosphatase, R2 = .22, P = .0002; aspartate amino transferase, R2 = .21, P = .0005; alanine amino transferase, R2 = .19, P = .001; gamma glutamyl aminotransferase, R2 = .12, P = .006). Docetaxel clearance was best described by the following equation: clearance = 21.51 + 217 ($1/T_{max}$) - 0.13 (ALT). The ability of this equation to a priori predict docetaxel clearance and achieve a target AUC will require prospective evaluation.

Clinical Pharmacokinetics

Weekly administration schedules

Weekly administration of docetaxel has demonstrated comparable efficacy together with a distinct toxicity profile with a reduction in acute toxicity and only mild myelosuppression [3-5,53]. The pharmacokinetics of weekly docetaxel administered at a dose of 35 mg/m^2 and on 3-weekly schedules for comparison are summarized in Table 4 [2]. Mean ± SD docetaxel clearance values were similar with weekly and 3-

weekly schedules (overall means, 25 ± 7.7 versus 23.7 ± 7.9 L/h/m^2; P = .5467). Values for half-life were also similar with both schedule of administration (overall means, 16.5 ± 11.2 and 17.6 ± 7.4 h; P = .6990). On average, Cmax values with weekly docetaxel 35 mg/m^2 over 30 min were comparable to those achieved with docetaxel 75 mg/m^2 over 1 h, but less than those achieved with 100 mg/m^2 over 1h. On the other-hand, estimated AUC during 3 weeks of treatment is larger following 35 mg/m^2 weekly (4.44 ± 1.24 μg/mL×h) compared to 60 mg/m2 (2.85 ± 1.40 μg/mL×h) and 75 mg/m^2 (3.05 ± 0.85 μg/mL×h) administered 3-weekly, but in the range of that achieved at 100 mg/m^2 (5.62 ± 2.12 μg/mL×h) given 3-weekly. Given the differences in the incidence of severe myelosuppresion between the two schedules of administration and the exposure-toxicity relationships defined previously for 3-weekly regimens (see section Pharmacokinetic-Pharmacodynamic Relationships), pharmaco-kinetic data showing similar exposure (AUC) over a 3-week period for both weekly and 3-weekly regimens suggests exposure-toxicity relationships for 3-weekly administration does not apply to weekly regimens.

Extended sampling schemes

The pharmacokinetics of docetaxel have been described utilizing extended plasma sampling schemes to days 8 to 22 post –treatment and a sensitive analytical method based on tandem mass spectrometry (Table 4) [2]. With extended sampling, clearance is approximately 18% lower and the terminal half-life is approximately 5-fold longer resulting in measurable concentrations for the entire dosing interval (Figure 2). At 35 mg/m^2, the mean ± SD docetaxel concentration on day 8 was 1.1 ± 0.51 nM; at 75 mg/m^2, concentrations on days 8, 15, and 22 were 1.8 ± 0.53 nM, 0.83 ± 0.31 nM, and 0.58 ± 0.099 nM, respectively [2]. These data demonstrate prolonged circulation of low nanomolar concentrations of docetaxel during each dosing interval, which may contribute to docetaxel mechanisms of action including suppression of microtubule dynamics and tumor angiogenesis [54-56].

Table 4. Comparative total docetaxel pharmacokinetic parameters for weekly and 3-weekly administration schedules using 24-h and extended sampling schemes[a]

Schedule	Concurrent Drug	n	Parameter			
			C_{max} (μg/mL)	AUC (μg/mL×h)	CL (L/h/m^2)	$t_{1/2,z}$ (h)
24-h Sampling						
Weekly						
35 mg/m^2	None	8	1.85 ± 0.73	1.32 ± 0.42	29.1 ± 10.2	15.6 ± 12.0
35 mg/m^2	Irinotecan	12	1.99 ± 0.52	1.59 ± 0.40	22.5 ± 4.2	17.1 ± 12.7
	ALL DATA				25.2 ± 7.7	16.5 ± 11.2
3-Weekly						
60 mg/m^2	Doxorubicin	10	1.55 ± 0.41	2.85 ± 1.40	25.0 ± 9.7	18.0 ± 9.3
75 mg/m^2	None	9	2.18 ± 0.71	3.05 ± 0.85	25.8 ± 6.3	17.5 ± 7.3
100 mg/m^2	None	7	4.15 ± 1.35	5.62 ± 2.12	19.6 ± 5.6	17.2 ± 6.2
	ALL DATA				16.5 ± 11.2	17.6 ± 7.4
Extended Sampling						
Weekly						
35 mg/m^2	None	6	N/A	1.63 ± 0.30	22.0 ± 3.8	61.3 ± 12.5
35 mg/m^2	Irinotecan	10	N/A	1.87 ± 0.37	19.4 ± 3.7	60.4 ± 24.6
3-Weekly						
60 mg/m^2	Doxorubicin	5	N/A	3.74 ± 0.77	16.6 ± 3.6	135 ± 21.9
75 mg/m^2	None	9	N/A	3.41 ± 0.98	23.2 ± 5.7	91.7 ± 32.1
100 mg/m^2	None	4	N/A	7.87 ± 2.90	14.4 ± 6.4	120 ± 80.5

[a] Data represent mean ± standard deviation. Data were obtained from reference [2]. Docetaxel administered as a 30-min infusion with weekly schedules and as a 1-h infusion with 3-weekly schedules.

Abbreviations: Mean values ± standard deviation; C_{max}, peak plasma concentration; AUC, area under the plasma concentration-time curve from time zero to infinity; CL, systemic clearance; $t_{1/2,z}$, half-life of the terminal disposition phase; n, number of patients studied; N/A, not applicable.

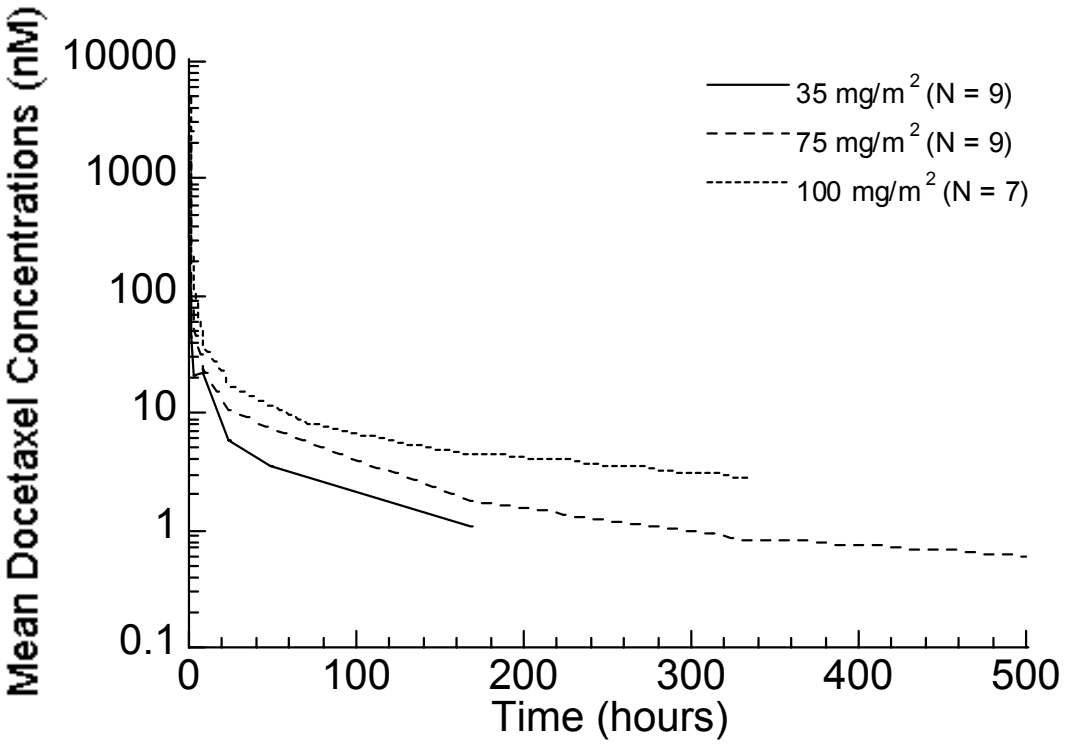

Figure 2. Mean docetaxel total plasma concentrations following administration of docetaxel 35 mg/m^2 (solid line); 75 mg/m^2 (dashed line); and 100 mg/m^2 (dotted line). The following equation converts docetaxel concentrations in units nM to μg/mL: concentration (nM) = concentration (μg/mL) × 1237.79. Data are from reference [2].

Elderly

The first evaluation to assess the influence of elderly age on the pharmacokinetics of docetaxel was from a large (N = 547) population pharmacokinetic analysis of 3-weekly docetaxel, which showed minimal effect of age on docetaxel clearance; a 71-year old patient would be expected to have a 6.7% decrease in docetaxel clearance [13]. More recently, a population pharmacokinetic analysis of sparse concentration-time data (2 to 3 samples) following administration of weekly docetaxel 40 mg/m^2 to 54 patients showed no effect of age on docetaxel clearance [52]. The effect of age on docetaxel pharmacokinetics has also been prospectively evaluated in 20 patients aged ≥ 65 years and 20 patients aged < 65 years following administration of 3-weekly docetaxel 75 mg/m^2 [51]. Docetaxel pharmacokinetic parameters including clearance, were similar in the elderly and younger age groups (mean ± SD, 30.1 ± 18.3 L/h versus 30.0 ± 14.8 L/h; P = .98). Combined, these results indicate that there is no

statistically significant change in the pharmacokinetics of docetaxel in older and younger patients. Consistent with the pharmacokinetic data, CYP3A activity assessed using the erythromycin test was similar in 34 patients aged ≥ 70 years compared to 82 patients aged < 70 years [12]. Docetaxel administered in weekly schedules at lower doses has been found to be both efficacious and generally well tolerated in elderly patients [57-59]. However, there is general reluctance to administer docetaxel 3-weekly to elderly patients due to the prevalence of neutropenia with docetaxel therapy [60], although this has not been adequately evaluated in a clinical trial as weekly schedules have.

Organ dysfunction

The pharmacokinetic profile of docetaxel has been evaluated in patients with varying degrees of hepatic impairment. A population pharmacokinetic analysis of patients receiving docetaxel 75 to 100 mg/m^2 identified a subset of patients with mild hepatic impairment defined as total bilirubin < 1.5 × ULN, elevated transaminases ≥ 1.5 to ≤ 3.5 × ULN concurrent with increased alkaline phosphatase ≥ 2.5 to ≤ 5 × ULN [14]. These patients had a 27% reduction in total docetaxel clearance. Prospective evaluation of docetaxel in patients with moderate (total bilirubin ≥ 1.5 × ULN to < 3.0 × ULN with any transaminase and alkaline phosphatase elevations) and severe (total bilirubin ≥ 3.0 × ULN with any transaminase and alkaline phosphatase elevations) hepatic impairment has been performed [61,62], resulting in an overall reduction in docetaxel clearance of approximately 50% in both groups. As described below (section Pharmacokinetic-Pharmacodynamic Relationships), reduced docetaxel clearance is associated with an increase in the incidence and severity of side effects. Consequently, docetaxel dose reductions are required in patients with liver function impairment.

Extravascular routes of administration

Oral Administration

Despite all kinds of dosing schedules tested to date, the total docetaxel dose that can be tolerated in any time period is approximately the same. Prolonged infusion times might theoretically improve the efficacy of docetaxel, as cytotoxicity of tubulin poisons is S-phase specific [63], although the relevance of this principle for docetaxel, given its prolonged terminal disposition half-life, is unclear. From in vitro models it was known that tumor cells are more responsive to increased exposure duration than to further escalation of drug concentrations above a plateau level for taxane drugs [63].

The availability of a suitable oral formulation of a taxane would allow for more convenient use of prolonged dosing schedules. In addition, the oral administration of drugs is usually considered to be more convenient and practical, drugs can be administered on an outpatient basis or at home, increasing convenience and patient quality of life, and possibly decreasing the costs by reducing hospital admissions [64]. Unfortunately, most taxanes, including docetaxel, have a low (less than 10%) and highly variable oral bioavailability [39]. In view of the narrow therapeutic window of docetaxel, this variable bioavailability may result in unanticipated toxicity or decreased efficacy when therapeutic plasma concentrations are not achieved. There is a number of important mechanisms that can explain the variable and/or low oral bioavailability, including high affinity for drug transporters in the gastrointestinal tract such as P-glycoprotein, which limits absorption, and high extraction of the drug by extensive metabolism in the intestine and/or liver (first-pass effect) by CYP3A [65]. Other important factors include structural instability and limited solubility of docetaxel in the gastrointestinal fluids, as well as increased potential for drug-drug and drug-food interactions, motility disorders or obstructive disorders, and existence of nausea and vomiting [66].

The overlap in substrate selectivity for ABCB1 and CYP3A, combined with their tissue localization, suggests that these two proteins cooperate and constitute a major absorption barrier against toxic xenobiotics such as docetaxel [67]. Cummins *et al*. have confirmed this and showed that ABCB1 can affect intestinal drug metabolism (especially by the isoenzyme CYP3A4) by controlling the access of a drug to the intracellular metabolizing enzyme system [68]. The proposed interplay between these proteins in the intestine and, consequently, the combined activity of CYP3A and ABCB1, may be major determinants of limited and/or variable oral bioavailability of shared substrate drugs.

Because of encouraging results obtained with paclitaxel administered orally in combination with ABCB1 inhibitors [69], studies in mice have also been performed recently with docetaxel. These studies performed in mice lacking functional ABCB1 confirmed that this protein plays a role in the low bioavailability of docetaxel. Furthermore, the AUC of oral docetaxel increased 9-fold by coadministration with cyclosporine, a competitive inhibitor of ABCB1 and CYP3A4 [38]. In addition, the coadministration of docetaxel and ritonavir, a very potent modulator of CYP3A4 function with minor ABCB1 inhibitory properties, was tested in a murine model. In this study, co-administration with ritonavir resulted in an increase in the apparent oral bioavailability of docetaxel from 4% to 183%, suggesting that extensive first-pass

metabolism is the predominant mechanism responsible for poor intestinal absorption of oral docetaxel in mice [38]. Based on these findings, it has been proposed that simultaneous inhibition of ABCB1 and CYP3A4 function may be a strategy to improve the systemic exposure to oral docetaxel.

A clinical proof-of-concept study of oral docetaxel was recently carried out in patients with solid tumors. Patients received one course of oral docetaxel at 75 mg/m^2 with or without a single oral dose of cyclosporine at 15 mg/kg. Pharmacokinetic results showed that coadministration of oral cyclosporine resulted in a 7.3-fold increase of the systemic exposure to docetaxel. The apparent bioavailability of oral docetaxel increased from 8% to 90% in the absence and presence of cyclosporine, respectively [70]. Although it is likely that this increase in systemic exposure can be explained by inhibition of both CYP3A4 and ABCB1 by cyclosporine, the contribution of each of these proteins is unknown. Interestingly, the effect of cyclosporine co-administration on the bioavailability of docetaxel was less pronounced in mice [38] compared with humans [70], but the reasons for the rather modest effect in mice are not clear.

The same investigators also performed a clinical phase II study in patients with advanced breast cancer given oral docetaxel in the presence of cyclosporine [71]. Cyclosporine 15 mg/kg was given 30 minutes prior to a weekly oral docetaxel dose of 100 mg, which produced a docetaxel AUC that was equivalent to a weekly intravenous dose of 40 mg/m^2. In addition, the interpatient and intrapatient variabilities in the AUC of docetaxel after oral administration were in the same range as that observed after intravenous administration of docetaxel (29 to 53%) [6]. However, the current availability of novel highly cytotoxic taxanes that are not substrates for ABCB1 and have good oral bioavailability, such as MAC-321 [72], 10-deoxy-10-C-morpholinoethyl-docetaxel [73], IDN 5390 [74], IDN-5109 [75], BMS-275183 [76], as well as other orally-available tubulin-interacting agents, such as epothilone and its derivatives [77] suggests that further evaluation of oral docetaxel is not warranted.

Intraperitoneal Administration

Intraperitoneal administration of docetaxel has also been studied recently and may have some potential advantages over the intravenous route. The major goal of intraperitoneal therapeutic strategies is to expose tumors within the peritoneal cavity to higher concentrations of antineoplastic agents for longer periods of time than can be achieved by systemic drug administration. Treatment with docetaxel given intraperitoneally is theoretically particularly attractive in patients with ovarian

carcinoma, since the drug has proven single agent activity in this disease [78].

Animal models evaluating the pharmacokinetics and tissue distribution of intraperitoneal versus intravenous docetaxel [79,81] have shown promising results and have led to the initiation of a number of clinical trials. De Bree *et al* have evaluated the pharmacokinetics and toxicity of docetaxel (75 mg/m^2) after continuous hyperthermic (41-43°C) peritoneal infusion in patients with gynecological malignancies [82,83]. In addition, a phase I trial was recently conducted in patients with peritoneal carcinomatosis in order to determine the pharmacokinetic characteristics and potential pharmacological advantage of intraperitoneal docetaxel [84]. Across all dose levels the mean AUC ratio ($AUC_{intraperitoneal}/AUC_{plasma}$) was approximately 200 in both studies, indicating high local drug concentrations and low systemic drug exposure. It is possible, as has been observed with paclitaxel [85], that with this route of drug administration, the presence of polysorbate 80 as an integral component of the clinical formulation may actually be advantageous as it prolongs exposure to the tumor cells and reduces transport across the peritoneal/blood barrier. Further phase II/III trials should determine if this favorable exposure ratio translates in an improvement of treatment outcome.

Drug interactions

Drug interactions may arise as a result of altered pharmacodynamics or pharmacokinetics of the drugs involved. In the case of the latter, this is usually due to modification of tissue disposition and metabolism of the drugs. These phenomena are of particular importance in cancer chemotherapy when cytotoxic agents are used, because of the increased risks of severe toxicity. Most of the data currently available to evaluate potential drug interactions with docetaxel come from clinical trials of docetaxel given in combination with one or more other anticancer agents. Although in most cases only limited information is available, some preliminary conclusions can be drawn.

Pharmacokinetic interaction studies with docetaxel have been performed with cisplatin either given alone or in combination with 5-fluorouracil [86], and indicated unaltered disposition profiles of both docetaxel and 5-fluorouracil at any dose level or sequence tested [87]. Likewise, no interactions have been reported when docetaxel is coadministered with carboplatin [89], epirubicin [90], doxorubicin [91], vinorelbine [92], gemcitabine [93], methotrexate [94], 5-fluorouracil [95], capecitabine [96], ifosfamide [97], estramustine [98], and irinotecan [2,99].

In contrast to the findings of Itoh *et al* [91], a recent pharmacokinetic evaluation involving a multivariate analysis in a large cohort of cancer patient revealed a 20%

decrease in the clearance of docetaxel in the presence of doxorubicin [50]. Although the mechanistic basis for this interaction is presently unclear, it is important to note that this interaction might be clinically significant in view of the notion that a 25% decrease in docetaxel clearance was previously shown to increase the odds for development of febrile neutropenia [14]. Furthermore, it provides an explanation for the severe hematological toxicity observed in regimens combining doxorubicin with docetaxel [50].

It has been suggested that an interaction or sequence-dependent effect is present for the combination of topotecan and docetaxel [100]. Administration of topotecan on days 1 and 4 and docetaxel on day 4 resulted in an approximately 50% decrease in docetaxel clearance [101]. As both drugs are metabolized by CYP3A, competition might occur when these drugs are given sequentially, and as a result, the clearance of docetaxel might be decreased. A similar interaction resulting in decreased clearance of docetaxel has been described in patients receiving concomitant administration of pegylated liposomal doxorubicin [102,103]. To date, no other cytotoxic drug has been shown to affect the pharmacokinetics of docetaxel.

Although not evaluated clinically, it is likely that anticonvulsants, phenytoin and phenobarbital in particular, will interact with docetaxel through induction of the docetaxel metabolic pathway. CYP3A4, in particular, has increased expression when patients are treated with these compounds. This type of interaction might be of major importance since it suggests that anticonvulsants will greatly reduce the potential antitumor effects of docetaxel. Besides potent inhibitors of CYP3A like ketoconazole [104], other well established CYP3A-inducing and inhibiting compounds (http://medicine.iupui.edu.flockhart/), dietary supplements and food products (e.g., grapefruit juice) and herbal preparations (e.g., St. John's wort and echinacea), harbor the potential to induce pharmacokinetic interactions with docetaxel.

In line with preclinical data obtained in mice lacking ABCB1 [38], specific inhibitors of P-glycoprotein like R101933 [105,106] have been demonstrated not to interfere with the plasma pharmacokinetics of docetaxel. It is noteworthy, however that certain modulators like GF120918 (elacridar) can increase the accumulation of docetaxel in normal brain tissue without simultaneously having significant effects on systemic drug exposure [107], suggesting that certain pharmacokinetic processes and side effects (e.g., central nervous system toxicity) cannot be predicted based on plasma concentration measurements alone.

Pharmacokinetic-Pharmacodynamic Relationships

Docetaxel's pharmacokinetic profile is characterized by substantial interpatient variability. In a population pharmacokinetic study of more than 600 patients receiving docetaxel 75 to 100 mg/m^2 that used a limited sampling scheme with the last sampling time point at 24 hours post-infusion, the median clearance was 36 L/h (5% to 95% percentile: 17-59 L/hr), representing approximately 3.5-fold variation in this population [14]. In a more recent study where patients received docetaxel 75 mg/m^2 and an extended sampling scheme to day 8 post infusion was utilized, approximately 10-fold variation in drug clearance was observed when patients with outlier values were not excluded from the analysis [51]. The observed large pharmacokinetic variability for docetaxel has important clinical consequences. In the aforementioned population pharmacokinetic study, decreased docetaxel clearance was associated with an increased frequency of grade 4 neutropenia and febrile neutropenia; a 50% decrease in docetaxel clearance increased the odds of developing grade 4 neutropenia and febrile neutropenia by 430% and 300%, respectively [14]. Even a 25% decrease in docetaxel clearance was associated with a 150% increase in the odds of developing febrile neutropenia [14]. In a subset of 180 patients with non-small cell lung cancer treated with docetaxel 100 mg/m^2 in phase II studies, docetaxel plasma AUC was the only significant predictor of severe toxicity including febrile neutropenia, infection, grade 3/4 mucositis, grade 3/4 diarrhea, and severe asthenia; in 13.9% of patients experiencing at least one severe adverse event, a high AUC was associated with increased probability of experiencing any of the severe toxicities [15]. Docetaxel exposure has also been related to treatment efficacy. AUC was a significant predictor of time to tumor progression in non-small cell lung cancer [14], and underdosing (e.g., lower docetaxel AUC due to increased clearance) was associated with a worse time to progression and time to death [108].

More recently, the association between exposure to unbound docetaxel and neutropenia in cancer patients was examined [18]. Docetaxel was administered once every 3 weeks at a dose of 75 mg/m^2 to 49 patients with normal liver function (N = 40) or mild elevations in liver function tests (N=9), or at 50 mg/m^2 to patients with moderate elevations in liver function tests (N=6). Patients with moderate liver impairment received a reduced dose of docetaxel to achieve AUC values similar to those with normal liver function or mild elevations in liver parameters receiving docetaxel 75 mg/m^2; as such, unbound and total AUC values were similar in the three groups and AUC values at the different dose levels were combined to assess the

relation between docetaxel unbound and total exposure and neutropenia. From this analysis, unbound docetaxel AUC was better correlated than total AUC with both percentage decrements in absolute neutrophil count (P=.002 versus P=.029) and worst grade of neutropenia (P=.013 versus P = .220), where higher exposure was associated with worse hematological toxicity. It was concluded that exposure to unbound docetaxel is more closely related to drug-induced hematologic toxicity than total drug and should be considered for future pharmacologic investigations.

Conclusion

Docetaxel, because of its broad spectrum of antitumor activity, is clearly one of the most important new anticancer drugs developed in the last few decades. The clinical pharmacokinetic behavior of docetaxel has been explored extensively in recent years, and the generated information has been of fundamental importance in the understanding of the clinical effects of this agent. In addition, a wealth of information has become available that has yielded valuable insight into the mechanism of action, the mechanisms of tumor resistance, toxicities, and considerations of dosage and schedule and route of drug administration. Many of these studies have been made possible by the development of selective analytical methodologies to specifically monitor the parent drug, with sufficient sensitivity to detect the compounds at concentrations achieved after therapeutic dosing. However, only through further investigations that may allow better definition of the biochemistry and pharmacokinetics of docetaxel can the rational optimization of therapy involving this agent be achieved. This need has become even more important in light of the current clinical use of docetaxel in combination with other antineoplastic drugs or agents specifically administered to modify docetaxel-induced toxicity profiles. In this respect, the use of mathematical models to predict systemic exposure measures for docetaxel by application of limited-sampling strategies or population pharmacokinetic models, coupled with continued investigations into the role of individual enzyme-expression levels and detection of enzyme and transporter polymorphisms, will allow more rational and selective chemotherapy with this agent.

Finally, the drawbacks presented by the presence of polysorbate 80 in the clinically used formulation of docetaxel have instigated extensive research to develop alternative delivery forms, and currently, several strategies are in progress to develop a surfactant-free formulation of docetaxel [109]. For example, a recent dose-finding study with a new submicronic polysorbate 80-free dispersion formulation of docetaxel

has shown a lower incidence and severity of hematological and non-hematological toxicity (fluid retention) at equimolar doses compared to the current formulation of docetaxel that contains polysorbate 80 [110]. Likewise, the absence of the pharmaceutical excipient Cremophor EL in a novel nanoparticle-based albumin-bound formulation of the related agent paclitaxel (i.e., ABI-007; Abrexane) permits drug administration without premedication routinely used for the prevention of hypersensitivity reactions, as well as increases in the maximum tolerated dose as compared to paclitaxel formulated in Cremophor EL [111]. It is suggested that continued investigations into the role of pharmaceutical vehicles in taxane-related drugs should eventually lead to a more rational and selective chemotherapeutic treatment with these agents.

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Chapter 3

Role of Body-Surface Area in Dosing of Investigational Anticancer Agents in Adults: 1991 – 2001

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Although the prescribed dose of anticancer agents is most commonly calculated using body-surface area as the only independent variable, it has been shown that this approach results in a large interpatient variability in drug exposure. We retrospectively assessed the pharmacokinetics of 33 investigational agents, which were tested in phase I trials between 1991 and 2001, as a function of body-surface area in 1650 adult cancer patients. Twelve of the drugs were administered orally, 19 were administered intravenously, and two were administered by both routes. Body-surface area-based dosing was statistically significantly associated with a reduction in interpatient variability in drug clearance for only five of the 33 agents: DHA-paclitaxel, 5-fluorouracil/eniluracil, paclitaxel, temozolomide, and troxacitabine. These results do not support the use of body-surface area in dose calculations and suggest that alternate dosing strategies should be evaluated. We conclude that body-surface area should not be considered to determine starting doses in future phase I studies of investigational agents.

In clinical oncology, the traditional method by which individualized anticancer drug doses are determined uses body-surface area, because use of this measurement is thought to reduce the interpatient variability of drug exposure, and hence, drug effects [1]. The use of body-surface area measurements arose from the extrapolation of drug doses used in experimental animals to those considered safe as starting doses for human cancer patients in phase I clinical trials [1]. However, a rigourous scientific rationale for body-surface area-based dosing of anticancer drugs in adults is lacking, especially when one considers that the difference in size between mice and humans is far greater than the difference in size between individual patients. Although the primary objective of phase I trials is to evaluate drug toxicity, antitumor activity is usually a secondary objective of such trials. Other measures, such as drug clearance, have also been used as a surrogate marker of drug effects. However, it has been widely recognized that large interpatient variability in drug clearance exists despite the use of body-surface area in drug-dose calculations [2]. Indeed, for most drugs that are used in clinical practice today, clearance cannot be reliably predicted by body-surface area because other factors involved in drug disposition may be more important for clearance [1,3-5]. For example, several recent studies have highlighted the importance of genetic polymorphisms in drug-metabolizing enzymes and drug transporter proteins in explaining inter-individual pharmacokinetic variability [6-9]. As a follow up to a preliminary report by Grochow et al. [10], we have assessed the pharmacokinetics of 33 investigational agents in adult cancer patients as a function of body-surface area in

order to provide a pharmacokinetic rationale for the appropriate selection of starting doses for phase I evaluation.

Data were obtained from 1650 patients who were treated with 33 anticancer drugs (involving 21 classes of agents) that were developed in phase I trials over a 10-year period at three institutions. Twelve of the drugs were administered orally, 19 were administered intravenously, and two were administered by both routes. Detailed clinical and pharmacokinetic profiles for these agents have been described elsewhere [5,11-68]. All patients were at least 18 years old and had normal organ function, except for those enrolled in two studies that involved patients with varying degrees of renal and hepatic impairment [65,66]. Drug clearance was calculated by using either non-compartmental or compartmental analysis [69], and was expressed either as liters per hour (L/h) or as L/h normalized to body-surface area in meters squared ($L/h/m^2$). Interpatient variation in drug clearance was calculated by dividing the standard deviation by the mean and expressed as a percentage (i.e., the coefficient of variation [CV]). We used the following arbitrarily-defined criteria to determine if body-surface area-based dosing was statistically significantly associated with a reduction in interpatient variation in clearance: (*i*) a linear regression coefficient (R) ≥ .50; (*ii*) $P <$ .01; and (*iii*) a relative reduction in the variability of clearance ≥ 15%, which was calculated as according to the following formula: {[CV for clearance (L/h) - CV for clearance ($L/h/m^2$)] / [CV for clearance (L/h)]} × 100. All three criteria had to be met for the reduction to be considered statistically significant.

The median body-surface area for the entire patient population was 1.86 m^2 (interquartile range = 1.68 - 2.00 m^2) and the mean body-surface area was 1.86 m^2 (range = 1.25 - 3.06 m^2). The CV for clearance, the correlation between body-surface area and clearance, and relative reduction in variability for clearance for each of the agents are listed in Tables 1 and 2. For all but five agents (i.e., DHA-paclitaxel, 5-fluorouracil/eniluracil, paclitaxel, temozolomide, and troxacitabine), body-surface area-based dosing was not associated with a statistically significant reduction in the interpatient variability in drug clearance.

In the case of drugs for which renal function plays a principal role in drug elimination, BSA-based dosing may decrease variability in drug clearance between patients. For example, troxacitabine, an L-nucleoside analogue, and 5-fluorouracil co-administered orally with the dihydropyrimidine-dehydrogenase inactivator eniluracil, are primarily excreted in the urine as unchanged drugs (~60% unchanged and 75% unchanged, respectively).

*Table 1. Variability in drug clearance of orally-administered agents**

Agent	CV (%) for drug clearance/f (L/h)	CV (%) for drug clearance/f (L/h/m^2)	No. patients	R†	*P*‡	RIV (%)	Drug class	Reference
9-AC§	53.7	55.0	41	.04	.81	0	topoisomerase I inhibitor	[11,12]
BN80915	151	148	17	.05	.85	2.0	topoisomerase I inhibitor	[13]
Capecitabine	31.3	36.5	30	.07	.71	0	oral fluoropyrimidine	[14]
CS-682‖	59.3	54.1	42	.32	.04	8.8	cytidine analogue	[15]
DMDC§	67.1	63.9	140	.16	.06	4.8	cytidine analogue	[16]
Eniluracil/5-FU	30.9	26.3	36	.57	<.001	15	oral fluoropyrimidine	[17]
MMI270B	68.1	62.8	46	.44	.002	7.8	MMP inhibitor	[18,19]
Phenylbutyrate	36.5	39.6	19	.13	.60	0	HDAC inhibitor	[20]
PKI166	82.8	86.0	24	.03	.89	0	TK inhibitor	[21]
SCH66336	95.6	96.6	26	.12	.55	0	FT inhibitor	[22]
Temozolomide	20.0	13.0	24	.88	<.001	35	alkylating agent	[23]
Topotecan§	47.0	44.5	54	.34	.01	5.3	topoisomerase I inhibitor	[24-31]
R115777	61.4	60.8	29	.17	.37	0.98	FT inhibitor	[32]
ZD9331	66.7	71.2	42	.09	.60	0	TS inhibitor	[33]

*CV = coefficient of variation; drug clearance/f = apparent oral clearance; L/h = liters per hour; L/h/m^2 = L/h normalized to body-surface area in meters squared; RIV = relative reduction in variability for clearance; 9-AC = 9-amino-camptothecin; DMDC = 2'-deoxy-2'-methylidenecytidine; 5-FU = 5-fluorouracil; MMP = matrix metalloproteinase; HDAC = histone deacetylase; TK = tyrosine kinase; FT = farnesyltransferase; TS = thymidylate synthase.

†regression coefficient from the relationship drug clearance/f (L/h) = [slope of line • body-surface area (m^2)] + [y-intercept]; ‡ *P* value from the regression analysis; § compartmental analysis; ‖ dose-normalized area under the curve of the active metabolite 2'-cyano-2'-deoxy-1-β-D-arabino-pentofuranosyl cytosine.

*Table 2. Variability in drug clearance of intravenously administered agents**

Agent	CV (%) for drug clearance (L/h)	CV (%) for drug clearance (L/h/m^2)	No. patients	R†	*P*‡	RIV (%)	Drug class	Reference
9-AC§	29.9	27.6	12	.31	.32	7.7	topoisomerase I inhibitor	[34]
BMS181174	33.2	27.2	15	.55	.04	18	antitumor antibiotic	[35]
Carzelesin	84.5	92.8	27	.14	.50	0	DNA minor groove binder	[36]
CI-958	40.5	38.3	38	.33	.04	5.4	DNA intercalator	[37]
DHA-paclitaxel§	29.1	21.9	11	.66	.03	24	antimicrotubule agent	[60]
DHA-paclitaxel §#	43.7	32.5	22	.61	.003	26	antimicrotubule agent	[60]
Docetaxel§	36.0	34.7	168	.33	<.001	3.5	antimicrotubule agent	[14,38-42]
EMD121974	25.7	28.0	36	.03	.87	0	antiangiogenic agent	[43]
EO9§								
phase I	54.5	54.4	31	.01	.94	0.2	bioreductive alkylating agent	[44]
phase II	170	162	72	.17	.16	4.7	bioreductive alkylating agent	[45]
Irinotecan§	31.8	33.9	85	.16	.14	0	topoisomerase I inhibitor	[5,46,47]
GI147211§	32.8	34.2	85	.06	.60	0	topoisomerase I inhibitor	[48]
MTA	39.0	32.8	34	.42	.01	16	TS inhibitor	[49]
NX 211§	98.9	95.4	29	.35	.07	3.5	topoisomerase I inhibitor	[50]
Paclitaxel§‖								
1-h inf	41.9	34.3	34	.57	<.001	18	antimicrotubule agent	[51,52]
3-h inf	28.5	23.1	40	.45	.003	19	antimicrotubule agent	[51,53]
PNU152243	59.2	62.4	13	.13	.66	0	anthracycline	[54]

Table 2. – continued –

Agent	CV (%) for drug clearance (L/h)	CV (%) for drug clearance (L/h/m^2)	No. patients	R†	*P*‡	RIV (%)	Drug class	Reference
PNU159548	46.2	42.8	24	.36	.09	7.4	alkycycline	[55]
PNU166196	30.8	28.5	23	.23	.30	7.5	DNA minor groove binder	[56]
SAM486A	57.6	55.2	60	.16	.21	4.2	polyamine inhibitor	[57,58]
SN-38§¶	60.9	67.6	85	.22	.04	0	topoisomerase I inhibitor	[5,46,47]
TAS-103	39.7	36.8	36	.36	.03	7.3	topoisomerase I & II inhibitor	[59]
Topotecan§	97.3	94.5	82	.24	.03	2.9	topoisomerase I inhibitor	[61-64]
Topotecan§**	82.5	80.6	55	.10	.46	2.3	topoisomerase I inhibitor	[65,66]
Troxacitabine	31.4	24.4	39	.66	<.001	22	L-nucleoside analogue	[67]
UCN-01§	79.4	79.9	20	.003	.99	0	PKC inhibitor	[68]
UCN-01§#	51.8	51.6	20	.16	.50	0.4	PKC inhibitor	[68]

*CV = coefficient of variation; L/h = liters per hour; L/h/m^2 = L/h normalized to body-surface area in meters squared; RIV = relative reduction in variability for clearance; 9-AC = 9-amino-camptothecin; DHA = docosahexaenoic acid; TS = thymidylate synthase; PKC, protein kinase C.

†regression coefficient from the relationship drug clearance (L/h) = [slope of line • body-surface area (m^2)] + [y-intercept]; ‡ *P* value from the regression analysis; § compartmental analysis; ‖unbound drug levels; ¶ irinotecan metabolite, dose-normalized area under the conentration-time curve (× 1000); # volume of distribution at steady-state in L versus L/m^2; ** patients with impaired renal or hepatic function.

Normalization of drug dose to body-surface area was associated with 22% reduction in the interpatient variation in troxacitabine clearance and a 15% reduction in the interpatient variation in 5-fluorouracil/eniluracil clearance [17,67]. Body-surface area-based dosing of MTA, a novel multi-targeted antifolate that is also mainly excreted in urine as unchanged drug (>70% unchanged), was also associated with a 16% reduction in interpatient variation in drug clearance [70]. The known association between body-surface area and glomerular filtration rate [71] may explain the observed relationship between body-surface area and clearance of renally-excreted agents. However, differences in body-surface area among patients account for only a small percentage of the total variability in drug clearance (i.e., ≤ 22%), which is consistent with results from a recent study that reported that body-surface area was poorly correlated with glomerular filtration rate (R < .22) [72]. Therefore, for agents that are excreted principally by the kidneys, dosing strategies that are based on the accurate assessment of glomerular filtration rate and not on body-surface area should be associated with decreased interpatient variability in clearance [73].

Body-surface area-based dosing may also be a preferred dosing strategy for drugs that are confined to blood volume because of the known relationship between body size and blood volume [74,75]. For example, we found that body-surface area was highly correlated with temozolomide clearance (R = .88, *P*<.001) and was associated with 35% of the variation in temozolomide clearance between patients. Temozolomide is an alkylating agent that undergoes pH-dependent breakdown to the active moiety 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide immediately following drug administration and is virtually isolated to the central compartment. Interpatient variability in DHA-paclitaxel clearance was reduced by 26% when the dose of that agent was normalized to body-surface area. DHA-paclitaxel has a low clearance (~ 0.11 L/h) and a small volume of distribution (~ 4 L), and is extensively (>99.6%), but non-specifically, bound to alpha$_1$-acid glycoprotein and albumin [76]. These characteristics indicate that DHA-paclitaxel is principally confined to blood volume, and that protein binding for this agent may dictate systemic exposure to total drug. By contrast, drugs that bind a single protein with high affinity but low capacity (e.g., as UCN-01 binds alpha$_1$-acid glycoprotein [77]) are more likely than DHA-paclitaxel to show wide variations in unbound (pharmacologically active) drug concentrations among patients. For agents with disposition characteristics like UCN-01, measurement of total drug concentrations in plasma is a poor surrogate for unbound drug [78], and accurate assessment of the relationship between body-surface area and clearance will require measurement of unbound drug concentrations. Thus, the protein-binding

characteristics of investigational agents across species should be characterized before those agents are evaluated in phase I trials.

Normalizing doses to body-surface area may also provide an advantage for drugs, such as paclitaxel, which are formulated in vehicle substances known to affect drug disposition. For example, previous work has shown that the distribution of paclitaxel in the blood depends on the duration of drug infusion and the dose of its formulation vehicle (Cremophor) [51], which is likely due to the preferential affinity of paclitaxel for Cremophor in the circulation [79]. It has been demonstrated that this vehicle has a distribution volume that approximates the blood volume, and that body-surface area is a statistically significant covariate for Cremophor clearance [80]. Thus the impact of body-surface area on the variability in paclitaxel pharmacokinetics is most likely associated with the affinity of paclitaxel for its vehicle in the circulation [81], the distribution of which is linked to total blood volume, and thus to body-surface area [75].

We found that body-surface area-based dosing did not decrease interpatient variability in clearance to a statistically significant extent for the majority of the anticancer agents we examined that underwent development in adult patients from 1991 through 2001. For the few agents for which clearance was statistically significantly associated with body-surface area, the relative reduction in variability for clearance was between 15% and 35%, which suggests that only up to one third of the total variability can be explained by body-surface area. These results, therefore, do not support the use of body-surface area-based dosing for most anticancer agents, and suggest that alternate dosing strategies should be considered for phase I evaluation of anticancer agents in adult humans. A non-body-surface area-based dosing strategy (e.g., one based on a fixed dose) was successfully implemented in the development of five of the orally-administered agents examined in the present study (i.e., phenylbutyrate [20], PKI166 [21], R115777 [32], SCH66336 [23], and ZD9331), demonstrating that administration of a fixed total dose is feasible for the development of both cytotoxic and non-cytotoxic anticancer agents. We therefore recommend that the practice of calculating starting drug doses on the basis of body-surface area in phase I trials should be abandoned and that future early clinical trials should instead evaluate the administration of fixed drug doses that are calculated on the basis of an average body-surface area of 1.86 m^2. For novel non-cytotoxic agents, dose refinement should be based on the achievement of an exposure that produces a biologic or molecular effect on a drug target that is associated with a desired therapeutic outcome or avoidance of a toxicologic outcome. For cytotoxic agents that have a narrow

therapeutic window, efforts should continue to focus on defining individual doses that are based on patient characteristics that are known to effect drug clearance (e.g., age, sex, renal function, use of concomitant medications). A combination of these strategies should yield more rational dosing schemes that can be implemented in oncology practice.

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Chapter 4

Factors Affecting Pharmacokinetic Variability Following Doxorubicin and Docetaxel-based Therapy

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Abstract

Current dosing strategies for anticancer drugs result in wide interindividual pharmacokinetic variability. Here, we explored the influence of age, body size, concomitant drugs, dose, infusion duration, and sex on the clearance for doxorubicin and docetaxel in 243 individual patients. Patients received doxorubicin (n=110) or docetaxel (n=152) as monotherapy or in combination chemotherapy regimens. The mean (±SD) clearance was 63.6±22.7 L/h for doxorubicin and 42.8±14.9 L/h for docetaxel. Normalization for body surface area (BSA) reduced the interindividual variability by only <1.7%. Doxorubicin clearance was significantly reduced when administered at doses >50 mg/m^2 or in combination with cyclophosphamide. Upper extremes of body size were associated with increased clearance for both drugs, whereas no consistent effect of age on clearance was discerned. Overall, these findings suggest that incorporation of variables in addition to BSA should be considered in routine dosing strategies for doxorubicin and docetaxel.

Introduction

There is often a marked variability in drug handling between individual patients, which may contribute to variability in the pharmacodynamic effects of a given drug dose. Hence, an identical drug dose may result in a therapeutic response with acceptable toxicity in one patient, and unacceptable and possibly life-threatening toxicity in another. A combination of physiological, genetic, and environmental factors is known to alter the relationship between the absolute dose and a drug's concentration-time profile. Over the last few decades, numerous studies have established relationships between systemic exposure to anticancer drugs and drug-induced toxicity or response. These relationships have subsequently been used to individualize chemotherapy regimens either *a priori* or *a posteriori*, for example as in case of carboplatin and methotrexate, respectively [1,2]. A variety of strategies is now being evaluated to improve the therapeutic index of other anticancer drugs, some of which include implementation of geno- or phenotyping individual patients for drug metabolizing enzymes, the use of biomodulating agents and/or modification of drug scheduling.

Docetaxel and doxorubicin are commonly used for the treatment of a variety of cancers. Although drug-induced toxicity is dose-dependent for both of these agents, the individual susceptibility to side effects varies considerably. As for most other

anticancer agents, the administered dose of docetaxel and doxorubicin is normalized by a patient's body surface area (BSA). However, for most anticancer agents clearance is poorly correlated to body-size measures and hence, the routine use of BSA as the only independent variable considered in drug dosing is questionable [3-12]. Previous studies have revealed significant relationships between interindividual variation in docetaxel and doxorubicin clearance and the likelihood of tumour response and/or toxicity [3]. However, the factors contributing to pharmacokinetic variability for these agents are largely unknown and unstudied. In an attempt to further optimize use of these agents, we characterized the pharmacokinetics of doxorubicin and docetaxel in a broad patient population under general clinical conditions, and explored demographic subpopulations or drug conditions for which dose adjustment may be needed.

Patients and methods

Patient selection and treatment

All patients studied had a malignant solid tumour and were treated on trials with doxorubicin monotherapy [13], doxorubicin-based combination therapy (with either cyclophosphamide [14], docetaxel [15], or paclitaxel [16], docetaxel monotherapy [17,18] [19-21], or docetaxel-based combination therapy (with either capecitabine [18], cisplatin [17], doxorubicin [15], doxorubicin with or without marimastat [22], methotrexate [19], or R101933 [20,21]). Doxorubicin was administered as a bolus (5 minutes), a short infusion (15 to 20 minutes), or as a 1- to 3-hour infusion at doses ranging from 40-75 mg/m^2, and docetaxel as a 1-hour infusion at doses 55-100 mg/m^2. For the purpose of this study, the drug administration schedule assigned for each agent was based on the co-administered drug and infusion duration (Table 1). Patients were included on these trials if they were over 18 years old and had adequate haematologic, hepatic, and renal function. Written informed consent was obtained from each patient according to institutional guidelines.

Pharmacokinetic sampling and analysis

Pharmacokinetic sampling schema and analytical methods employed have been previously described for total drug concentrations [13-24]. All sampling schema involved intensive serial plasma sampling for up to 24-48 hours post infusion. Pharmacokinetic studies were performed during one cycle of therapy (cycle 1 or 2).

Table 1. Drug administration schedules

Drug and Administration Schedule (Infusion Duration)	Administration Schedule Number	Number of Patients	No. of Courses	
			Course #1	Course #2
Doxorubicin				
A 60 mg/m^2 (20-min) followed by CYT 600 mg/m^2 (1-h)	1	19	19	
A 40-60 mg/m^2 (20-min) followed 1 h later by TXT 60 – 100 mg/m^2 (1-h)	2	22	22	
A 50-75 mg/m^2 (5-min)	3	39	39	
A 60 mg/m^2 (5-min) followed 15 min later by TAX (3-h) 150 – 200 mg/m^2	4	7	5	2
A 60 mg/m^2 (3-h) coadministered with TAX (3-h) 200 mg/m^2	5	9	9	
A 60 mg/m^2 (5-min) followed 24 h later by TAX (3-h) 150 mg/m^2	6	6	6	
TAX (3-h) 200 mg/m^2 followed 15 min later by A 60 mg/m^2 (5-min)	7	8	4	4
TOTAL		**110**		
Docetaxel				
TXT 60 – 100 mg/m^2 (1 h)	1	27	27	
A 40-60 mg/m^2 (20-min) followed 1 h later by TXT (1-h) 60 – 100 mg/m^2	2	19	19	
A 60 mg/m^2 (5-min) followed 1 h later by TXT (1-h) 60 mg/m^2	3	10	10	
TXT 55 – 100 mg/m^2 (1-h) immediately followed by CIS (3-h) 50 – 100 mg/m^2	4	57	57	
TXT 75 – 100 mg/m^2 (1-h) coadministered with oral CAP 825 to 1250 mg/m^2 BID	5	32	32	
TXT 75 – 85 mg/m^2 (1-h) administered on day 2 with MTX (5-min) 30 – 50 mg/m^2 administered on day 1 and 15	6	7	7	
TOTAL		**152**		

Abbreviations: capecitabine (CAP), cisplatin (CIS), cyclophosphamide (CYT), docetaxel (TXT), doxorubicin (A), methotrexate (MTX), once daily (QD), paclitaxel (TAX), twice daily (BID).

Clearance values for doxorubicin and docetaxel were estimated for individual patients by the method of weighted least-squares regression using either a 2-compartment or 3-compartment linear model as implemented in ADAPT II [25], WinNonlin (Pharsight Corp., Mountain View, CA, USA), or Siphar (InnaPhase, Philadelphia, PA, USA). Previous investigations evaluating drug exposure-effect relationships for doxorubicin and docetaxel have shown that area under the total plasma concentration curve (AUC) is more closely correlated with the principal toxicity neutropenia than other pharmacokinetic parameters [26,27]. AUC is a function of both the drug dose and clearance by the equation clearance=dose/AUC; therefore, drug clearance was selected as the parameter for evaluation of pharmacokinetic variability.

Body-size measures including BSA, lean body mass (LBM), ideal body weight (IBW), adjusted ideal body weight (AIBW), and body mass index (BMI) were calculated as described previously [28-30]. For statistical and graphical analysis, values for BSA were grouped as: lower quartile (25% quantile), interquartile range, and upper quartile (75% quantile). Values for BMI were grouped as: ≤25 (normal), 25-29 (overweight), 30-34 (obese), and ≥35 (morbidly obese). The four BMI groups were used to graphically display individual clearance values; due to the small numbers in the BMI 30-34 and ≥35 groups, the data were combined for these two groups for ANOVA. Values for age were grouped as: <65 years old, 65-69 years old (borderline elderly), and ≥70 years old (elderly).

Statistical analysis

Interindividual differences in clearance values were evaluated by the coefficient of variation (CV). The relative reduction in variability (RIV) for clearance was calculated as described previously [9]. Univariate linear regression analysis was used to assess the relationship between body size indices, age, and drug clearance. One-way analysis of variance (ANOVA) was used to compare the differences in clearance as a function of drug administration schedule (a combination of concomitant drug and infusion duration), categorical age, categorical BSA, categorical BMI, and sex followed by a Tukey-Kramer's multiple comparison test. Univariate linear least-squares regression analysis and ANOVA were performed using JMP Statistical Discovery Software, version 4.0.4 (SAS Institute, Cary, NC, USA).

Due to the study design, it was necessary to account for potential confounding variables and the correlation between individuals from the same protocol. Random effects multiple linear regression models were used to assess the influence of dose, concomitant drugs, infusion duration, age, sex, and BSA (predictor variables) on drug

clearance (outcome) where a random effect was included to account for correlation between individuals within an administration schedule. This approach assumes independence between individuals from different protocols, but assumes that outcomes from individuals in the same protocols are correlated. First, the associations between predictor variables were assessed to detect possible multi-colinearity in regression models: categorical variables were compared using Fisher's exact test, continuous variables were compared using Pearson and Spearman correlation, categorical were compared to continuous using ANOVA. This analysis was exploratory in nature and necessary due to the observational nature of the dataset. Random effects models were then fit to the data iteratively and interactions between predictors were also considered. Regression coefficients, standard errors of the coefficients, and the associated P-values were determined. The coefficients represent the expected difference in clearance for a one-unit difference in a predictor, adjusted for the other predictors in the linear regression model. Multiple linear regression modelling was performed using the software STATA, version 7.0 (Stata Corporation, Cary, NC, USA). The *a priori* level of significance was set at $P < 0.05$.

Results

Patient demographics are summarized in Table 2.

Doxorubicin pharmacokinetics

The mean plasma clearance of doxorubicin in the entire group was 63.6±22.6 L/h (range, 16.6-125 L/h) with a CV of 35.5% (Table 3), similar to previous findings.(31) Compared to the other drug administration schedules, doxorubicin clearance was reduced by approximately 30% when co-administered with cyclophosphamide (47.9±25.6 L/h versus 66.9±20.6 L/h; P=0.0007) (Figure 1A); consequently, these nineteen patients were excluded from subsequent analysis. A positive association was observed between BSA and doxorubicin clearance (r=0.34; P=0.0015); a separate analysis in males and females revealed a stronger correlation in males (r=0.64; P=0.0002) than females (r=0.01; P=0.95). However, when normalized to BSA, the RIV for clearance was 0.8%. Consequently, only for males did normalization of clearance to BSA result in a substantial reduction in variability of clearance (RIV 18.7%). A trend for increasing doxorubicin clearance as BSA values increased from the lower quartile to the upper quartile was observed; clearance was 20% higher in patients with BSA values in the upper quartile (BSA>1.97 m^2) (63.1±22.6 L/h versus 64.7±17.3 L/h versus 76.6±24.2 L/h; P=0.061) (Figure 2A).

Table 2. Patient demographics

Patient Characteristics	Doxorubicin Therapy	Docetaxel Therapy
Total Number of Patients	110[d]	152[d]
Age [years (range)]	52.5[a] (27-78)	53.1[a] (21-75)
< 65	94[b]	128[b]
65 – 69	9[b]	16[b]
≥ 70	7[b]	8[b]
Sex (male:female)	34:76	69:83
Height [cm (range)]	165.9[a,c] (140-193)	170.9[a] (149-193)
Weight [kg (range)]	74.1[a,c] (38-117)	73.3[a] (39.3-109.2)
BSA [m^2 (range)]	1.83[a,c] (1.26-2.42)	1.86[a] (1.28-2.38)
25% Quantile	1.67	1.71
Median	1.78	1.84
75% Quantile	1.97	2.00
LBM [kg (range)]	53.9[a,c] (34.1-72.3)	54.2[a] (31.8-78.8)
IBW [kg (range)]	59.1[a,c] (34.1-87.3)	64.5[a] (42.3-87.3)
AIBW [kg (range)]	62.6[a,c] (41.9-86.6)	66.7[a] (41.5-90.6)
BMI (range)	26.6[a,c] (15.5-43.6)	25.1[a] (16.4-40.5)
< 25	40[b]	80[b]
25 – 29	37[b]	50[b]
30 – 34	15[b]	18[b]
≥ 35	7[b]	3[b]
Clearance (L/h)	63.6[a] (16.6-124.5)	42.8[a] (13.8-84.4)
Infusion Duration	0.47 (0.07-3)	1.05[a] (0.92-1.50)
Bolus	57[b]	
Short Infusion	43[b]	
Long Infusion	10[b]	

[a]Results expressed as mean value. [b]Data are the number of patients. [c]Height, BSA, LBM, IBW, AIBW, and BMI were available for 99 of 110 patients; weight was available for 104 of 110 patients. [d]Nineteen patients received both doxorubicin and docetaxel in combination therapy and are represented in both data sets; the total number of individual patients studies were 243.

Similarly, clearance was 22% higher in patients with BMI values ≥30 kg/m^2 (63.6±19.9 L/h versus 65.7±17.1 L/h versus 78.9±27.3 L/h; P=0.045) (Figure 3A). Although doxorubicin clearance was reduced by 13% in females compared to males (63.3±19.2 L/h versus 73.0±22.2 L/h; P=0.032), no sex differences were noted when clearance was normalized to BSA (37.0±12.4 L/h/m^2 versus 37.3±9.37 L/h/m^2; P=0.90). Age was not associated with reductions in doxorubicin clearance (P=0.88) (Figure 4A); however, the number of patients in the studied population that were aged 65-69 years (n=4) or ≥70 years (n=6) may not be adequate to detect a difference if one exists.

Table 3. Mean (± standard deviation) clearance of doxorubicin and docetaxel as a function of body-size measures

Body-size measure	Doxorubicin[1]		Docetaxel	
	% CV	Clearance[2]	% CV	Clearance[2]
None (L/h)	30.8	66.9 ± 20.6	34.8	42.8 ± 14.9
BSA (L/h/m^2)	30.6	37.1 ± 11.3	34.2	23.0 ± 7.9
LBM (L/h/kg)	32.0	1.3 ± 0.4	35.0	0.8 ± 0.3
IBW (L/h/kg)	33.9	1.2 ± 0.4	35.8	0.7 ± 0.2
AIBW (L/h/kg)	32.4	1.1 ± 0.4	35.4	0.7 ± 0.2
BMI (kg/m^2)	33.3	2.6 ± 0.9	36.8	1.7 ± 0.6
Height (L/h/cm)	30.0	0.4 ± 0.1	36.0	0.3 ± 0.1
Weight (L/h/kg)	35.1	0.9 ± 0.3	36.7	0.6 ± 0.2

[1]Excludes patients enrolled to drug administration schedule #1. n=91 for none, n = 86 for weight, n = 82 for all others. [2]mean ± SD

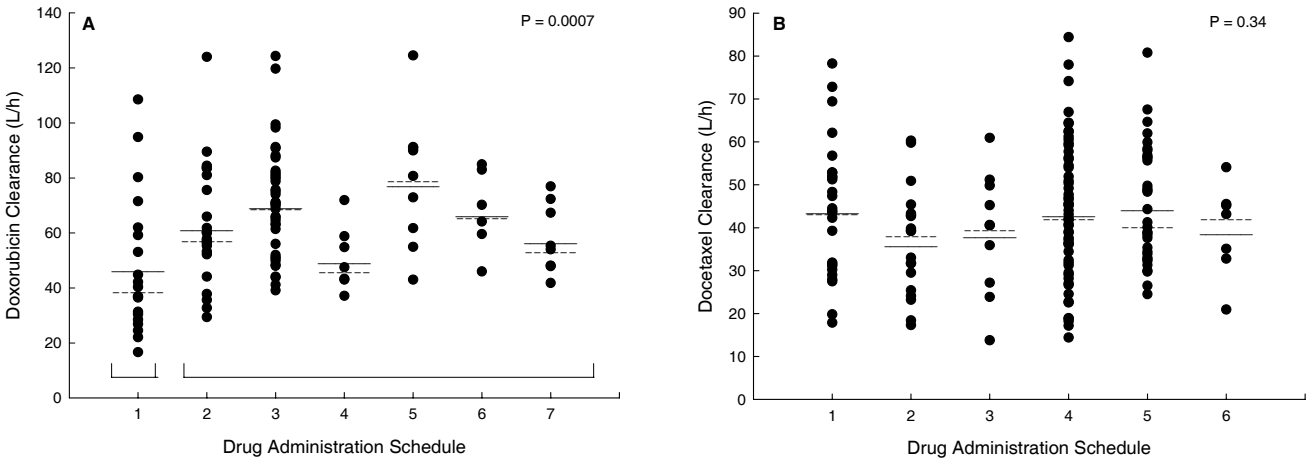

Figure 1. Clearance as a function of drug administration schedule for (A) doxorubicin and (B) docetaxel. The solid line represents the mean, while the dashed line represents the median.

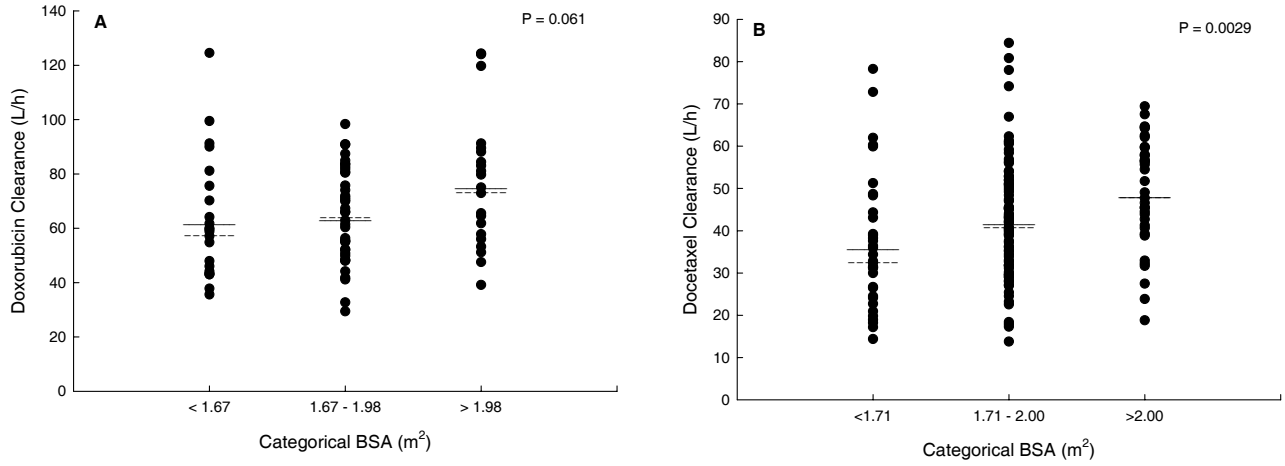

Figure 2. Clearance as a function of body surface area (BSA) for (A) doxorubicin and (B) docetaxel. The solid line represents the mean, while the dashed line represents the median.

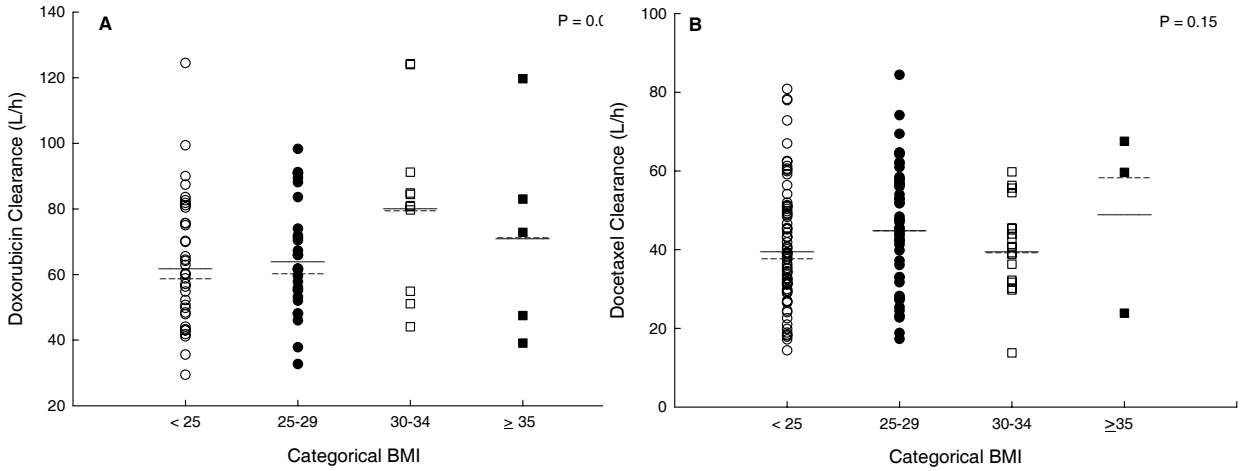

Figure 3. Clearance as a function of body mass index (BMI) for (A) doxorubicin and (B) docetaxel, where ○ is < 25, ● is ≥ 25-29 years, □ is 30-34, and ■ is ≥ 35. The solid line represents the mean, while the dashed line represents the median.

Multiple linear regressions helped to determine that concomitant medication and sex, and concomitant medication and infusion duration were strongly confounded. Therefore, concomitant medication was not included in the final multiple linear regression model. In addition, preliminary modelling indicated that infusion duration

was not significantly associated from clearance so it was not included in the final model. Interactions were found between sex and BSA, and sex and dose. As a result, the associations between predictor variables and doxorubicin clearance were stratified by sex and summarized in Table 4. At doxorubicin doses greater than 50 mg/m^2, there was a trend for decreased clearance, although this did not reach a level of significance (coefficient=-10.63; P=0.056). Assuming an average clearance of 63 L/h, this would represent a 17% decrease in clearance when doxorubicin is administered at doses >50 mg/m^2. BSA was positively associated with clearance in males (coefficient=66.42; P<0.001) but not in females (coefficient=-7.46; P=0.61), and age was not associated with altered drug clearance (coefficient=-0.05; P=0.82).

Table 4. Multiple regression model for doxorubicin clearance

	Coefficient	Standard Error	P value
Males (n = 29):			
Intercept	-50.03	26.80	0.11
High dose[1]	-10.63	4.49	0.056
BSA	66.42	9.72	<0.001
Age	-0.05	0.21	0.82
Females (n = 69):			
Intercept	83.15	31.15	0.04
High dose	-10.63	4.49	0.056
BSA	-7.46	13.84	0.61
Age	-0.05	0.21	0.82

[1]High dose is binary with a dose greater than 50 mg/m^2 coded as 1, and less than or equal to 50 coded as 0.

Docetaxel pharmacokinetics

The mean plasma clearance of docetaxel was 42.8±14.9 L/h (range, 13.8-84.4 L/h) with a CV of 34.8% (Table 3), and was similar among the different drug administration schedules (P=0.34) (Figure 1B). A positive correlation was noted between BSA and docetaxel clearance (r=0.30; P=0.0002); a separate analysis revealed a stronger correlation in males (r=0.35; P=0.0032) than in females (r=0.18; P=0.11). After normalization of clearance for BSA, the mean value was 23.0±7.87

L/h/m^2 with an associated CV of 34.2%, indicating a negligible RIV for clearance of 1.7%. However, the mean clearance increased as BSA values increased from the lower to the upper quartile, and was significantly higher by 33% in patients with BSA values >2.0 m^2 (36.8±15.9 L/h versus 42.6±14.4 L/h versus 49.0±13.0 L/h; P=0.0029) (Figure 2B). Clearance was not higher in patients with BMI ≥30 kg/m^2 (40.9±15.0 L/h versus 46.1±15.2 L/h versus 42.3±13.3 L/h; P=0.15) (Figure 3B). Docetaxel clearance was reduced on average by 11% in females compared to males (40.6±14.7 L/h versus 45.6±14.8 L/h; P=0.040), although no sex differences were noted when clearance was normalized to BSA (22.9±8.44 L/h/m^2 versus 23.2±7.19 L/h/m^2; P=0.88). Advanced age was not associated with reduced docetaxel clearance in 16 patients between the ages of 65-69 and 8 patients aged ≥70 years (P=0.45) (Figure 4B).

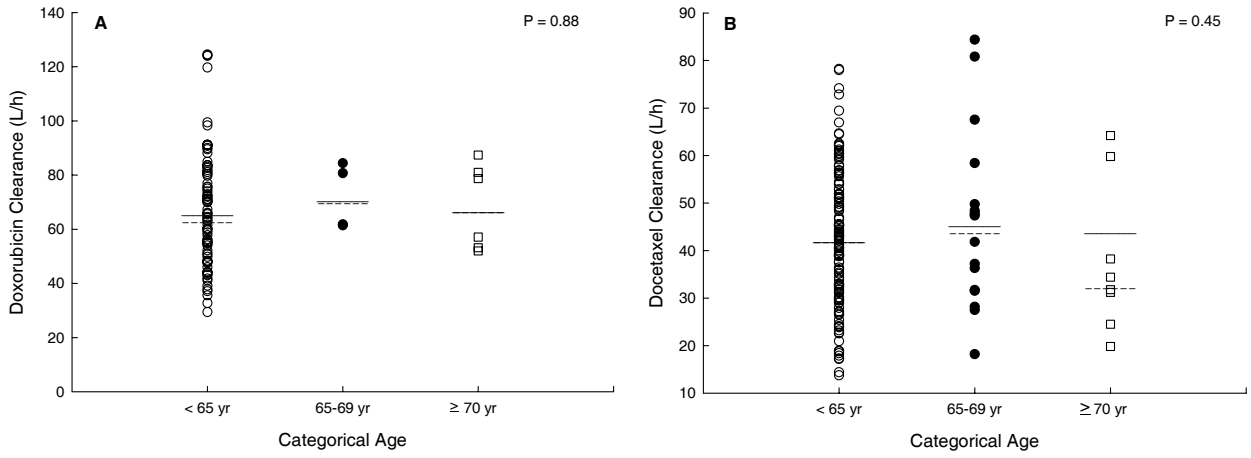

Figure 4. Clearance as a function of categorical age for (A) doxorubicin (data from drug administration schedule 1 is not included) and (B) docetaxel, where ○ is < 65 years old, ● is ≥ 65 and < 70 years old, and □ is ≥ 70 years old. The solid line represents the mean, while the dashed line represents the median.

Using multiple linear regression analysis, an interaction between sex and BSA was observed, and therefore results are stratified for males and females. The association between predictor variables and docetaxel clearance is summarized in Table 5. BSA was associated with docetaxel clearance in males only (coefficient=28.11; P=0.039). Concomitant treatment with doxorubicin and capecitabine was associated with decreased and increased docetaxel clearance, respectively (for doxorubicin, coefficient=-8.57; P=0.014; for capecitabine, coefficient=1.48; P=0.010). Assuming an

average docetaxel clearance of 42.8 L/h, the coefficient of –8.57 indicates that co-treatment with doxorubicin results in a 20% decrease in clearance. No association was observed between age and docetaxel clearance (P=0.67).

Discussion

The traditional method of individualizing anticancer drug dosage in adult patients is by using BSA [3-12,32,33]. The usefulness of normalizing anticancer drug doses to BSA has been questioned, since it has been shown that for some agents there is no relationship between BSA and anticancer drug clearance [3-12,32,33]. In these cases, the use of BSA-adjusted dosing results in the administration of a standard dose multiplied by an arbitrary number (i.e., the ratio of the patient's BSA to an average BSA). These considerations have led to a desire for better tools to individualize chemotherapy, and to new ways of evaluating and treating patients. In the present study, exploratory relationships were assessed between disposition characteristics of doxorubicin and docetaxel and a number of common patient and drug-related variables.

Doxorubicin clearance was decreased by approximately 17% at doses >50 mg/m^2, consistent with a previous report suggesting nonlinear disposition [34]. In contrast, some investigators have observed the absence of dose- and time-dependency, possibly on the basis of sparsity of data sets [35]. It was also observed that doxorubicin clearance was reduced (30%) when co-administered with cyclophosphamide, which is in line with a previous report [36]. This interaction has been attributed to reduced formation of the doxorubicin 7-deoxyaglycone metabolite, and, hence, decreased elimination of the parent drug [37]. The administration of doxorubicin doses >50 mg/m^2 when combined with cyclophosphamide could result in greatly reduced doxorubicin clearance. These observations provide mechanistic support for recent observations in an ongoing adjuvant breast cancer clinical trial (NSABP B-30, see: www.nsabp.pitt.edu), where excessive toxicity, including the occurrence of toxic deaths, was noted in the original TAC regimen combining doses of doxorubicin 60 mg/m^2, docetaxel 60 mg/m^2, and cyclophosphamide 600 mg/m^2. However, similar events were not observed in another breast cancer adjuvant trial that used TAC doses of 50, 50, 500 mg/m^2, respectively [38]. In contrast to cyclophosphamide, taxanes in the tested schedules had no apparent effect on the clearance of doxorubicin by univariate analysis but could not be tested in multiple linear regression analysis because of confounding predictor variables. We could therefore not confirm previous

observations describing effects of docetaxel or paclitaxel on doxorubicin pharmacokinetics [15,16].

Similar to most other chemotherapeutic agents, in the entire population studied, dose calculations based on BSA did not significantly reduce interpatient variability in doxorubicin clearance. However, doxorubicin clearance was approximately 20% higher in patients with BSA >1.97 m^2 (P=0.061) and BMI ≥30 kg/m^2 (P=0.045). It is noteworthy in this context that a previous study involving 21 patients indicated that drug clearance was reduced by almost 50% in 7 obese females, where obesity was defined as greater than 130% of IBW [39]. This observation was not confirmed in the present investigation both for females and males in a broader patient population. Doxorubicin clearance was unaltered in 10 elderly patients, consistent with previous findings in 9 elderly patients [40]. However, another study involving 56 patients, 7 of whom were aged ≥70 years, showed a linear association between age and clearance, although complete overlap in clearance values was observed in patients between the ages of 40-80 years [41].

In the case of docetaxel, a multiple regression analysis revealed a 20% decrease in clearance in the presence of doxorubicin. Although the mechanistic basis for this interaction is unknown, it may be clinically significant considering the notion that a 25% decrease in docetaxel clearance significantly increases the odds for development of febrile neutropenia [42,43]. It provides a further explanation for the severe haematological toxicity observed in regimens combining doxorubicin with docetaxel.

Similar to doxorubicin, normalization of docetaxel clearance to BSA resulted in negligible reduction in variability in clearance. However, also similar to doxorubicin, docetaxel clearance was increased by approximately 33% in patients with BSA >2.0 m^2 (P=0.0029); this association was not observed, however in obese patients with BMI values ≥30 kg/m^2 (P=0.15) despite 15 of 21 patients being identical in the 2 subcategories. It thus remains unclear if docetaxel clearance is altered in obese patients. It is still debatable whether the interpatient variability of docetaxel clearance has a clinically meaningful relationship with BSA. Although Bruno and colleagues reported that BSA is a significant covariate for docetaxel clearance [43], the only clinically relevant variables that impact significantly upon clearance of this drug are altered levels of transaminases and alkaline phosphatase [42]. Thus, although docetaxel clearance may be weakly related to BSA, this measure does not contribute substantially to explaining interindividual pharmacokinetic variability.

In the present population, docetaxel clearance does not appear to be significantly reduced in elderly patients, which lends further support to a population

pharmacokinetic analysis indicating that age is not a significant covariate for clearance [42,43]. In contrast, a previous investigation in a group of 226 patients with equal histopathologic conditions has shown a significant decline in the content of the main docetaxel-metabolizing enzyme (i.e., cytochrome P450 3A) in patients after 70 years of age [44]. A confirmatory multi-institutional trial that includes phenotyping for cytochrome P450 3A is currently being conducted in patients of different age groups.

Although recent investigations have provided evidence against the use of BSA in anticancer drug dosing [3-12], there may be some situations, in the absence of more accurate and validated dosing strategies, where BSA-based dosing is relevant. For example, the present investigation observed an increase in drug clearance at the upper extremes of body-size; for the studied agents, under these circumstances, normalizing drug dose to BSA could account for some of the variability in clearance. In addition, the data does not justify the capping of BSA at 2.0 m^2 or using ideal-body weight in the formula for BSA when calculating drug dose for obese patients. Alternative weight descriptors for dose adjustment of these anticancer agents in obese patients are being evaluated as recently described [45].

In conclusion, the current analysis confirms a number of findings previously described by conventional pharmacological analyses in smaller numbers of patients. The current statistical evaluation also has eliminated several candidate covariates from further consideration as important determinants of drug disposition. It is difficult to make specific recommendations for dosing changes of doxorubicin- or docetaxel-containing chemotherapeutic regimens on the basis of the current findings. Although monitoring of plasma levels and dosage adjustment may be necessary to optimize anticancer efficacy in patients, therapeutic drug monitoring is not routinely available for these agents. Regardless, the described data continue to increase our knowledge on these clinically important drugs, and provide the basis for designing future, prospective investigations aimed at evaluating alternative and improved dosing regimens.

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Chapter 5

Factors Affecting Cytochrome P-450 3A Activity in Cancer Patients

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Submitted

Abstract

Background: Variable response to cancer chemotherapy is related, in part, to interindividual variation in expression of the enzyme cytochrome P-450 3A (CYP3A). The aim of this study was to identify the demographic, physiologic, and inheritable factors that influence CYP3A activity in a large population of patients with cancer.

Methods: A total of 134 patients (62 females; age range, 26 to 83 years) underwent the erythromycin breath test as a phenotyping probe of CYP3A. Genomic DNA was screened for 6 variants of suspected functional relevance in the CYP3A4 and CYP3A5 genes.

R*esults:* CYP3A activity varied up to 14-fold in this population, and was not significantly influenced by age, sex, and body size measures. Likewise, no variants in the CYP3A4 and CYP3A5 genes was a significant predictor of CYP3A activity. CYP3A activity was reduced by approximately 50% in patients with concurrent elevations in liver transaminases and alkaline phosphatase or elevated total bilirubin; in a multivariate analysis, liver function combined with the concentration of the acute-phase reactant, alpha-1 acid glycoprotein, explained approximately 18% of overall variation in CYP3A activity.

Conclusion: These data suggest that baseline demographic, physiologic and genetic factors have only a minor impact on phenotypic CYP3A activity in patients with cancer. Consideration of additional factors, including the inflammation marker C-reactive protein as well as concomitant use of other drugs, food constituents, and complementary and alternative medicine with inhibitory and inducible effects on CYP3A, is needed to reduce variation in CYP3A and response to chemotherapeutic treatment of cancer.

Cancer chemotherapy is characterized by wide variation in response among patients. This is due, in part, to pharmacokinetic variability. The most widely used strategy to decrease pharmacokinetic variability is to normalize a drug dose to the patient's body-surface area (BSA). Because BSA-based dosing strategies do not reduce interindividual variability in anticancer drug pharmacokinetics [1], other measures to predict drug disposition and effects are needed. As cytochrome P-450 3A (CYP3A) is involved in the metabolism of approximately 50% of all prescribed drugs [2], including many anticancer agents, phenotyping strategies to predict an individual's CYP3A activity prior to cytotoxic chemotherapy treatment is one approach for dose individualization. Various noninvasive *in vivo* probes for evaluating CYP3A activity

have been described and several have been shown to correlate with drug clearance [3]. The most widely tested and accepted CYP3A probes are midazolam and erythromycin, although selection of the ideal CYP3A phenotyping probe remains controversial [4,5].

Little is known regarding factors affecting CYP3A activity in cancer patients. Rivory and colleagues noted an association between the inflammatory response and CYP3A activity, which was assessed using the erythromycin breath test (ERMBT), in 40 patients with advanced cancer [6]. CYP3A activity was inversely correlated with both inflammatory markers C-reactive protein and α-1 acid glycoprotein (AAG), with the former accounting for 44% of interpatient variation. In the current study, the influence of patient characteristics, including age, body size, liver function and sex, the acute phase reactant AAG, and CYP3A4 and CYP3A5 genotype on CYP3A activity, as assessed using the ERMBT, was explored in 134 patients with advanced cancer.

Methods

Patients

Patients were enrolled to one of 2 clinical protocols where the ERMBT was administered at baseline (see below) prior to initiation of chemotherapy treatment. Patients had advanced cancer (histologically or cytologically confirmed malignancy). Criteria for inclusion of patients into this study were: 1) age ≥ 18 years; 2) performance score (PS) ≤ 3 according to the Eastern Cooperative Oncology Group criteria; and 3) creatinine ≤ 2.0 × the institutional upper limit of normal (ULN). Patients with varying degrees of liver impairment were included in this study and grouped according to the following: liver function group 1, total bilirubin < 1.5 × ULN and no elevations in aspartate aminotransferase (AST), alanine aminotransferase (ALT) or alkaline phosphatase as described for groups 2, 3A, and 3B; liver function group 2, total bilirubin < 1.5 × ULN, elevations in AST and/or ALT > 1.0 × ULN concurrent with alkaline phosphatase ≥ 2.5 × ULN, or AST and/or ALT ≥ 1.5 × ULN concurrent with alkaline phosphatiase > 1.0 × ULN, or isolated elevations of AST and/or ALT or alkaline phosphatase ≥ 5.0 × ULN; liver function group 3A, total bilirubin < 1.5 × ULN, concurrent elevations in AST and/or ALT ≥ 1.5 × ULN concurrent with alkaline phosphatase ≥ 2.5 × ULN; group 3B, total bilirubin ≥ 1.5 × ULN with any elevations in liver transaminases or alkaline phosphatase.

Patients were not eligible for the clinical trial conducted in Baltimore, MD, Washington D.C., and Rotterdam and Leiden, the Netherlands (study protocol 1) if they were concurrently taking phenytoin, carbamazepine, barbiturates, rifampin,

phenobarbital, St. John's wort, and/or ketoconazole. Patients enrolled to the clinical trial in Sydney, Australia (study protocol 2) were eligible if they were concurrently taking medications that were known to induce or inhibit CYP3A activity. The dose, frequency and duration of all concomitant drugs were recorded. The clinical protocols were approved by the local institutional review boards (Baltimore, MD, Washington, DC, Rotterdam and Leiden, the Netherlands, and Sydney, Australia), and all patients provided written informed consent before enrollment.

Pretreatment evaluations included in this study were performance status, height (HT), weight (WT), and the following serum chemistries: serum creatinine, alkaline phosphatase, AST, ALT, total bilirubin, and AAG. Body surface area (BSA) was calculated using Mosteller's formula, $BSA = [HT(cm) \times WT(kg) \div 3600]^{0.5}$ [7]. Body mass index (BMI) was calculated using the formula, $BMI = [WT(kg) / (HT/100)^2]$.

Erythromycin Breath Test (ERMBT)

For study protocol 1, the ERMBT dose and collection balloons were obtained from Metabolic Solutions. The dose consisted of 0.04 mg [^{14}C-N-methyl]-erythromycin, containing 3 μCi of radioactivity, dissolved in 4.5 mL of 5% dextrose solution. The dose was administered as an intravenous injection over approximately 1 min. Breath samples were collected in balloons post-injection at 5, 10, 15, 20, 25, 30 and 40 min. Samples were shipped to Metabolic Solutions (Nashua, NH) for measurement of breath carbon dioxide. The data was reported as the flux of $^{14}CO_2$, expressed as a percentage of dose exhaled per min, at each collection time point assuming a CO_2 output of 5 mmol/min per m^2 of BSA [8].

For study protocol 2, ERMBT was performed as described previously [9]. Briefly, 4 μCi of ^{14}C-erythromycin (N-methyl-^{14}C, 55 mCi/mmol, NEN Life Science Products Inc, Boston, MA) was administered as an intravenous injection and breath samples were collected into gas-tight balloons (Pytest, Ballard Medical Products, UT) post-injection at 5, 10, 15, 20, 25, 30 and 40 min. Breath samples were processed by bubbling the collected gas through a capture solution consisting of hyamine hydroxide 10X (Packard, Sydney, NSW, Australia) in 50:50 (v/v) methanol:ethanol to which a trace of phenolphthalein was added. After the addition of scintillation solution (Ultima Gold, Packard, Sydney, NSW, Australia) and counting, the data was reported as the flux of $^{14}CO_2$, expressed as a percentage of dose exhaled per min, at each collection time point assuming a CO_2 output of 5 mmol/min per m^2 of BSA [8].

The conventional ERMBT parameter, the flux at 20 min (C_{20min}) was the observed value. The area under the flux curve from time zero to 40 min ($AUC_{0\text{-}40min}$) was

determined using the linear trapezoidal method. The ERMBT parameter, $1/T_{max}$, was determined as described previously [9,10]. A mono-exponential equation was fitted to the % dose ^{14}C exhaled/min-time data and the time of the maximum % dose ^{14}C exhaled/min (T_{max}) was estimated. For some patients, the profiles had not reached a maximum and were still increasing at 40 min. In these cases, T_{max} was set at 50 min, as previously recommended [6].

Genotyping Procedures

DNA was isolated from 3 to 5 mL of whole blood using a QIAamp DNA Blood Midikit or from 1 mL plasma using a QIAamp UltraSens Virus Kit (Qiagen, Valencia, CA). DNA was amplified using polymerase chain reaction (PCR) based techniques. Restriction fragment length polymorphisms (RFLP) analysis was used to identify variations in the CYP3A4 (CYP3A4*1B, CYP3A4*6, CYP3A4*17, and CYP3A4*18) and CYP3A5 (CYP3A5*3C and CYP3A5*6) genes as previously described [11]. For the CYP3A5*6 assay, samples were first analyzed for the CYP3A5*3C variant in duplicate. Subsequently, only samples with at least one wild type allele (*1/*1 and *1/*3 genotypes) were then analyzed for the CYP3A5*6 variant. Samples with the CYP3A5*3C genotype *3/*3 were assigned the wild type genotype for CYP3A5*6.

Statistical Considerations

ERMBT parameters were summarized as the mean, 95% confidence level, and range. Values for age were grouped as < 70 and ≥ 70 (elderly) years. Values for BSA and AAG were grouped as: lower quartile, interquartile range, and upper quartile. Values for BMI were grouped as: < 25 (normal weight), 25 – 29.9 (overweight), ≥ 30 (obese). For continuous variables, nonparametric tests were used to compare mean values between different groups. When 3 or more groups were compared, a trend test was used [12]. Univariate correlation analysis was performed using the software program JMP version 3.2.6 (SAS Institute, Carey, NC). Although this analysis was mainly exploratory in intent, an adjustment was used to evaluate the significance of the multiple comparisons (5 demographic characteristics, 2 genotypes). Probability values (two-sided) of less than 0.007 were regarded as statistically significant, and those less than 0.05 were considered a trend.

Multiple linear regression models were then used to assess the influence of age, body size (BSA, BMI), liver function group, sex, AAG, and CYP3A4 and CYP3A5 genotype (predictor variables) on ERMBT parameters (outcome variables). Age, body size and AAG were included as continuous variables; liver function group, sex, and

CYP3A4 and CYP3A5 genotype were included as categorical variables. Regression coefficients, 95% confidence intervals, and the associated P-values were determined from the multiple linear regression modeling. Stepwise backward elimination was performed to systematically exclude the least significant factors until the P-value was < .05. Multiple linear regression modeling was performed using the software program Stata version 8.2 (Stata Corp., College Station, TX). For this analysis, the *a priori* level of significance was set at P < .05.

Table 1. Patient characteristics

Characteristics	Number of Patients
Age (years)	61 (26 - 83)[a]
< 70	99
≥ 70	35
Body surface area (m^2)	1.85 (1.39 – 2.52)[a]
Body mass index (kg/m^2)	25 (17 - 44)[a]
< 25	65
25 – 29.9	49
≥ 30	20
Race/ethnicity	
Black	7
White	127
Sex	
Females	62
Males	72
α-1 acid glycoprotein (x ULN)	1.08 (0.33 – 2.36)[a]
Liver function group	
1	102
2	14
3A	12
3B	6
Primary Tumor	
Lung	34
Breast	33
Head and Neck	20
Genitourinary	17
Gastrointestinal	10
Unknown	9
Melanoma	7
Angiosarcoma	4

[a]Data are values for median (range)

Results

Patients

A total of 134 patients with cancer (62 females and 72 males) was included in this study (Table 1). The median age was 61 years (range, 26 to 83 years), and 35 patients were 70 years or older. The majority of the population was of European descent and 7 patients were African American. Twenty patients were obese with a BMI ≥ 30 kg/m^2 and 32 patients had elevated liver function tests as defined for liver function group 2 (N = 14), liver function group 3A (N = 12) and liver function group 3B (N = 6).

CYP3A Phenotype

The ERMBT parameters were similar between the 2 study protocols: C_{20min}, 0.047 versus 0.048 % dose/min (P = .5615); $AUC_{0-40min}$, 1.57 versus 1.69 % dose/min (P = .3294); and $1/T_{max}$, 0.062 versus 0.061 min^{-1} (P = .2036). C_{20min} and $AUC_{0-40min}$ were highly correlated (R^2 = .9657, P < .0001), whereas C_{20min} and $1/T_{max}$ were less strongly correlated (R^2 = .2578, P < .0001). In this population of cancer patients, interpatient variation in CYP3A activity was 50-fold and 10-fold as determined by values for $AUC_{0-40min}$ and $1/T_{max}$, respectively (Figure 1 and Table 2); this extent of interpatient variation was observed in patients with normal liver function as defined for liver function group 1. In consideration of patients with ERMBT parameter values at the extreme of the population, values for $AUC_{0-40min}$ and $1/T_{max}$ for 95% of the population varied 14-fold (0.240 – 3.30 % dose/min) and 5-fold (0.020 - 0.10 min^{-1}), respectively.

CYP3A Genotype

Genotype and allele frequencies for 4 variants of CYP3A4 and 2 variants of CYP3A5 are summarized in Table 3. All patients were wild type for CYP3A4*6 (n = 120), CYP3A4*17 (n = 116), and CYP3A4*18 (n = 122). For CYP3A5*6, no patients were homozygous variant, 2 were heterozygous variant, and 119 patients were wild type; the 2 heterozygotes were African American. Because of the low frequency, genotypes for CYP3A4*6, CYP3A*17, CYP3A4*18, and CYP3A5*6 were not included for genotype-phenotype association analysis as shown below. For CYP3A5*3C, 2 patients was wild type (*1/*1 genotype), 19 patients were heterozygous variant (*1/*3 genotype), and 100 patients were homozygous variant (*3/*3 genotype); 6 of 6 African Americans and 15 of 115 white subjects carried at least one *1 allele, respectively.

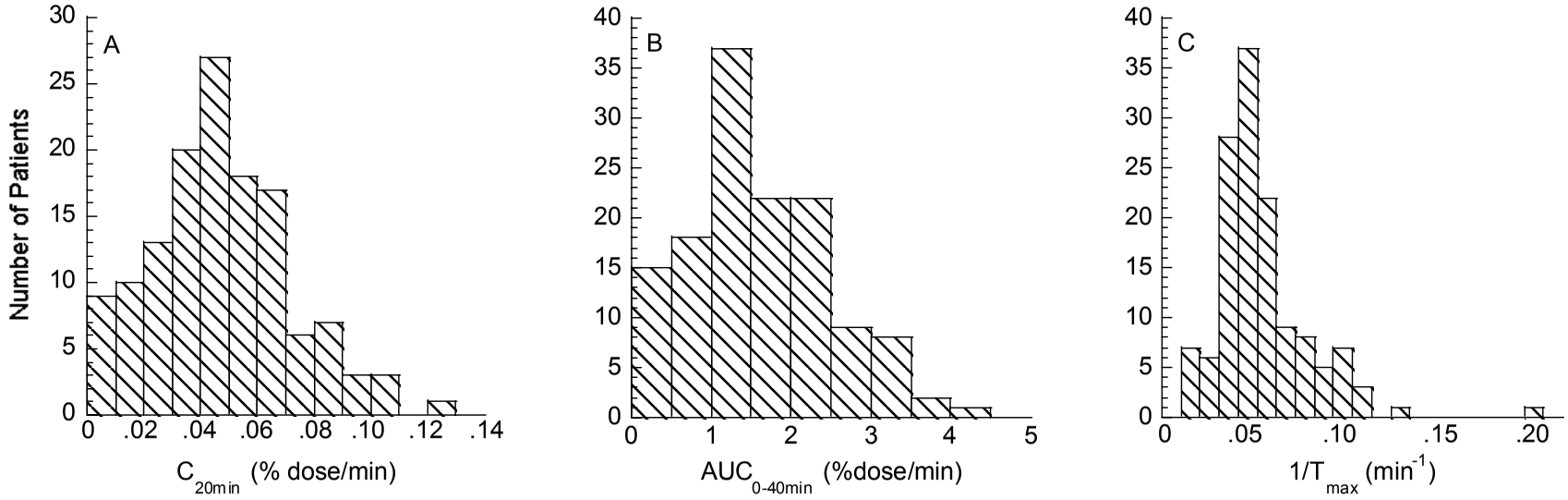

Figure 1. Distribution of ERMBT parameters in 134 cancer patients: (A) C20min, (B) AUC0-40min, and (C) 1/Tmax.

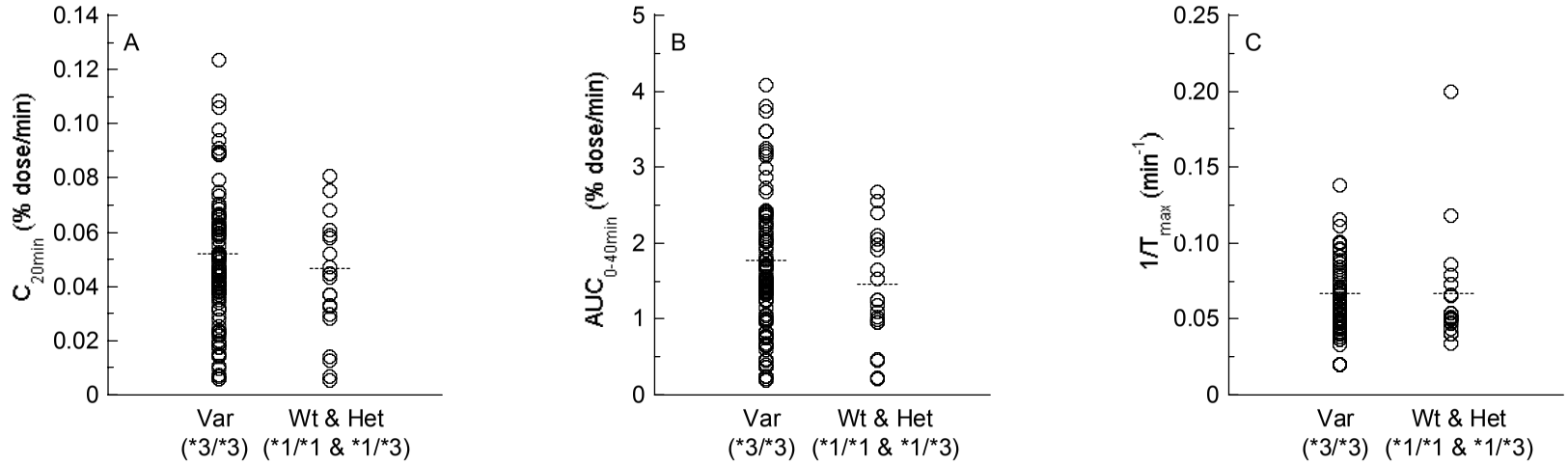

*Figure 2. Genotype-phenotype associations between CYP3A5*3C genotype and the ERMBT parameters (A) C_{20min}, (B) $AUC_{0-40min}$, and (C) $1/T_{max}$ in 103 cancer patients with normal liver function (groups 1 and 2). Lines are mean values*

Table 2. Liver function tests and ERMBT parameters

Parameter	All Patients n = 134	Liver Function Group			
		Group 1 n = 102	Group 2 n = 14	Group 3A n = 12	Group 3B n = 6
Liver Function Tests[a]					
AST (× ULN)		0.71 (0.10 – 3.2)	2.4 (0.40 – 4.8)	3.4 (1.5 – 11)	9.6 (0.40 – 27)
ALT (× ULN)		0.56 (0.10 – 3.4)	2.1 (0.30 – 6.6)	1.8 (0.60 – 3.9)	2.4 (0.20 – 4.5)
AP (× ULN)		0.88 (0.30 – 4.5)	2.9 (1.1 – 9.9)	5.0 (2.5 – 15)	4.3 (0.70 – 8.5)
Total bilirubin (× ULN)		0.40 (0.10 – 1.1)	0.52 (0.20 – 1.4)	0.76 (0.40 – 1.4)	2.4 (1.5 – 5.2)
		P < .001[c]			
C_{20min} (% dose/min)[b]	0.047 (0.043 – 0.051; 0.0021 – 0.12)	0.050 (0.046 – 0.055; 0.0021 – 0.12)	0.055 (0.039 – 0.071; 0.0071 – 0.11)	0.027 (0.016 – 0.038; 0.0060 – 0.052)	0.021 (0 – 0.045; 0.0055 – 0.062)
		P < .001[c]			
$AUC_{0-40min}$ (% dose/min)	1.61 (1.46 – 1.76; 0.082 – 4.09)	1.71 (1.54 – 1.87; 0.82 – 4.09)	1.89 (1.30 – 2.47; 0.23 – 3.81)	0.90 (0.56 – 1.25; 0.20 – 1.63)	0.71 (0 – 1.48; 0.20 – 2.02)
		P < .001[c]			
$1/T_{max}$ (min^{-1})	0.061 (0.057 - 0.066; 0.020 – 0.20)	0.065 (0.060 – 0.070; 0.020 – 0.20)	0.062 (0.049 – 0.076; 0.020 – 0.12)	0.041 (0.032 – 0.050; 0.020 – 0.066)	0.046 (0.028 – 0.064; 0.020 – 0.069)

Abbreviations: n, number of samples; ERMBT, erythromycin breath test, AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase. [a]Data are mean (range); [b]Data are mean (95% confidence interval for mean; range); [c]P values from trend test.

Table 3. Genotype and allele frequencies for CYP3A4/5 genes

Polymorphism	Nomenclature	Description	Genotype Frequencies			n	Allele Frequencies[c]	
			Wt	Het	Var		p	q
CYP3A4 -392A>G[a]	CYP3A4*1B	Promoter	0.90 (n = 106)	0.10 (n = 12)	0.0 (n = 0)	118	0.95	0.05
CYP3A4 831 InsA (exon 9)	CYP3A4*6	Stop codon at 285 site	1.0	0.0	0.0	120	1.0	0.0
CYP3A4 566T>C (exon 7)	CYP3A4*17	F189S[b]	1.0	0.0	0.0	116	1.0	0.0
CYP3A4 878T>C (exon 10)	CYP3A4*18	L293P	1.0	0.0	0.0	122	1.0	0.0
CYP3A5 6986G>A (intron 3)	CYP3A5*3C	Splicing effect	0.02 (n = 2)	0.16 (n = 19)	0.83 (n = 100)	121	0.10	0.90
CYP3A5 14690G>A (exon 7)	CYP3A5*6	Splicing effect	0.98 (n = 119)	0.02 (n = 2)	0.0 (n = 0)	121	0.99	0.01

Abbreviations: n, number of samples; Wt, wild-type sequence; Het, heterozygous variant sequence; Var, homozygous variant sequence.

[a]Number represents position in nucleotide sequence (exon or intron).

[b]Number represents amino acid codon.

[c]Hardy-Weinberg notation was used for allele frequencies (p and q).

For CYP3A4*1B, 106 patients were wild type, 12 patients were heterozygous variant, and no patients were homozygous variant; 4 of 6 African Americans were heterozygous variant.

Predictors of CYP3A Activity

CYP3A activity was reduced by approximately 50% in patients in liver function groups 3A and 3B ($P < .001$ for trend) (Table 2). Consequently, for univariate association analysis between patient characteristics, CYP3A4/5 genotype and CYP3A activity, patients in liver function groups 3A and 3B were excluded (Table 3). CYP3A activity was similar in patients aged 70 years or older compared to those less than 70 years of age ($P > .3726$), at the upper extreme of BSA values ($P > .092$ for trend) and in obese patients ($P > .162$ for trend). CYP3A, as determined from the parameter $AUC_{0\text{-}40min}$, was higher in females compared to male patients (1.92 versus 1.56 % dose/min, $P = .0063$); the ERMBT parameters C_{20min} and $1/T_{max}$ showed a trend for difference according to sex ($P = .0120$ and .0491, respectively). Analysis of AAG values in the 3 quartile groups showed that only the ERMBT parameter $1/T_{max}$ was associated with AAG (lower quartile, mean = 0.077 min^{-1}; interquartile range, mean = 0.065 min^{-1}; upper quartile, mean = 0.055 min^{-1}; $P = .001$ for trend). There was a trend for lower CYP3A activity in patients with AAG concentrations in the upper quartile compared to patients with AAG values below the 75% quantile (C_{20min}, 0.053 versus 0.042 % dose/min, $P = .0481$; $AUC_{0\text{-}40min}$, 1.82 versus 1.43 % dose/min, $P = .0560$; $1/T_{max}$, 0.067 versus 0.055 min^{-1}, $P = .0057$). CYP3A activity was not associated with CYP3A5*3C genotype ($P > .2954$) or CYP3A4*1B genotype ($P > .1680$).

As BSA and BMI were highly correlated ($R^2 = .3794$, $P < .0001$), only BSA was included as a body size indicator in the multivariate models. In addition, because of the small number of observations and similar values for CYP3A activity in liver function groups 3A and 3B, these 2 groups were combined for multiple linear regression analysis. Observations for BSA, sex, liver function group and AAG concentration were available for 126 patients. When CYP3A4 and CYP3A5 genotype were added to the model, observations were available for 115 individuals; because no association was noted between CYP3A4*1B and CYP3A5*3C genotype and ERMBT parameters values ($P > .2719$, Table 4), CYP3A4 and CYP3A5 genotypes were not included in the multiple linear regression analysis. After stepwise backward deletion, age, BSA, sex and were removed from the final model for the 3 ERMBT outcome variables (C_{20min}, $AUC_{0\text{-}40min}$, and $1/T_{max}$).

Table 4. CYP3A activity as a function of patient characteristics and CYP3A4/5 genotype frequencies.[a]

Characteristic	No. of Patients	ERMBT Parameter		
		C_{20min} (% dose/min)	$AUC_{0-40min}$ (% dose/min)	$1/T_{max}$ (min^{-1})
Age (years)		P=.3726[b]	P=.5810[b]	P=.6750[b]
< 70	82	0.049 (0.040 – 0.050)	1.68 (1.50 – 1.86)	0.065 (0.059 – 0.071)
≥ 70	34	0.055 (0.046 – 0.063)	1.83 (1.51 – 2.15)	0.063 (0.056 – 0.070)
Body surface area (m^2)		P=.106[c]	P=.092[c]	P=.318[c]
Lower quartile (< 1.71)	29	0.053 (0.045 – 0.061)	1.82 (1.52 – 2.11)	0.061 (0.052 – 0.070)
Interquartile range	58	0.053 (0.046 – 0.060)	1.81 (1.56 – 2.06)	0.066 (0.058 – 0.073)
Upper quartile (> 2.04)	29	0.044 (0.038 – 0.050)	1.48 (1.25 – 1.70)	0.065 (0.058 – 0.072)
Body mass index (kg/m^2)		P=.220[c]	P=.162[c]	P=.349[c]
< 25 (normal weight)	56	0.053 (0.047 – 0.060)	1.83 (1.60 – 2.06)	0.064 (0.057 – 0.070)
25 – 29.9 (overweight)	43	0.049 (0.041 – 0.056)	1.66 (1.40 – 1.92)	0.063 (0.054 – 0.073)
≥ 30 (obese)	17	0.047 (0.035 - 0.058)	1.57 (1.17 – 2.00)	0.069 (0.059 – 0.078)
Sex		P=.0120[b]	**P=.0063**[b]	P=.0491[b]
Females	63	0.055 (0.049 – 0.062)	1.92 (1.70 – 2.15)	0.070 (0.062 – 0.078)
Males	53	0.047 (0.041 – 0.053)	1.56 (1.35 – 1.77)	0.060 (0.055 – 0.065)

Table 4. – continued –

α-1 acid glycoprotein (×ULN)		P=.170[c]	P=.161[c]	**P=.001[c]**
Lower quartile (< 0.73)	27	0.051 (0.051 – 0.061)	1.76 (1.39 – 2.14)	0.077 (0.064 – 0.091)
Interquartile range	56	0.057 (0.051 – 0.063)	1.93 (1.72 – 2.14)	0.064 (0.059 – 0.069)
Upper quartile (> 1.38)	27	0.042 (0.033 – 0.051)	1.43 (1.12 – 1.74)	0.055 (0.047 – 0.063)
Genotype				
CYP3A5*3C		P = .3573[b]	P = .2954[b]	P = .8928[b]
Wt and Het (*1/*1 and *1/*3)	18	0.046 (0.036 – 0.055)	1.52 (1.18 – 1.86)	0.069 (0.050 – 0.088)
Var (*3/*3)	85	0.052 (0.047 – 0.057)	1.77 (1.59 – 1.94)	0.063 (0.058 – 0.068)
CYP3A4*1B		P = .6330	P = .4496	P = .2719
Wt	91	0.051 (0.046 – 0.056)	1.75 (1.57 – 1.92)	0.064 (0.058 – 0.069)
Het	10	0.047 (0.034 – 0.060)	1.52 (1.05 – 2.00)	0.069 (0.052 – 0.086)

[a]Data are values for mean (95% confidence interval for mean)

[b]P value from nonparametric Wilcoxon test

[c]P value from trend test

AAG and liver function group 3A/B were predictors of CYP3A activity, where the multivariate model explained approximately 18% of overall variation in CYP3A activity (Table 4). Both AAG and liver dysfunction were negatively correlated with CYP3A activity. Liver impairment had the most profound effect on CYP3A activity (coefficient = -.798; P < .001); assuming an average $AUC_{0\text{-}40min}$ value of 1.61 % dose/min, this represents a 50% reduction in CYP3A activity, which is consistent with that observed from the univariate association analysis (Table 2).

Table 5. Multiple regression models for ERMBT parameters[a]

	n	Coefficient	95% Confidence Interval	P value
C_{20min} (% dose/min)				
Intercept		.0621	.0512 to .0729	< .001
α-1 acid glycoprotein (× ULN)	126	-.0107	-.0200 to -.00140	**.024**
Liver function group				
1[b]	99			
2	11	.0134	-.00097 to .0277	.067
3A & 3B	16	-.0228	-.0349 to -.0107	**< .001**
Overall model		R^2 = .1765, P < .0001		
$AUC_{0\text{-}40min}$ (%dose/min)				
Intercept		2.14	1.75 to 2.53	< .001
α-1 acid glycoprotein (× ULN)	126	-.385	-.717 to -.0534	**.023**
Liver function group				
1[b]	99			
2	11	.465	-.0471 to .976	.075
3A & 3B	16	-.798	-1.23 to -.365	**< .001**
Overall model		R^2 = .1720, P < .0001		
$1/T_{max}$ (min^{-1})				
Intercept		.0833	.0722 to .0944	< .001
α-1 acid glycoprotein (× ULN)	126	-.0174	-.0269 to -.00786	**< .001**
Liver function group				
1[b]	99			
2	11	.00580	-.00888 to .0205	.436
3A & 3B	16	-.0192	-.0316 to -.00677	**.003**
Overall model		R^2 = .1799, P < .0001		

Abbreviations: n, number of patients; [a]ERBMT parameter value is lower with a negative coefficient value; [b]Included as baseline category.

Discussion

There has been great interest to evaluate genotyping methods as a tool to predict CYP3A activity in individuals, as this is generally less cumbersome than phenotyping methods and require only one single blood sample. In adults, CYP3A4 and CYP3A5 are the most important among the four CYP3A subfamily members for CYP3A-mediated drug metabolism [13-15], and because of the genetic diversity in the genes encoding these proteins [16], genotyping for CYP3A4 and CYP3A5 variants may be useful for prediction of total hepatic CYP3A activity. The CYP3A5 protein isoform is known to be expressed in only 10 to 30% of Caucasians due to a splice variant in intron 3 of the CYP3A5 gene at nucleotide position 6986 (CYP3A5*3C) [17,18]. Approximately 85 to 95% of the White population and 35 to 45% of the Black population are homozygous for CYP3A5*3C and thus deficient in CYP3A5 [17-19]. Another splice variant (CYP3A5*6), which is observed in black populations also, results in lack of CYP3A5 expression [17,18]. Genetic differences may also explain 60-90% of the observed variation in CYP3A4-mediated drug metabolizing capacity between patients [16]. Over 30 single nucleotide polymorphisms (SNPs) in CYP3A4 have been published, representing alleles CYP3A4*1 to CYP3A4*19, most of which are very rare and unlikely to impact on CYP3A4 activity in vivo. The best characterized variant, a promoter variant with an A to G transition at nucleotide -392 (CYP3A4*1B), was shown *in vitro* to have increased transcriptional activity [16,20] This variant exhibits interethnic variation with allele frequencies of 2-10% in subjects of European descent and 35-84% in subjects of African descent [17,20-37]. Three other CYP3A4 polymorphisms (CYP3A4*6, CYP3A4*17, and CYP3A4*18) have been shown *in vitro* to result in functional changes in CYP3A activity [38,39].

In the present study, no association was noted between the CYP3A5*3C and CYP3A4*1B variants and CYP3A activity. This is similar to findings in healthy subjects using the CYP3A phenotyping probes midazolam [29,34,40,41], erythromycin [34,35], and nifedipine [35,42]. Since the majority of patients studied here were of European descent, one cannot rule out that genetic differences in CYP3A5 and CYP3A4 may contribute to CYP3A activity in African Americans, considering the frequencies of CYP3A5*3C and CYP3A4*1B in this racial/ethnic population. This is not supported, however, by one study involving healthy subjects that included a population where the majority of subjects were African American (34 of 57 subjects); no association between CYP3A5*3C and CYP3A4*1B genotype and CYP3A activity was observed using the ERMBT and by assessment of midazolam

clearance [34]. Due to the lack of CYP3A4*6, CYP3A4*17, and CYP3A4*18 variants in the present study, these polymorphisms most likely have no relevance to CYP3A activity in Caucasian populations.

Even though CYP3A4 variants did not explain variation in CYP3A activity in this predominantly Caucasian population, a consensus is building that human variation in CYP3A4 activity may be caused by polymorphisms in transcription factors that regulate CYP3A4, such as pregnane X receptor (PXR) and constitutive androstane receptor (CAR) [43]. Several variants in human PXR gene, NR1I2, have been identified with altered transactivation activity [18,44]. However, these variants are extremely rare in Black and White populations (0 - 1.6%) and it is unlikely that they explain the variation in CYP3A4 expression.

In the current study, CYP3A activity was unaltered in patients with mild elevations in liver function tests (group 2), but was reduced by approximately 50% in patients with moderate to severe liver impairment as defined for liver function groups 3A and 3B. Interestingly, categorization of liver function tests for group 3A was described by Bruno *et al* for prediction of docetaxel clearance [45,46]; patients with total bilirubin $< 1.5 \times$ ULN but elevations in liver transaminases ($\geq 1.5 \times$ ULN) concurrent with elevated alkaline phosphatase ($\geq 2.5 \times$ ULN) where shown to have reduced docetaxel clearance by 25%. The same categorization of liver function tests to describe liver impairment was also associated with reduced CYP3A activity in the present study. However, liver function tests are not accurate in prediction of CYP3A activity as patients with the lowest CYP3A activity had normal liver function (group 1). The use of the ERMBT as a phenotypic probe has been questioned as conflicting reports on its ability to predict the total body clearance of probe drugs have been reported [4,47], although a study in 20 patients with sarcoma showed that the ERMBT predicted docetaxel clearance in those with greatly reduced drug clearance [48]. Nevertheless, despite the limitations of the ERMBT, results of the present study demonstrate that patients with low breath levels of $^{14}CO_2$ have low CYP3A function and are likely to have reduced CYP3A-mediated drug clearance.

Because of large variations in concentrations of the acute phase reactant protein AAG in cancer patients (7-fold), it has been hypothesized that decreased hepatic clearance by CYP3A in some individuals might be a consequence of an inflammatory response [49]. In the present study, AAG was associated with CYP3A activity in both univariate and multiple regression analysis. However, combined with liver function, only 18% of CYP3A variation was explained by these 2 variables. Previously, a better correlation was observed between C-reactive protein and CYP3A activity compared to

AAG [6]. Assessment of C-reactive protein, a more specific marker of inflammation, may have accounted for more of the variation in CYP3A activity in this population of cancer patients.

In the present study, BSA and BMI were not correlated with CYP3A activity providing further support that BSA has no relevance to anticancer drug dosing [1]. However, alterations in anticancer drug clearance have been noted in obese patients and those at the upper extreme of body size, such as for doxorubicin and docetaxel [50]. Other factors in addition to CYP3A activity, such as changes in volume of distribution, may contribute to altered drug disposition in obese patients [51]. Prospective pharmacokinetic and pharmacodynamic evaluations of anticancer drugs in obese patients are required to determine the effect of altered body size and composition on drug disposition and determination of alternative doseing strategies.

The influence of age on the expression and activity of drug-metabolizing enzymes remains controversial with reports describing either a decline in activity or no change in activity in elderly patients [52-54]. In the current study, CYP3A activity was shown to be similar in patients aged less than 70 years (n = 99) and 70 years or older (n = 35). Prior *in vitro* studies have suggested an age related decline in CYP3A activity [55]. However, our results are consistent with an *in vivo* study that applied the ERMBT as a phenotyping probe of CYP3A-mediated drug clearance where no decrease in CYP3A activity was observed as a function of age in 39 older hypertensive men [54].

Many drugs that are substrates of CYP3A show higher clearance in women than in men [56-58]. In concordance with this observation, previous studies have shown approximately 20 to 25% higher CYP3A activity in females than males using the ERMBT [8,34,52]. However, it has been suggested that this observation is due to one limitation of the ERMBT, the assumption that individuals (both females and males) produce 5 mmol of CO_2/min per m^2 at rest [10]. Reanalysis of previously published data evaluating the calculation of CO_2 output in different populations revealed an approximately 20% lower rate of CO_2 production in females, which is consistent with the 20 to 25% difference observed in ERMBT results between the two sexes [10]. In the present study, females were found to have approximately 15 to 20% higher CYP3A activity than males in univariate analysis, but the association was not significant in multiple regression analysis. Interestingly, a recent study of 94 surgical liver samples found 2-fold higher CYP3A4 protein content and higher expression of CYP3A4 messenger RNA transcripts in female compared with male samples [59]. Regardless of apparent sex-related differences in CYP3A activity, the same range of wide interpatient variation in CYP3A activity was observed in both female and male

cancer patients (Table 4), indicating that dosing strategies for drugs cleared by CYP3A should focus on the individual and not necessarily sex.

One additional factor to consider is the influence of drug-interactions on interpatient variation in CYP3A activity. There is considerable motivation for understanding adverse drug interactions with anticancer agents because of their narrow therapeutic index. Usually, such interactions arise as a result of altered pharmacokinetics of the drugs involved. Careful examination of the concomitant drug profiles for drugs that are substrates, inhibitors or inducers of CYP3A did not reveal an association with results of the ERMBT in the entire population or upon analysis of values in the lower and upper quartiles. In addition, many botanical dietary supplements contain pharmacologically active phytochemicals, and to date, numerous herb-drug interactions have been described in the medical literature [60,61], and are most commonly pharmacokinetic in nature. Availability of this type of information is particularly relevant to the treatment of cancer patients who are known to take a wide variety of complementary and alternative medicine (CAM) concomitantly with their chemotherapeutic regimen [62]. However, little information is available from prospective evaluation of drug interactions between CAM and anticancer agents to help guide the use of CAM in cancer patients receiving chemotherapy [63]. To complicate matters further, the current study protocol conducted in the United States and the Netherlands included a list of CAM to review with each patient prior to study enrollment; however, of 80 patients enrolled on this protocol, no patients were documented to be taking CAM. Since various studies have documented the extensive use of CAM in cancer patients, and that fewer than 40% of patients disclose their herbal supplement usage to health care providers [64], ways to ensure accurate and complete documentation of CAM use by patients enrolled to clinical trials is urgently needed.

In conclusion, the current investigation has identified liver function and AAG concentration in plasma as significant predictors of CYP3A activity in patients with cancer. However, these two factors combined explained only 18% of overall variation in CYP3A activity. Consideration of additional factors, including C-reactive protein as well as the concomitant use of other drugs, food constituents, and CAM with inhibitory and inducible effects on CYP3A, may account for variation in CYP3A activity in cancer patients. Furthermore, the influence of genetic polymorphisms in transcription factors that regulate the inhibition and induction of CYP3A expression by co-administered agents is currently not known. In light of the present results noting no significant genotype-phenotype associations, CYP3A phenotyping strategies should

provide the most clinically relevant information reflecting the combined effects of genetic, environmental, and endogenous/ physiological factors on drug disposition and effects.

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Chapter 6

Clinical Pharmacokinetics of Unbound Docetaxel: Role of Polysorbate 80 and Serum Proteins

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Abstract

Objective: Our objectives were to study the extent of docetaxel binding to plasma in the presence and absence of its excipient, polysorbate 80 (Tween 80), in vitro and to evaluate the pharmacokinetics of unbound docetaxel in vivo.

Methods: Equilibrium-dialysis was used for determination of the fraction unbound docetaxel (f_u), and was applied to study the pharmacokinetic behavior of unbound docetaxel in 23 cancer patients receiving an intravenous infusion of the drug formulated in polysorbate 80 (Taxotere).

Results: Polysorbate 80, added at clinically relevant concentrations (up to 1.0 μL/mL), increased f_u in vitro by 13% (7.84 ± 0.0752% versus 6.95 ± 0.0678%, $P < 0.00001$). Similarly, f_u calculated on the basis of the observed AUC values [f_u(AUC)] in vivo was 12% higher than f_u in pretreatment samples [f_u(pre)] (6.00 ± 1.03% versus 5.49 ± 1.01%, $P = 0.38$). Of various serum proteins evaluated, only α_1-acid glycoprotein was significantly related to f_u ($P < 0.0018$), with higher f_u in the presence of lower protein levels. Total docetaxel clearance was related to α_1-acid glycoprotein ($R^2 = 0.13$, $P = 0.58$), f_u(pre) ($R^2 = 0.15$, $P = 0.39$) and f_u(AUC) ($R^2 = 0.29$, $P = 0.0048$).

Conclusion: This study demonstrates that the plasma binding of docetaxel is influenced by both α_1-acid glycoprotein and its formulation vehicle. Further investigation is required to resolve the potential clinical significance of these observations.

Introduction

The use of nonionic surfactants like Cremophor EL and polysorbate 80 (Tween 80) as pharmaceutical formulation vehicles has become an area of increasing interest in the development of poorly water-soluble agents and has significant implications for their pharmacokinetic behavior [1,2]. There are several factors contributing to the complexity of the pharmacological handling of drugs delivered by such vehicles after intravenous administration, including the fact that the circulating drug is present in 3 distinguishable forms (i.e., vehicle associated, protein-bound, and protein-unbound) and that clearance occurs as a result of various processes with different elimination rates (i.e., distribution of vehicle micelles carrying the drug, leaking of drug from the micelles, and clearance of unbound drug). Therefore it has been argued that pharmacokinetic studies with such agents limited to the analysis of total drug concentrations in plasma are not informative enough and may even be misleading,

because the pharmacodynamic effects are mainly related to the level of free drug in plasma [3]. A very small number of reports have addressed this issue for anticancer agents formulated in nonionic surfactants, including the antimicrotubule agent paclitaxel (Taxol), which is formulated for clinical use in a mixture of Cremophor EL-ethanol. These studies have demonstrated that the principal fraction of drug present in the circulation remains entrapped within the hydrophobic interior space of the carrier system [4,5]. In contrast to paclitaxel, the only other approved taxane, docetaxel (Taxotere), is formulated in a vehicle containing polysorbate 80 (Tween 80), and clinical data on binding interactions for this agent are essentially lacking [6]. Docetaxel was shown previously to bind extensively in vitro to serum proteins, including α_1-acid glycoprotein (AAG), albumin and lipoproteins, in a concentration-independent manner [7], with high AAG levels being associated with both a decrease in the unbound drug fraction in vitro and a decrease of total docetaxel clearance in vivo [7,8]. Although these combined effects were thought not to result in marked changes in systemic exposure to unbound docetaxel [9], a restriction of drug distribution in patients with high AAG or polysorbate 80 levels cannot be ruled out and could explain the decreased hematologic toxicity observed in these patients [10,11]. To further clarify the role of polysorbate 80 and serum proteins in docetaxel pharmacology, we now describe the development and validation of a novel assay for separation of unbound and bound (i.e., polysorbate 80 plus protein-associated) drug in plasma, as well as its implementation in a retrospective analysis of clinical samples from cancer patients treated with 1-hour docetaxel infusions.

Methods

Chemicals and reagents

Reference standards of docetaxel and paclitaxel were kindly supplied by Aventis (Vitry-sur-Seine Cedex, France) and Bristol-Meyers Squibb (Woerden, the Netherlands), respectively. An aliquot of the docetaxel powder was used to synthesize generally-labeled [^{3}H]docetaxel (specific activity, 7.2 Ci/mmol; concentration, 2 mCi in 2 mL of ethanol), and was purchased from Moravek Biochemicals Inc. (Brea, Ca). The product was found to be radiochemically pure (~96.5%) as determined by reversed-phase high-performance liquid chromatography, and did not contain any detectable levels of contaminating tritiated water (not shown). Polysorbate 80 was obtained from Sigma (St. Louis, Mo), acetonitrile and methanol from Biosolve (Valkenswaard, the Netherlands), ammonium acetate and formic acid from Baker

(Deventer, the Netherlands), dimethyl sulfoxide, n-butyl chloride and tetrahydrofuran from Rathburn (Walkerburn, UK), and absolute ethanol from Merck (Darmstadt, Germany). Phosphate-buffered saline, pH 7.4 (PBS) was from Oxoid (Basingstoke, UK), and Emulsifier-Safe scintillation cocktail from Packard Biosciences BV (Groningen, the Netherlands). Water was filtered and deionized by a Milli-Q-UF system delivered by Millipore (Milford, Ma) and used throughout.

Assay methods

Analytical measurement of total docetaxel concentrations was performed as described previously [12], whereas concentrations of [^{3}H]docetaxel in aqueous solutions or biological samples were analyzed for total radioactivity (^{3}H-cpm) by liquid-scintillation counting on a 1409 counter (Wallac Oy, Turku, Finland), until a preset time of 20 minutes was reached. Determination of the fraction unbound (f_u) docetaxel in human plasma was performed using a previously published method for unbound paclitaxel [13]. In the final optimized method, 260-µL aliquots of plasma were dialyzed against an equal volume of PBS containing a [^{3}H]docetaxel tracer (i.e., a 25,000-fold dilution of the ethanolic stock in PBS) over a membrane with a 12,000-14,000-dalton molecular weight cut-off (Spectrum Medical, Houston, Tx). Dialysis experiments were performed using 2-mL polypropylene Safe-Lock vials (Eppendorf, Hamburg, Germany) as dialysis chamber in a humidified atmosphere of 5% CO_2 at 37°C [14]. After the dialysis period, 150 µL of the PBS fraction (containing unbound docetaxel) and 25 µL of the plasma fraction (containing both bound and unbound drug) were transferred to separate 2-mL vials. To the latter, 125 µL of PBS were added, followed by addition of 2 mL of scintillation cocktail to all samples, vigorous mixing, and liquid-scintillation counting. The f_u, expressed as a percentage, was calculated as follows:

$$f_u = [(^3\text{H-cpm PBS} - {}^3\text{H-cpm blank}) / (^3\text{H-cpm plasma} - {}^3\text{H-cpm blank})] \times 100 \quad \text{(Eq. 1)}$$

The equilibrium dialysis method was validated by use of 2 quality-control samples prepared from plasma obtained from a healthy volunteer. One quality-control sample did not contain docetaxel, and one contained unlabeled docetaxel at a concentration of 1 µg/mL, which represents a clinically relevant concentration achieved at the end of a 1-hour docetaxel infusion. The within-run precision and between-run precision were calculated after analysis of the samples in quintuplicate on 3 separate occasions, as described previously [12]. The recovery of the radiolabel was calculated by comparing

the total amount of [^{3}H]docetaxel observed in the plasma and PBS fractions after dialysis to those when added to PBS.

To eliminate any extent of quenching, a fixed volume of 25 µL of human plasma was mixed with 125 µL of PBS before quantitation in all further experiments. The time to equilibrium of docetaxel between proteins and plasma water was reached within 48 hours after start of incubation. The f_u docetaxel was not significantly different with or without the addition of unlabeled docetaxel, with mean (± SD) values of 6.81 ± 0.312% and 7.01 ± 0.487%, respectively. This suggests that within a clinically relevant range the docetaxel concentration has no influence on the unbound fraction, which is consistent with previous findings [7]. With the final method, values for within-run and between-run precisions were less than <7.04%. It was confirmed that the total drug recovery was equal to amount of [^{3}H]docetaxel added to PBS (i.e., >97.5% recovery). The effect of polysorbate 80 on docetaxel binding was evaluated in triplicate at final polysorbate 80 concentrations in the expected clinically relevant range of 0.010 to 1.0 µL/mL.

AAG concentrations in plasma samples were measured by use of a turbidimetric assay on a Hitachi 911 clinical chemistry analyzer (Roche, Basel, Switzerland). The reagents, calibrator standard, and the normal range controls were obtained from INstruchemie (Delfzijl, the Netherlands), whereas liquicheck I (Biorad, Veenendaal, the Netherlands) was used as low-range control material. The assay showed linearity between 0.10 and 3.00 g/L, and the between-run variation coefficients ranged from 2.0 to 2.8%.

Patient samples

Plasma samples were obtained from 23 patients participating in a clinical trial in which docetaxel was administered in combination with intravenous methotrexate. The eligibility and exclusion criteria and detailed clinical profiles have been documented elsewhere [15]. Docetaxel was preceded by an intravenous bolus of methotrexate and was given as a 1-hour intravenous infusion (mean, 1.02 ± 0.112 hours; range, 0.73 – 1.27 hours) on day 1 (n = 4) or day 2 (n = 19) at dose levels of 75 (n = 19) or 85 mg/m^2 (n = 4). Blood samples for pharmacokinetic analysis were drawn from a vein in the arm contralateral to that used for drug administration. Samples were obtained at the following time points: immediately before docetaxel administration, at 30 minutes after the start of infusion, at the end of the infusion (typically at 1 hour), and at 1, 3, 5 and 23 hours after the end of infusion. Blood was collected in glass tubes containing lithium heparin as anticoagulant and was immediately centrifuged to separate plasma,

which was stored at –80°C until analysis. A previous analysis has shown that the pharmacokinetic profile of docetaxel is unaffected by methotrexate in the dose range studied (30-50 mg/m^2), and is similar to single agent data [6,15]. The study protocol was approved by the Ethics Board of the Erasmus MC (Rotterdam, the Netherlands), and written informed consent before study entry was obtained from all patients.

Pharmacokinetic analysis

The individual AUC values of total and unbound docetaxel were calculated by noncompartmental analysis up to the last measurable time point (T_{last}) by use of the linear-trapezoidal rule. The AUC values were extrapolated to infinity by dividing T_{last} by the terminal disposition rate constant (λ_z), determined from the slope of the terminal phase of the concentration-time profile. Clearance was calculated by dividing the dose administered (in mg/m^2) by the observed AUC, and the unbound fraction on the basis of the observed AUC values [f_u(AUC)] was calculated as follows:

$$f_u(AUC) = \text{AUC unbound docetaxel} / \text{AUC total docetaxel} \qquad \text{(Eq. 2)}$$

Pharmacokinetic parameters were estimated using the software package Siphar v4.0 (InnaPhase, Philadelphia, Pa), and statistical analyses were done with Number Cruncher Statistical System 2001 (NCSS, Kaysville, UT). The cut-off level for statistical significance was taken at 0.05.

Table 1. Extent of binding of docetaxel to human plasma, as a function of polysorbate 80

Polysorbate 80 µl/ml	Fraction unbound mean ± SD (%)	Percentage of reference value[1]
none	6.95 ± 0.0678	100%
0.010	6.34 ± 0.0241	91% *
0.025	6.50 ± 0.182	93% *
0.050	6.32 ± 0.281	91% *
0.10	6.44 ± 0.198	93% *
0.25	6.94 ± 0.154	100%
0.50	7.55 ± 0.189	109% **
1.0	7.84 ± 0.0752	113% **

1 None is considered as the reference value (i.e., no polysorbate 80 added)

* Significantly lower than none; ** Significantly higher than none (one-way ANOVA, followed by Duncan's multiple range test); Data are presented as mean values ± SD of triplicate observations [except for none, 0.010, and 0.50 µL/mL (duplicate each)].

Table 2. Patient demographic data (n=23)

Characteristic	Median	Range
Baseline screening		
Age (years)	47	27-66
Sex (male/female)*	10 / 13	
BSA (m^2)	1.79	1.50-2.18
Height (cm)	173	152-192
Weight (kg)	69	53-97
WHO performance status	1	0-2
Pre-treatment hematology		
Hemoglobin (mM)	7.2	5.8-9.8
Hematocrit (%)	35	31-42
Leukocytes ($\times 10^9$/L)	8.3	4.9-22
Neutrophils ($\times 10^9$/L)	5.6	2.9-20
Platelets ($\times 10^9$/L)	352	171-545
Pre-treatment clinical chemistry		
AST (units/L)	21	12-75
ALT (units/L)	15	3-76
ALP (units/L)	93	37-178
GGT (units/L)	41	13-98
Total bilirubin (μM)	6	3-12
Serum creatinine (μM)	86	66-130
AAG (g/L)	1.40	0.58-2.06
Albumin (g/L)	36	30-41
High-density lipoprotein (mM)	1.2	0.8-1.8
Low-density lipoprotein (mM)	3.2	1.7-5.3

Abbreviations: n, total number of patients studied; BSA, body-surface area; WHO, World Health Organization; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; AAG, α_1-acid glycoprotein.

* Data indicate number of patients.

Results

In vitro results

As predicted by earlier experiments [7], docetaxel was found to bind extensively to human plasma in vitro in the absence of polysorbate 80. In the presence of polysorbate 80, the f_u docetaxel significantly changed in an apparent concentration-dependent, biphasic manner; at low concentrations of polysorbate 80, a small decrease in the f_u docetaxel was observed, whereas at higher concentrations, an increase was noted (Table 1). The highest f_u relative to the reference value was observed at a polysorbate 80 concentration of 1.0 µL/mL (Table 1), which caused an increase of 13% (7.84 ± 0.0752% versus 6.95 ± 0.0678%, P < 0.00001, 1-way ANOVA).

In vivo results

Equilibrium-dialysis was applied to plasma samples of 23 patients treated with docetaxel given as a 1-hour infusion (Table 2). The concentration-time profile of unbound docetaxel followed the same general pattern as that of total docetaxel levels. The overall mean value for unbound docetaxel clearance was relatively consistent in all patients (Table 3), suggesting a moderate degree of interindividual variability (coefficient of variation, 22.7%).

Table 3. Summary of docetaxel pharmacokinetics (n=23)[*]

Parameter	Median	Mean ± SD	Range
CL total (L/h/m^2)	17.9	18.9 ± 5.50	10.7 – 33.0
CL unbound (L/h/m^2)	300	315 ± 71.4	188 – 446
$fu_{(pre)}$ (%)	5.38	5.49 ± 1.01	3.93 – 7.93
$fu_{(Cmax)}$ (%)	6.18	6.37 ± 1.50 [**]	3.91 – 10.3
$fu_{(AUC)}$ (%)	6.05	6.00 ± 1.03 [***]	4.12 – 8.06

Abbreviations: n, total number of patients studied; CL, apparent clearance; $fu_{(pre)}$, fraction unbound docetaxel in pre-treatment plasma samples; $fu_{(Cmax)}$, fraction unbound docetaxel at the peak concentration; $fu_{(AUC)}$, fraction unbound docetaxel based on the unbound drug to total drug AUC ratio; AUC, area under the plasma concentration-time curve.

[*] Based on non-compartmental analysis; [**] P = 0.012 versus $fu_{(pre)}$; [***] P = 0.038 versus $fu_{(pre)}$ (paired T-test).

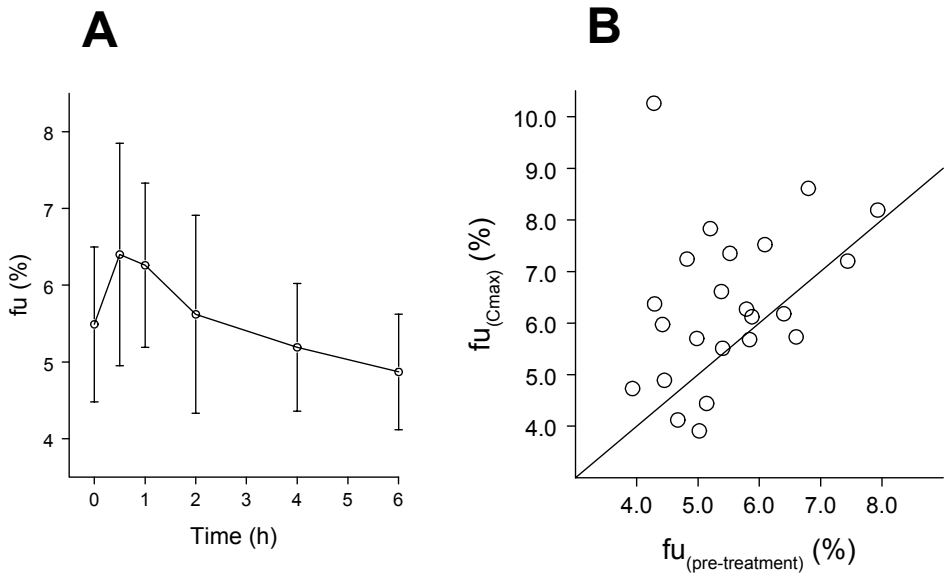

Figure 1. (A) Pattern of the fraction unbound docetaxel over time in all studied patients (mean ± SD), and (B) a scatterplot of the fraction unbound docetaxel in pre-treatment plasma samples [fu(pre-treatment)] and at docetaxel peak concentration [fu(Cmax)] in those patients. The solid line represents the line of identity.

The f_u docetaxel in pretreatment plasma samples (i.e. the in vitro assessment of binding capacity) was also moderately variable, and ranged from 3.93% to 7.93% (coefficient of variation, 18.4%). This fraction was time-dependent and increased during the infusion (Figure 1A), resulting in a 12% higher f_u on the basis of the AUC ratio of unbound to total drug, as compared with that obtained in individual pre-treatment plasma samples (6.00 ± 1.03% versus 5.49 ± 1.01%, P = 0.038) (Table 3). The estimate of the highest f_u in vivo (i.e., the fraction observed at peak polysorbate 80 concentrations) was on average 16% higher as compared with the pretreatment values (6.37 ± 1.50% versus 5.49 ± 1.01%, P = 0.012) (Table 3 and Figure 1B). Of the various serum proteins analyzed in pretreatment samples, only AAG concentration was significantly correlated with the observed f_u docetaxel at pretreatment both in univariate (P = 0.0015) and multivariate regression analyses (P = 0.0018), with higher

f_u values in the presence of lower AAG concentrations (Table 4 and Figure 2). Relationships were also observed between AAG concentrations and clearance of total docetaxel (R^2 = 0.13, P = 0.058), the f_u docetaxel in pretreatment samples (R^2 = 0.15; P = 0.039), and the f_u docetaxel determined based on the AUC ratio of unbound to total drug (R^2 = 0.29; P = 0.0048) (Figure 3).

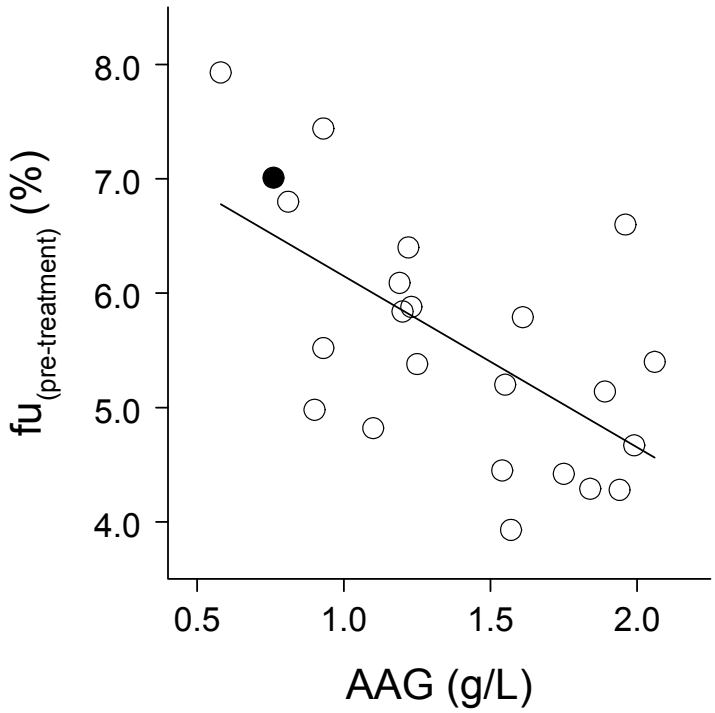

Figure 2. Relationship between AAG and the fraction unbound docetaxel in pre-treatment plasma samples [fu(pre)]. Open symbols indicate individual patient data, and the closed symbol represents the quality-control sample without docetaxel. The solid line is the fit from linear regression analysis.

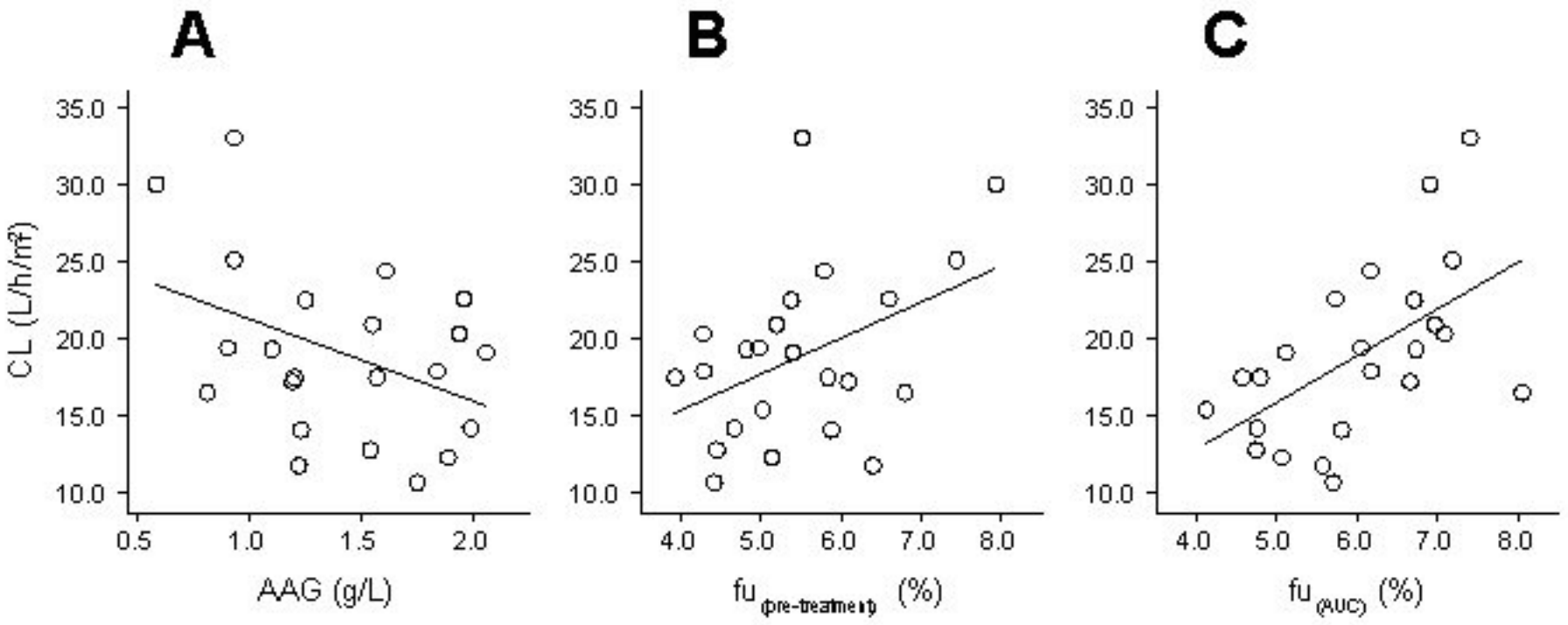

Figure 3. Relationships between (A) AAG concentration, (B) the fraction unbound docetaxel in pre-treatment samples [fu(pre-treatment)], and (C) the AUC ratio of unbound to total drug [fu(AUC)] and the clearance of total docetaxel.

Table 4. Relationships between patient characteristics and docetaxel pharmacokinetics

Characteristics			R^{2*}	P^{**}
AAG (g/L)	vs	$fu_{(pre)}$ (%)	0.36	0.0015
Albumin (g/L)	vs	$fu_{(pre)}$ (%)	<0.01	0.73
High density lipoprotein (mM)	vs	$fu_{(pre)}$ (%)	<0.01	0.30
Low density lipoprotein (mM)	vs	$fu_{(pre)}$ (%)	<0.01	0.41
AAG (g/L)	vs	CL total (L/h/m^2)	0.13	0.058
$fu_{(pre)}$ (%)	vs	CL total (L/h/m^2)	0.15	0.039
$fu_{(AUC)}$ (%)	vs	CL total (L/h/m^2)	0.29	0.0048

Abbreviations: AAG, α_1-acid glycoprotein; $fu_{(pre)}$, fraction unbound docetaxel in pre-treatment plasma samples; $fu_{(AUC)}$, fraction unbound docetaxel based on the unbound drug to total drug AUC ratio; AUC, area under the concentration-time curve; CL, apparent clearance.
* Regression coefficient for a least-squares linear-regression analysis; ** Probability value for a least-squares linear-regression analysis.

Discussion

The standard analytical methods for measuring concentrations of drugs (including docetaxel) in plasma determine concentrations of drug bound to plasma proteins or other macromolecules (e.g., formulation vehicles), as well as free drug dissolved in plasma water [3]. For this reason, the relationship between total drug concentration in plasma and treatment outcome (i.e., toxicity and efficacy) will only be good if the degree of binding of the agent is constant over time and in the clinically relevant concentration range,or if so little drug is protein-bound that changes in binding result in insignificant changes in unbound concentration. Previous in vitro studies demonstrated that docetaxel is mainly bound to albumin and AAG, an acute-phase reactant, the concentration of which is highly variable in cancer patients [16], and that this latter protein is the main determinant of variability in docetaxel plasma binding [7]. Because only the unbound drug fraction is directly available for cellular partitioning and disposition processes, AAG concentrations might be a predictor of docetaxel pharmacokinetics. Indeed, Bruno et al. [8] have shown that AAG was a significant covariate on total docetaxel clearance in a population pharmacokinetic

model, in which high AAG levels were related to low docetaxel clearance. Likewise, AAG was shown previously to be a predictor of the severity of drug-induced neutropenia, in which low AAG levels were related to increased severity of neutropenia [10], as well as efficacy and survival in patients with non-small-cell lung cancer, in whom high AAG levels were related to worse outcome [11].

To further study the clinical pharmacological behavior of docetaxel, a reliable method for the determination of unbound docetaxel fractions in human plasma samples was developed and validated, based on experience with a previous equilibrium dialysis assay for measurement of the f_u paclitaxel in patient samples [13]. Despite the longer period of incubation needed to reach equilibrium when the radiolabeled docetaxel tracer is added to the buffer compartment, this method is less laborious and time-consuming because of the simultaneous addition of [^{3}H]docetaxel-containing PBS to all samples. Moreover, by estimating the recovery of the total amount of radioactivity added, the accuracy of the procedure can be evaluated. For example, in cases in which hemolytic plasma samples are to be analyzed, these can be diluted in blank plasma prior to the liquid-scintillation counting after dialysis, because slightly discolored samples will result in lower counts and, thus, an overestimation of the f_u docetaxel.

The in vitro binding of docetaxel to human plasma in the absence of polysorbate 80 was approximately 93% (unbound drug fraction, 6.95 ± 0.0678%), which is similar to the 92 – 94% reported previously with ultrafiltration techniques [7]. This degree of binding to serum proteins appears to be higher than that of the related agent paclitaxel, which is 87% bound in vitro [13] and 85% in vivo [17] in the absence of its formulation vehicle Cremophor EL. In contrast to earlier observations [7], we found that polysorbate 80 at clinically relevant concentrations increases the f_u docetaxel from 6.95% to 7.84% in vitro and on average from 5.49% to 6.37% at docetaxel peak concentration in vivo. This increase in vitro was observed at concentrations above those used by Urien et al. in a previous study (i.e. 0.20 µL/mL) [7].

Recent investigations into the pharmacokinetic behavior of polysorbate 80 after docetaxel administration have shown peak levels of 0.30 mg/mL (i.e. ~0.28 µL/mL) at a dose of 35 mg/m^2 administered as a 30-min intravenous infusion, as measured using liquid chromatography coupled with tandem mass-spectrometric detection [18]. The decline in polysorbate 80 levels after the end of infusion was rapid, with concentrations falling below 0.10 µL/mL within 30 minutes and below 0.010 µL/mL within 3 hours after the end of infusion. Overall, these results suggest that concentrations of polysorbate 80 during drug infusion in the current study, in which

docetaxel was administered at doses of 75 and 85 mg/m^2, might be high enough to have an impact on the unbound fraction of docetaxel in patients. This is in keeping with recent observations that for docetaxel a minor deviation from linear distribution pathways exists that may be related to the presence of the nonionic surfactant [19].

The mechanistic basis for the altered binding of docetaxel in the presence of high polysorbate 80 concentrations is as yet unclear. It is possible, however, that with time polysorbate 80 is able to form complexes with proteins so that the binding of docetaxel to plasma proteins becomes saturable on single sites [20]. Alternatively, it cannot be excluded that this effect is the result of a displacement interaction caused by some (degradation) components of polysorbate 80. Similar observations have been reported for the binding of several other drugs that bind with high affinity but low capacity to AAG in the presence of structurally-related mixed-micellar systems [21]. Interestingly, the effect of polysorbate 80 appears to be biphasic both in vitro and in vivo, with a slight decrease in the f_u at low concentrations of the vehicle. The mechanism underlying this phenomenon is unknown. However, it is possible that two competing polysorbate 80-mediated mechanisms affect the f_u docetaxel in opposite directions; that is, micellar encapsulation of docetaxel (resulting in a reduced f=u docetaxel) and binding-displacement by polysorbate 80 degradation products (resulting in an increased f_u docetaxel). The former process might be predominating at low polysorbate 80 concentrations, whereas the latter may become increasingly important as a result of a proportionally increased release of polysorbate 80 degradation products, which interfere with the normal binding of docetaxel to plasma proteins. Most important is that the net effect over the entire sampling interval in vivo was a moderate increase in the f_u docetaxel (from 5.49% to 6.00%). This overall increase is consistent with clinical data from a dose-finding study with a new submicronic polysorbate 80-free dispersion formulation of docetaxel (XRP6976L; Aventis), which suggests a lower incidence and severity of the dose-limiting hematologic toxicity at equimolar doses compared with the current polysorbate 80-containing formulation of docetaxel [22].

Given that docetaxel is bound in plasma to albumin, AAG and high-density lipoproteins [7], we investigated the impact of the pretreatment levels of these proteins on drug disposition. A significant inverse relationship was observed between AAG concentration and f_u docetaxel in pretreatment samples, with higher fractions in the presence of lower AAG concentrations, as has also been found in previous in vitro experiments [7]. No correlations were observed with other investigated proteins in both univariate and multivariate analyses. Similar to data from a population model for docetaxel pharmacokinetics [8], AAG concentration was related to clearance of total

docetaxel. Furthermore, the f_u docetaxel in pretreatment samples was correlated with total clearance, and the correlation improved when the AUC ratio of unbound to total drug was related to the total clearance. This suggests that AAG concentration is not the sole predictor of the f_u during docetaxel treatment. Interestingly, the f_u docetaxel did not alter during the infusion in all patients, which might be explained by the high degree of interindividual variability in the rate of serum esterase-mediated metabolic breakdown of polysorbate 80 [23,24].

In conclusion, this study confirms previous observations regarding the involvement of AAG in the binding of docetaxel, and demonstrates that the f_u docetaxel in human plasma is also influenced by the presence of its formulation vehicle, polysorbate 80, in a concentration-dependent, biphasic manner. Future studies will focus on the potential clinical significance of this latter observation in addition to the mechanisms underlying the altered docetaxel plasma binding in the presence of polysorbate 80.

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Chapter 7

Simultaneous Analysis of Docetaxel and the Formulation Vehicle Polysorbate 80 in Human Plasma by LC/MS/MS

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Abstract

An analytical procedure is described for the simultaneous determination of the anticancer agent docetaxel (Taxotere) and its formulation vehicle polysorbate 80 (Tween 80) in human plasma samples. Sample pretreatment involved a double liquid-liquid extraction step with a mixture of acetonitrile/*n*-butyl chloride (1/4, v/v). Separation of the compounds of interest, including the internal standard paclitaxel, was achieved on a reversed-phase Waters X-Terra MS column (50 × 2.1 mm internal diameter) packed with a 3.5-µm octadecyl stationary phase, using isocratic elution. Detection of docetaxel and polysorbate 80 was performed using tandem MS detection with electrospray ionization. Validation results indicated that the method is accurate and precise, and has lower limits of quantitation of 0.500 nM (~0.4 ng/ml) and 1.00 µg/ml for docetaxel and polysorbate 80, respectively. The method was subsequently used to measure concentrations of docetaxel and polysorbate 80 in plasma samples in support of a project to assess the influence of polysorbate 80 concentrations on the disposition and toxicity profile of docetaxel in cancer patients receiving Taxotere.

Introduction

Docetaxel (Taxotere) is an antineoplastic agent that acts by disrupting the microtubular network, and is one of the most active agents in the treatment of locally advanced or metastatic breast and non-small cell lung cancer [1,2]. Elimination routes of docetaxel are mediated by the cytochrome P450 (CYP) 3A isoforms, notably CYP3A4 and CYP3A5 [3], and the membrane transporter P-glycoprotein (ABCB1) [4]. It has been suggested that variability in expression of these proteins accounts for the substantial interindividual differences in drug clearance [5], and the severity of drug-induced neutropenia as well as efficacy and survival in patients treated with standard doses of docetaxel [6]. For clinical use, docetaxel is formulated in the nonionic surfactant polyoxyethylene-20-sorbitan monooleate (polysorbate 80; Tween 80). In recent years, substantial evidence has been generated that polysorbate 80 is a biologically and pharmacologically active compound [7]. It has been shown that polysorbate 80 has intrinsic antitumor activity [8], and its use has been implicated in the occurrence of severe anaphylactoid hypersensitivity reactions and cumulative fluid retention associated with docetaxel therapy [9]. More recently, it was demonstrated that polysorbate 80 interferes with the normal binding of docetaxel to serum proteins in a concentration-dependent manner, and can modulate the pharmacokinetics of

docetaxel in vivo [10]. As part of a project to further assess the importance of polysorbate 80 with respect to the disposition and toxicity profile of docetaxel, we report here on the development and validation of an analytical method that allows the simultaneous determination of docetaxel and polysorbate 80 in human plasma samples.

Materials and methods

Chemicals

Docetaxel (lot #9915420; purity, 99.5%; Figure 1A) was obtained from Aventis Pharma (Vitry-sur-Seine Cedex, France). Polysorbate 80 (lot #99H01002; Fig. 1B) and the internal standard paclitaxel (lot #061K1158) were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Formic acid (98%, v/v in water) was obtained from J. T. Baker (Phillipsburg, NJ, USA), acetonitrile (HPLC grade) and methanol (HPLC Grade) from EM Science (Gibbstown, NJ, USA), and *n*-butyl chloride from Burdick & Jackson (Muskegon, MI, USA). Deionized water was obtained from a Milli-Q-UF system (Millipore, Milford, MA, USA) and used throughout in all aqueous solutions. Drug-free (blank) human plasma originated from Pittsburgh Blood Plasma, Inc. (Pittsburgh, PA, USA).

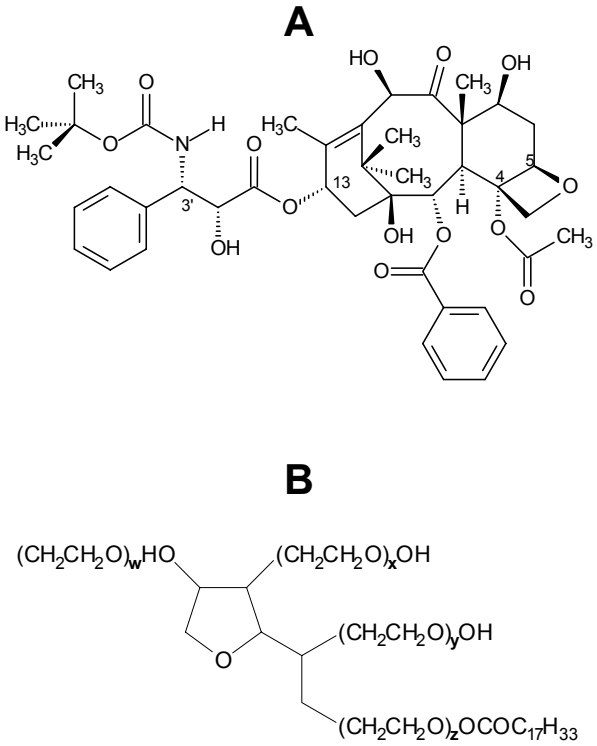

Figure 1. Chemical structures of (A) docetaxel and (B) polysorbate 80 (w + x + y + z ~ 20).

Drug solutions

Stock solutions of docetaxel (M_r 807) at a concentration of 1 mM were prepared in duplicate by dissolving 8.07 mg in 1.0 mL of 50% methanol/water (50/50, v/v). Stock solutions of polysorbate 80 (M_r 1309) at a concentration of 10 mg/ml were prepared in duplicate by diluting 100 mg in 10 ml of methanol/water (50/50, v/v). Stock solutions of docetaxel and polysorbate 80 were stored in glass vials at –20ºC, and found to be stable under these conditions for at least 246 and 62 days, respectively. Likewise, dilutions of the stock solutions in 50% methanol did not demonstrate significant loss of docetaxel or polysorbate 80 when stored for up to 6 h at room temperature (data not shown). The stock solutions were diluted further in blank human plasma on each day of analysis to prepare 10 calibration samples containing docetaxel and polysorbate 80, respectively, at the following combinations of analyte concentrations: 0.500 nM and 1.00 µg/ml (duplicate), 0.500 nM and 2.00 µg/ml, 1.00 nM and 2.00 µg/ml, 5.00 nM and 10.0 µg/ml, 10.0 nM and 30.0 µg/ml, 50.0 nM and 50.0 µg/ml, 75.0 nM and 75.0 µg/ml, and 100 nM and 100 µg/ml (duplicate). Quality control (QC) samples were prepared independently in blank plasma at 5 different concentrations for docetaxel and polysorbate 80, respectively: 0.500 nM and 1.00 µg/ml, 2.00 nM and 3.00 µg/ml, 20.0 nM and 20.0 µg/ml, 80.0 nM and 80.0 µg/ml, and 8000 nM and 1000 µg/ml.

Sample preparation

Prior to extraction, frozen plasma samples were thawed in a water bath at ambient temperature. A 1-ml aliquot of plasma was extracted in a screw-cap glass tube (16 × 125 mm) with 4 ml of a mixture of acetonitrile/*n*-butyl chloride (1/4, v/v) and paclitaxel (20 nM), the internal standard. The tube was capped and mixed vigorously for 10 s on a vortex-mixer, and for 5 min on automated multi-tube shaker, followed by centrifugation at 2,000 g for 10 min at ambient temperature. The top organic layer was transferred to a disposable borosilicate glass culture tube (13 × 100 mm). After a repeat of the solvent extraction, the combined organic extracts were combined and evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue was reconstituted in 100 µl of acetonitrile/water (50:50, v/v) by vortex mixing (30 s) and ultrasonication (5 min). The sample was transferred to a 250-µl polypropylene autosampler vial, sealed with a Teflon crimp cap, and a volume of 50 µl was injected onto the HPLC instrument for quantitative analysis using a temperature-controlled autosampling device operating at 10°C.

Equipment and conditions

Chromatographic analysis was performed using a Waters Model 2690 chromatographic system (Milford, MA, USA). Separation of the analytes from potentially interfering material was achieved at ambient temperature using a Waters X-Terra MS column (50 × 2.1 mm internal diameter) packed with a 3.5-µm octadecyl stationary phase, protected by a stainless steel guard column packed with 3.5-µm RP18 material (20 × 2.1 mm internal diameter; Waters). The mobile phase used for the chromatographic separation was composed of acetonitrile/water (80/20, v/v) containing 0.1% of formic acid, and was delivered isocratically at a flow rate of 0.15 ml/min. The column effluent was monitored using a Micromass Quattro LC triple-quadrupole MS detector (Beverly, MA, USA). The instrument was equipped with an electrospray interface, and controlled by the Masslynx version 3.4 software (Micromass), running under Microsoft Windows NT on a Compaq AP200 computer with a Pentium III processor. The samples were analyzed using an electrospray probe in the positive ionization mode operating at cone voltages of 15 V, 38 V, and 20V for docetaxel, polysorbate 80, and paclitaxel, respectively. Samples were introduced into the interface through a heated nebulizer probe (350°C). The spectrometer was programmed to allow the $[MH]^+$ ions of docetaxel at *m/z* 808.5, polysorbate 80 at *m/z* 1309.6, and paclitaxel at *m/z* 854.5 to pass through the first quadrupole (Q1) and into the collision cell (Q2). The collision energy was set at 10 eV for docetaxel, at 35eV for polysorbate 80, and at 8.0 eV for paclitaxel. The product ions for docetaxel (*m/z* 527.2), polysorbate 80 (*m/z* 419.4), and paclitaxel (*m/z* 509.4) were monitored through the third quadrupole (Q3). Argon was used as collision gas at a pressure of 0.0027 mBar, and the dwell time per channel was between 0.3 and 0.5 s.

Calibration

Calibration curves for docetaxel and polysorbate 80 were computed using the ratio of the peak area of the analyte and internal standard by using a least-squares linear regression analysis with uniform weighting for docetaxel or using a non-linear power-regression analysis for polysorbate 80. Selection of the weighting option was based on assessment of the correlation coefficient and accuracy of the standards at the lower and upper end of the calibration curve**.** The parameters of each calibration curve were used to compute back-calculated concentrations and to obtain values for the QC samples and unknown samples by interpolation.

Method validation

Method validation runs were performed under Good Laboratory Practice according to the Food and Drug Administration guidance (see: http://www.fda.gov/cvm/guidance/published.htm; accessed July 13, 2004) on five consecutive days using freshly prepared samples. Each of the runs included a calibration curve processed in duplicate, and QC samples at five different concentrations in quintuplicate. The lower limit of quantitation (LLOQ) of the assay was assessed by determining the concentration of docetaxel and polysorbate 80 at which the signal to noise ratio was greater than 5, and the values for precision and accuracy were less than 20%.

The accuracy and precision of the assay were assessed by the mean relative percentage deviation (DEV) from the nominal concentrations and the within-run and between-run precision, respectively. The accuracy for each tested concentration was calculated as:

$$DEV = 100 \times \{([\text{analyte}]_{\text{mean}} - [\text{analyte}]_{\text{nominal}})/[\text{analyte}]_{\text{nominal}}\}$$

Estimates of the between-run precision were obtained by one-way analysis of variance (ANOVA) using the run day as the classification variable. The between-groups mean square (MS_{bet}), the within-groups mean square (MS_{wit}), and the grand mean (GM) of the observed concentrations across runs were calculated using the software package JMP version 4 (SAS Institute, Cary, NC, USA). The between-run precision (BRP), expressed as a percentage relative standard deviation, was defined as:

$$BRP = 100 \times (\sqrt{((MS_{bet} - MS_{wit})/n)}/GM)$$

where n represents the number of replicate observations within each run. For each concentration, the estimate of the within-run precision (WRP) was calculated as:

$$WRP = 100 \times (\sqrt{(MS_{wit})}/GM)$$

The specificity of the method was tested by visual inspection of chromatograms of extracted human plasma samples from 6 different donors for the presence of interfering peaks. The extraction efficiency of the assay was measured by comparison of extracted plasma samples and aqueous samples at concentrations of 2.00, 20.0, and 80.0 nM, and 3.00, 20.0, and 80.0 μg/ml for docetaxel and polysorbate 80, respectively. The extraction efficiency of the internal standard, paclitaxel, was tested at a concentration of 20 nM.

The stability of docetaxel and polysorbate 80 in plasma was tested at the concentrations of the low and high QC samples following 3 freeze-thaw cycles at –80ºC. The long-term (up to 91 days at -20°C) and short-term storage stability (6 h at room temperature) of both agents in plasma, and autosampler stability (24 h at room

temperature) of both agents extracted from plasma and reconstituted with 50 % acetonitrile was assessed in triplicate. Results are expressed as the percent recovery relative to the initial (nominal) concentration at time zero. Stability was defined as less than 10% loss of the initial concentration.

Analysis of patient samples

Blood samples were obtained from two patients with cancer who were treated with docetaxel administered as a 1-h intravenous infusion at a dose of 30 or 75 mg/m^2. Docetaxel was formulated in vials containing 20 mg (0.5 ml) or 80 mg (2.0 ml) of the active ingredient and 570 mg and 2080 mg of polysorbate 80, respectively. The protocol was approved by the Institutional Review Board of The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins (Baltimore, MD, USA), and the patients provided written informed consent.

The samples were collected in heparin-containing tubes before drug administration, at 30 min after start of infusion, at 4 min before the end of infusion, and at 10 and 30 min, 1, 3, 7, 24, 48, and approximately 168 (day 8), 336 (day 15), and 504 h (day 22) after the end of infusion. Samples were processed immediately by centrifugation for 10 min at 3,000 g at ambient temperature. The plasma supernatant was collected and stored frozen at –70ºC until analysis.

For quantitation of docetaxel and polysorbate 80 in patient plasma, QC samples at low, medium, and high concentrations were assayed in duplicate and were distributed among the calibrators and unknown samples in the analytical run; no more than 33% of the QC samples were greater than ± 15% of the nominal concentration. Samples with docetaxel or polysorbate 80 concentrations greater than the assay upper limits of quantitation of 100 nM and 100 µg/ml were diluted with blank human plasma prior to extraction and quantitation. Depending on the docetaxel dose level and the time point of blood collection relative to the end of infusion, plasma samples were pre-diluted with blank human plasma at volume ratios between 1:10 and 1:100.

Results and discussion

In recent years, several analytical methods based on reversed-phase HPLC have been reported for the quantitative determination of docetaxel in human plasma either based on UV detection [11-17] or MS detection [18-20]. So far, most have relied on laborious solid-phase extraction techniques and lack in sensitivity, precluding drug measurement at sub nanogram-per-milliliter levels associated with drug doses used in

weekly regimens. The present paper describes an alternative procedure, which is based on a previous assay for polysorbate 80 and uses a double liquid-liquid extraction step followed by reversed-phase HPLC and tandem MS detection [21]. The method is sufficiently sensitive to be applied to samples from patients receiving low doses of docetaxel (e.g., 25 to 35 mg/m^2), simultaneously detects polysorbate 80, and yet avoids the use of solid-phase extraction for sample clean-up.

Detection and chromatography

The mass spectra of docetaxel and polysorbate 80 showed protonated molecules ([MH$^+$]) at *m/z* 808.5 and *m/z* 1309.6, respectively (Figures 2 and 3). The prominent ions observed at *m/z* 527.2 and *m/*z 419.4 were selected for subsequent monitoring in the third quadrupole. The mass spectrum of the internal standard paclitaxel showed a [MH$^+$] at *m/z* 854.5, and the high collision energy gave one major product at *m/z* 509.4, consistent with previous observations [22].

Representative chromatograms of blank and spiked human plasma samples are shown in Figures 4 and 5. The mean (± standard deviation) retention times for docetaxel, polysorbate 80, and paclitaxel under the optimal conditions were 1.90 ± 0.03 min, 3.88 ± 0.03 min, and 1.90 ± 0.03 min, respectively, with an overall chromatographic run time of 7 min. The selectivity for the analysis is shown by symmetrical resolution of the peaks, with no significant chromatographic interference around the retention times of docetaxel, polysorbate 80, and the internal standard in drug-free specimens, obtained from a total of six different individuals.

Linearity of detector responses

The calculated peak-area ratios of docetaxel to paclitaxel versus the nominal concentration of the analyte displayed a linear relationship in the tested range (i.e., 0.500 nM to 100 nM). The measurement variance over this range increased proportionally with the docetaxel concentration, as detected by a one-sided *F*-test at an α-value of 5%. Because of this heteroscedasticity a weighting factor was used, which is inversely proportional to the variance at the given concentration level (x). After applying the peak area ratio in combination with a weighting factor of $1/x$, a mean least-squares linear-regression correlation coefficient of greater than 0.995 was obtained in all analytical runs. The statistical evaluation of the coefficients of the ordinary least-squares line indicated small bias in the slope and in the intercept further indicating minor matrix effects and blank problems, respectively.

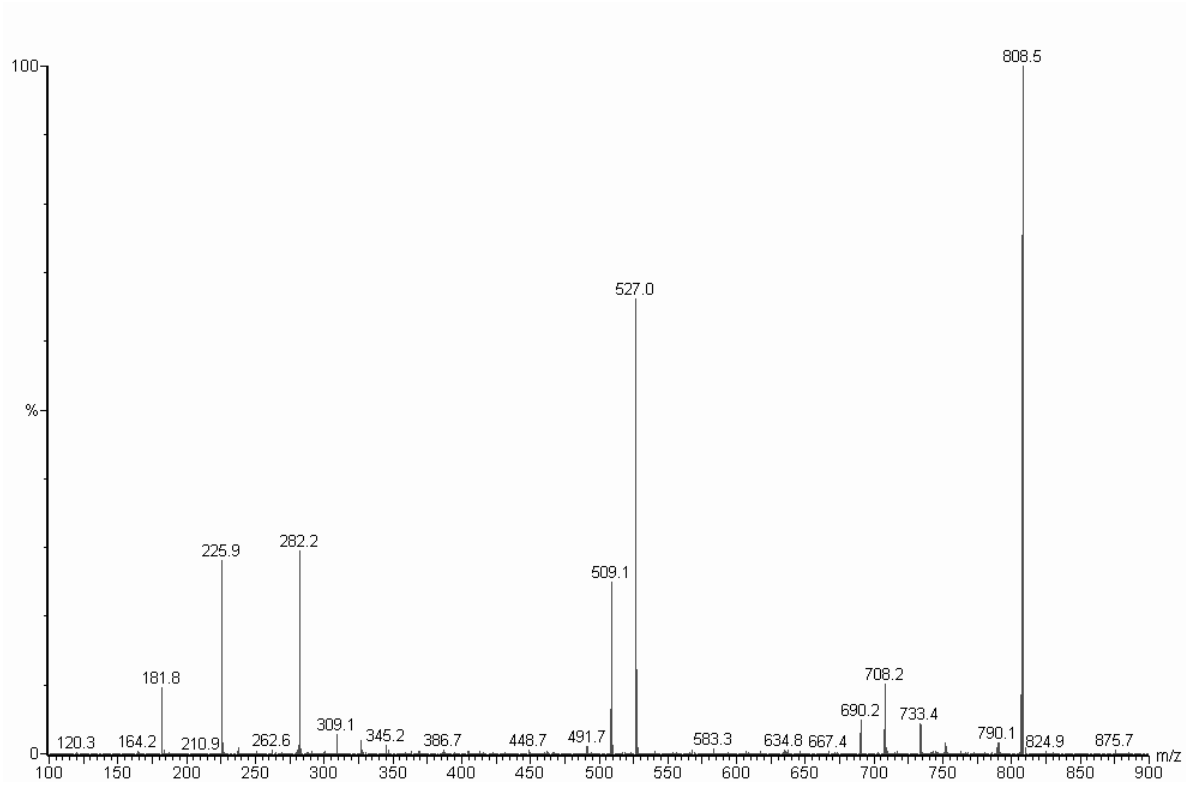

Figure 2. Mass spectrum of docetaxel with monitoring at m/z 808.5 → 527.2.

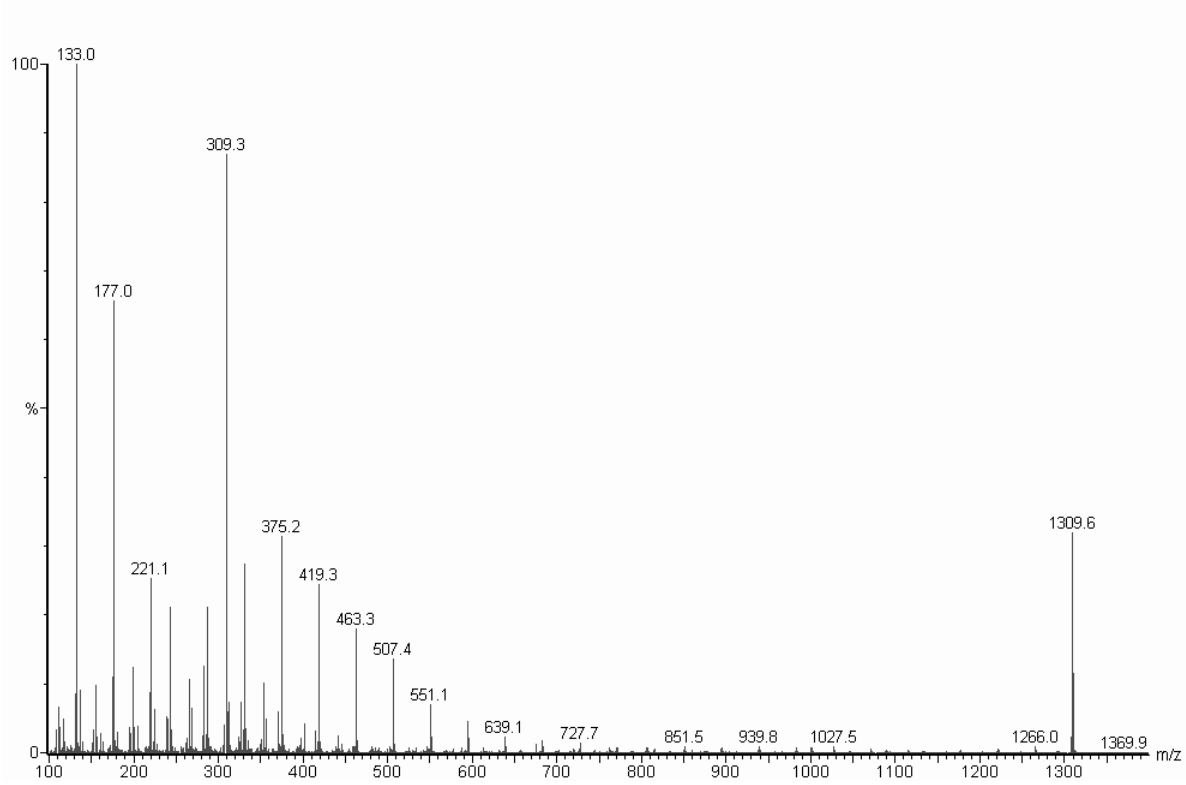

Figure 3. Mass spectrum of polysorbate 80 with monitoring at m/z 1309.6 → 419.4.

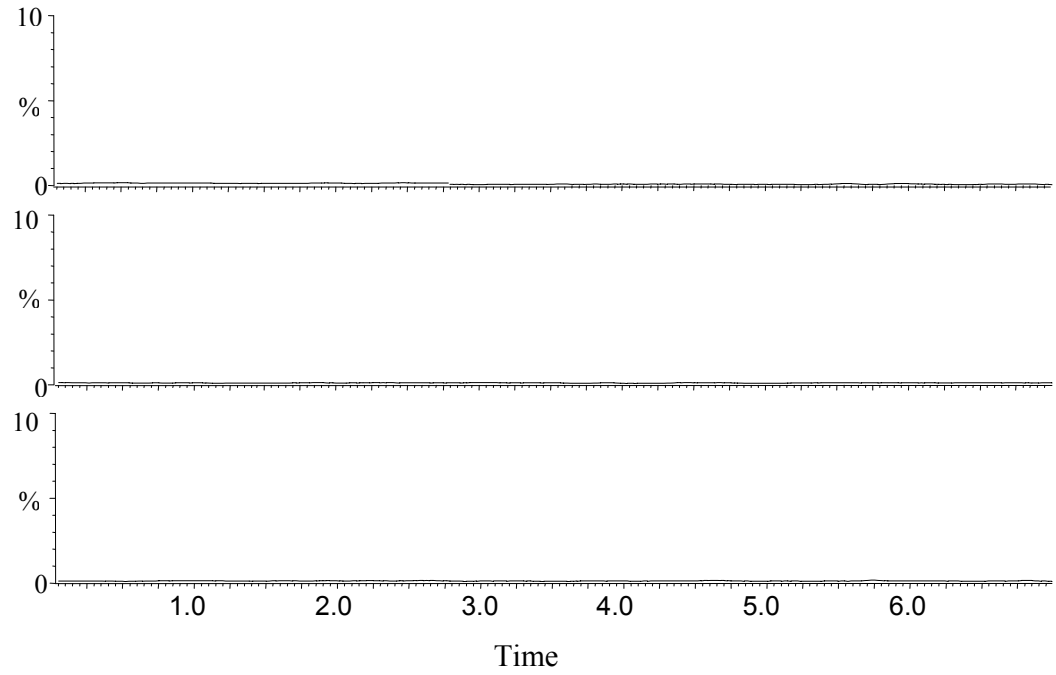

Figure 4. Mass chromatograms of pre-treatment plasma from three different individuals. Polysorbate 80, paclitaxel, and docetaxel were monitored at m/z 1309.6 → 419.4, 854.5 → 509.4, and 808.5 → 527.2, respectively. The retention times of polysorbate 80, paclitaxel, and docetaxel where approximately 1.90 ± 0.03 min, 3.88 ± 0.03 min, and 1.90 ± 0.03 min, respectively.

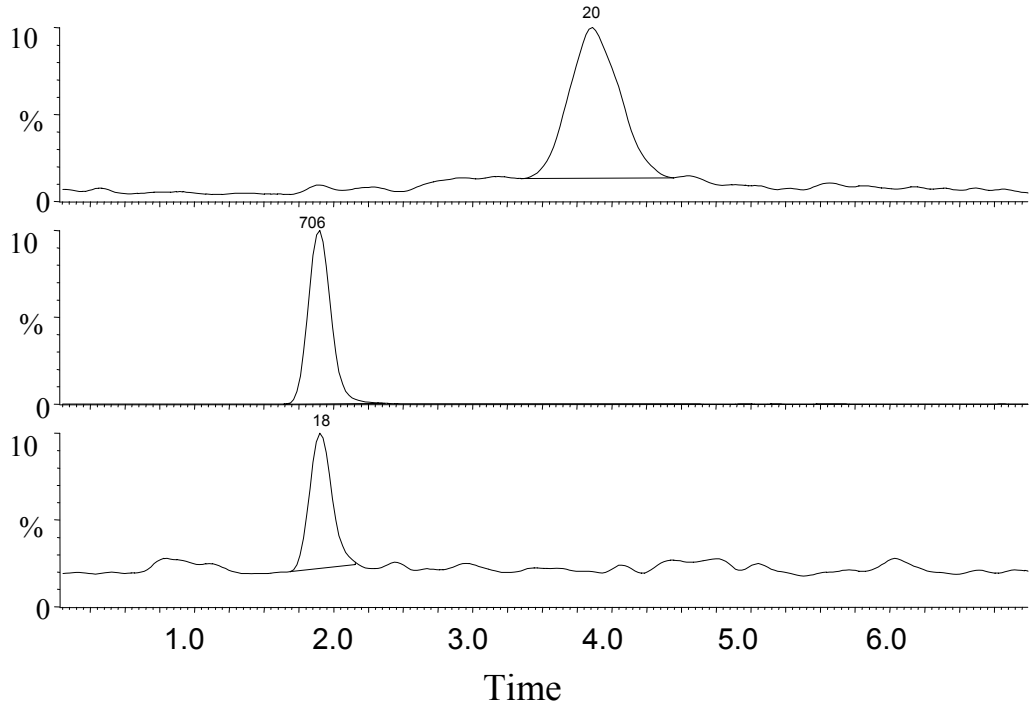

Figure 5. Mass chromatograms of plasma spiked with polysorbate 80 (1.00 μg/ml; top panel), the internal standard paclitaxel (20.0 nM; middle panel), and docetaxel (0.500 nM; bottom panel). Polysorbate 80, paclitaxel, and docetaxel were monitored at m/z 1309.6 → 419.4, 854.5 → 509.4, and 808.5 → 527.2, respectively. The retention times of polysorbate 80, paclitaxel, and docetaxel where approximately 1.90 ± 0.03 min, 3.88 ± 0.03 min, and 1.90 ± 0.03 min, respectively.

The lack-of-fit test indicated that the detector response factors for polysorbate 80 in the range of 1.00 µg/ml to 100 µg/ml deviated significantly from linearity, which is related to a concentration-dependence of the extraction efficiency (see below). Hence, for polysorbate 80, calibration curves were fit using a nonlinear power regression analysis, as described previously [21].

For each point on the calibration curves for docetaxel and polysorbate 80, the concentrations back-calculated from the equation of the regression analysis were always within acceptable limits for accuracy and precision (Table 1). A linear regression of the back-calculated concentrations versus the nominal values provided a unit slope and an intercept not significantly different from zero. The distribution of the residuals showed random variation, was normally distributed and centered around zero. The bias was not statistically different from zero, and the 95% confidence intervals included zero (not shown).

Table 1. Back-calculated concentrations from calibration curves

Nominal concentration[a]	Accuracy (%)	Precision (%) Within-run	Between-run
Docetaxel			
0.500	99.20	14.1	[b]
1.00	97.60	6.18	2.42
5.00	100.7	4.87	3.78
10.0	103.8	5.05	[b]
50.0	100.4	4.37	5.75
75.0	100.3	7.67	[b]
100	99.22	5.44	[b]
Polysorbate 80			
1.00	107.6	4.32	9.77
2.00	98.05	16.0	[b]
5.00	93.30	17.1	[b]
10.0	101.7	5.19	6.96
30.0	101.6	5.93	3.95
50.0	99.63	6.50	[b]
75.0	99.54	8.53	[b]
100	100.6	3.59	0.53

[a] In units of nM for docetaxel and µg/ml for polysorbate 80; [b] No significant additional variation was observed as a result of performing the assay in different runs.

The lower limit of quantitation (LLOQ) for docetaxel and polysorbate 80 was established at 0.500 nM (~0.4 ng/ml) and 1.00 µg/ml, which concentrations were associated with a mean (± standard deviation) signal-to-noise ratio of 5.37 ± 1.60 and 13.2 ± 4.14, respectively. At these concentrations, the values for precision did not exceed 15%, and the accuracy was less 104% for both analytes (Table 2). This quantitation limit for docetaxel represents a 25 to 50-fold increase in sensitivity compared to analytical assays based on HPLC with UV detection [11-17], and is similar to a recent procedure based on solid-phase extraction followed by HPLC with single-quadrupole MS detection using positive mode electrospray ionization [19]. The present LC/MS/MS assay based on liquid-liquid extraction provides the advantage of being less labor intensive and expensive relative to methods using solid phase extraction, without compromising assay sensitivity.

Table 2. Assessment of accuracy, precision and recovery

Nominal concentration[a]	Accuracy (%)	Precision (%) Within-run	Between-run	Recovery (%)
Docetaxel				
0.500	104.0	19.8	3.22	[b]
2.00	97.20	14.1	8.38	92.50
20.0	96.70	6.86	2.30	90.48
80.0	97.83	4.47	5.20	95.66
8000[c]	101.1	4.02	8.06	
Polysorbate 80				
1.00	103.2	15.9	12.8	[b]
3.00	99.07	8.80	0.71	102.4
20.0	94.20	8.68	6.10	81.56
80.0	104.4	5.00	5.74	81.16
1000[c]	104.7	14.8	19.3	[b]

[a] In units of nM for docetaxel and µg/ml for polysorbate 80; [b] Not done; [c]Sample diluted 1:100 with plasma prior to extraction

Accuracy, precision and recovery

For non LLOQ QC samples spiked with docetaxel, the within-run and between-run variability (precision), expressed as the percentage relative standard deviations, were always less than 10%, whereas the mean predicted concentration (accuracy) was

within 5% of the nominal value at the various concentrations analyzed in quintuplicate on five separate occasions (Table 2); for polysorbate 80, precision was less than 18% and accuracy was less than 7%. The extraction efficiency of docetaxel was concentration-independent and averaged 92.9%, whereas for paclitaxel the overall recovery was 94.8%. For polysorbate 80, the extraction efficiency slightly decreased with an increase in the spiked concentration (Table 3). As compared to a previous procedure for polysorbate 80 [21], the recovery was substantially improved over the range studied from 50-60% to 80-100% as a result of the use of a double extraction procedure with acetonitrile/*n*-butyl chloride.

Table 3. Assessment of stability[a]

Condition	Docetaxel (nM)		Polysorbate 80 (μg/ml)	
	2.00	80.0	3.00	80.0
Freeze-thaw stability				
Cycle 1	101.0	101.8	128.3	108.0
Cycle 2	81.14	102.4	131.9	106.6
Cycle 3	83.98	102.4	131.9	104.3
Long-term stability				
T = 91 days	118.4	107.4	97.0	116.5
Short-term stability				
T = 1 h	94.48	105.4	99.89	100.9
T = 2 h	95.64	102.1	91.66	98.47
T = 4 h	92.44	102.6	76.89	90.62
T = 6 h	93.31	98.63	71.03	79.40
Autosampler stability				
T = 2 h	102.8	97.82	96.63	90.93
T = 4 h	107.8	97.16	115.4	99.35
T = 6 h	93.66	94.62	96.39	102.6
T = 24 h	94.57	96.78	95.90	92.32

[a] Expressed as mean percentage change from time zero (nominal concentration).

Analyte stability

QC samples undergoing 3 freeze-thaw cycles showed a concentration-dependent degradation for docetaxel, with a loss of approximately 20% at the lowest

concentration (Table 3). This needs to be taken into consideration when multiple analytical measurements or re-analyses are required on the same frozen material. In frozen plasma samples, stability was assessed over a three month period, and no significant degradation was observed for either docetaxel or polysorbate 80. Plasma spiked with docetaxel or polysorbate 80 allowed to stand at room temperature for up to 6 hours indicated that docetaxel was stable during this time period. However, a concentration-dependent instability of polysorbate 80 was noted at 4 and 6 hours for polysorbate 80 (Table 3). This is most likely related to enzyme-mediated breakdown of the surfactant, as has been noted previously [23], and suggests that rapid freezing of clinical samples is indicated in order to obtain reliable pharmacokinetic data. In acidic extracts, both analytes could last at least 24 hours on the autosampler without any significant degradation, allowing for more than 200 samples to be analyzed simultaneously within a single chromatographic run.

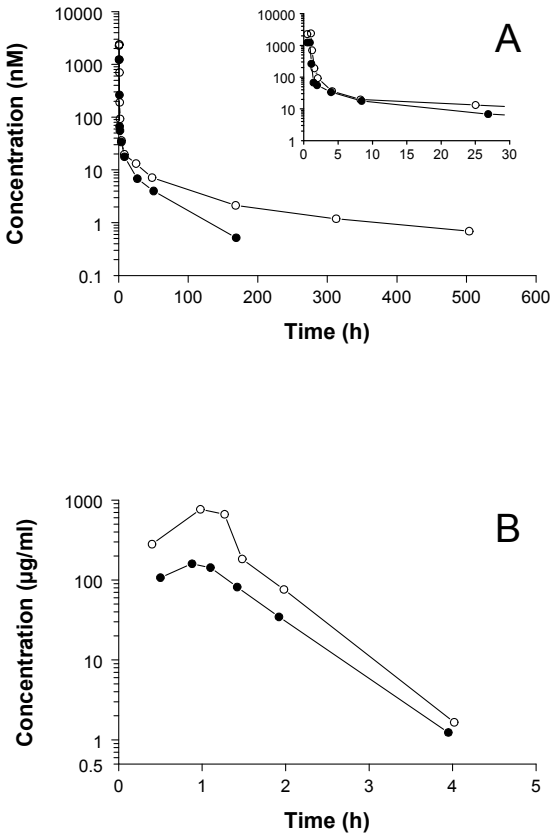

Figure 6. Plasma concentration-time profiles of (A) docetaxel and (B) polysorbate 80 in a cancer patients treated with docetaxel formulated in polysorbate 80 at a dose of 30 mg/m^2 (docetaxel, 68 mg; polysorbate 80, 1768 mg; closed symbols) or at a dose of 75 mg/m^2 (docetaxel, 129 mg; polysorbate 80, 3354 mg; open symbols).

Plasma concentration-time profiles

The suitability of the developed method for clinical use was demonstrated by the determination of docetaxel and polysorbate 80 in plasma samples from two patients with cancer treated with docetaxel at a dose level of 30 or 75 mg/m^2 (Figure 6). The assay allowed plasma concentrations of docetaxel to be detected for more prolonged time periods (up to 3 weeks after treatment) compared with less sensitive methods based on HPLC with UV detection, which typically only allow detection of circulating levels up to 24 h at a lower limit of quantitation of 5-10 ng/ml. In contrast to docetaxel, polysorbate 80 concentrations in plasma of both patients declined in an apparent mono-exponential fashion, and remained undetectable beyond 4 h after the start of drug administration.

In conclusion, a novel assay for simultaneous measurement of docetaxel and its formulation vehicle polysorbate 80 in human plasma samples was developed. The method was validated according to the Food and Drug Administration bioanalytical guidance, and met the pre-defined acceptance criteria for precision and accuracy [24]. The described method permits the analysis of patient samples to concentrations of 0.500 nM and 1.00 μg/ml for docetaxel and polysorbate 80, respectively, which is sufficiently sensitive to allow pharmacokinetic monitoring after intravenous administration of docetaxel at low doses used in weekly dosing regimens. The method is currently being used to further study the influence of circulating polysorbate 80 levels on the clinical pharmacology of docetaxel in patients with cancer.

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Chapter 8

Determination of Fraction Unbound Docetaxel Using Micro-Equilibrium Dialysis

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Docetaxel is a semisynthetic taxane derived from needles of the European yew (*Taxus baccata*), and is currently approved for the treatment of breast cancer and non-small-cell lung cancer [1,2]. The drug has been shown to bind extensively to plasma proteins, including α_1-acid glycoprotein (AAG), albumin, and lipoproteins [3]. Interindividual variability in AAG concentrations has been associated with altered docetaxel clearance, severity of neutropenia, and overall survival in patients with non-small-cell lung cancer [4,5]. Recent data also indicate that the plasma binding of docetaxel is further influenced by the presence of its formulation vehicle polysorbate 80 in a concentration-dependent biphasic manner [6], although the clinical significance of this observation in addition to that of AAG is currently unknown. Regardless, these findings indicate that unbound docetaxel concentrations may better correlate with treatment outcome (i.e., toxicity and efficacy) than with total drug. In an effort to better understand the clinical pharmacology of docetaxel, a rapid and reproducible assay based on microequilibrium dialysis was developed for measuring the fraction unbound (fu) docetaxel in patient plasma samples.

Equilibrium dialysis was performed on a plate rotator (Model 74-2334; Harvard Apparatus, Holliston, MA) at 37°C in a humidified atmosphere of 5% CO_2 using 96-well microdialysis plates (Harvard Apparatus; Holliston, MA; www.harvardapparatus.com). The dialysis compartments in each well are separated by membrane. In preliminary experiments, both regenerated cellulose membranes and polyethylsulfone membranes with a 5- or 10-kDa cutoff were evaluated. Eventually, a cellulose membrane with a 5-kDa cutoff was used for further experiments, because it provided the most consistent results and minimal accidental leaking. Experiments were carried out with 250-µl aliquots of plasma containing a tracer amount of [*G*-^{3}H]docetaxel (specific activity, 7.2 Ci/mmol; Moravek Biochemicals, Brea, CA) against an equal volume of phosphate-buffered saline (PBS; pH 7.4). Next, the individual chambers in the dialysis plate were physically separated and aliquotted. Drug concentrations in 125 µl-aliquots of both compartments were measured by liquid scintillation counting for 1 minute following the addition of 5 ml Bio-Safe II scintillation fluid (Research Products International, Mount Prospect, IL) on a Model LS6000IC counter (Beckman Instruments, Inc., Columbia, MD). The time to equilibrium was assessed in triplicate at 2, 3, 4, 6, 8, 16, 24, 48, and 72 hours after start of the experiment. As fu measurements were to be made on patient samples that contained variable amounts of drug and excipient, fu was also determined in plasma samples over the entire anticipated concentration range of docetaxel (i.e., 0, 1, 10, 100, 1,000, and 10,000 ng/ml; Aventis Pharma, Vitry-sur-Seine Cedex, France) [7] and

polysorbate 80 (i.e., 0, and 0.5 mg/ml; Sigma Co., St. Louis, MO) [8]. Unlabeled docetaxel or polysorbate 80 were added to the plasma-containing compartment to mimic the composition of samples obtained from patients treated with docetaxel (i.e., with varying concentrations of docetaxel and polysorbate 80).

During the establishment of the method, quadruplicate plasma samples with differing docetaxel fu values, depending on the spiked polysorbate 80 concentrations, were subject to repeated analysis on 6 consecutive days to assess reproducibility. Preliminary experiments indicated that volume shifts during the dialysis period were negligible (< 10%); hence the results were used directly without applying a correction factor.

It was confirmed in all equilibrium dialysis experiments that the total drug recovery from the fractions was equal to the amount of [*G*-^{3}H]docetaxel added to the plasma samples (mean recovery, 95%; $P > 0.05$ *versus* hypothesized mean of initial value). This recovery was assessed for each paired PBS and plasma sample taken after dialysis by calculating the ratio of the measured radioactivity and the amount of radioactivity added to each plasma sample prior to dialysis. Equilibrium for docetaxel transport across the membrane was established within 4 hours, which is considerably faster than the 48-hour period required in our previous method [6]. In the absence of polysorbate 80, the mean (± SD) docetaxel fu was 8.6% ± 0.5%, which is comparable to previous data obtained using conventional equilibrium dialysis or ultrafiltration [3,6]. In the presence of polysorbate 80 at a concentration of 0.5 mg/ml, the mean docetaxel fu was increased to 9.4% ± 0.80% (relative increase, 8.5%). As predicted based on previously published data on docetaxel binding to plasma proteins [3], docetaxel concentration had no influence on fu as determined by one-way analysis of variance ($P = 0.88$; $N = 16$), with an overall coefficient of variation of 7.93%. This suggests that the overall binding of docetaxel to plasma proteins is concentration-independent and nonsaturable in the clinically achievable range.

The mean relative SD of all samples was less than 4.18%, assuring high discriminatory power in the detection of changes in docetaxel fu in the presence of variable AAG and/or polysorbate 80 levels in patient samples. With the final method, the within-run and between-run variability were always less than 15%.

The assay was next applied to a prospective analysis to define concentration-time profiles of unbound docetaxel in 5 patients with cancer receiving single-agent docetaxel (Taxotere; Aventis Pharma), which was administered as a 1-hour continuous intravenous infusion at a dose of 75 mg/m^2. The unbound concentration was calculated as the product of fu and the total plasma concentration, determined using a validated

Table 1. Fraction unbound docetaxel in plasma of 5 patients with cancer (docetaxel dose, 75 mg/m^2

Time (h)*	(Mean)	SD (%)	Range (%)
0	3.93	0.496	3.34 - 4.50
0.50	4.85	1.06	3.66 - 6.25
1.0	5.38	1.64	3.10 - 7.54
1.2	4.48	1.11	3.67 - 6.11
1.5	4.54	0.491	3.98 - 5.03
2.0	4.77	1.13	3.55 - 6.23
4.0	4.52	0.964	3.46 - 5.84
8.0	4.77	1.35	3.19 - 6.78
25	4.56	2.02	2.47 - 7.61

*Denotes time after the start of a 1-hour continuous intravenous infusion

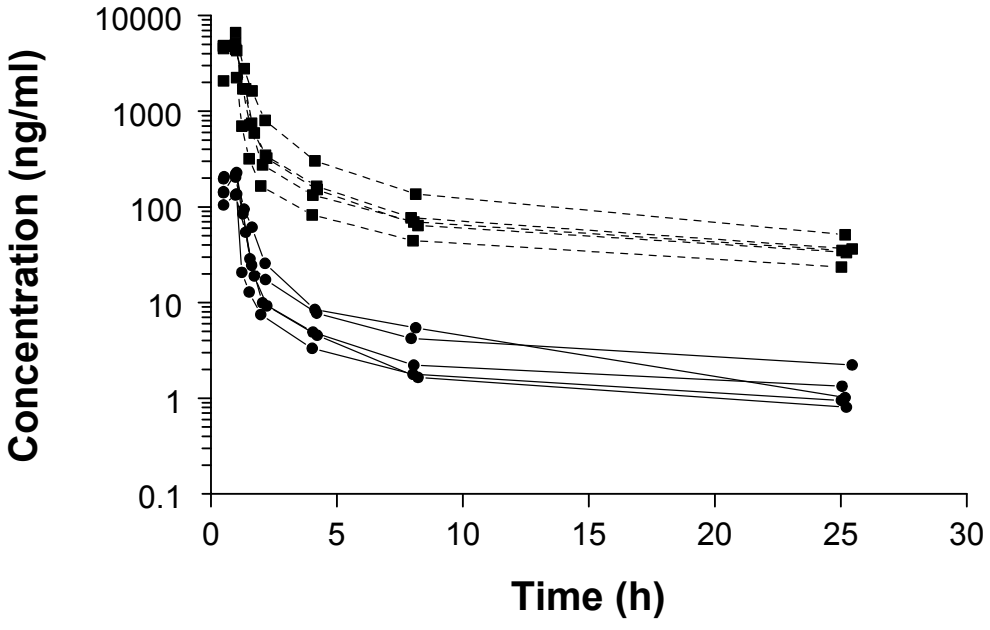

Figure 1. Concentration-time profiles of total docetaxel (ng/ml) (squares) and unbound docetaxel (ng/ml) (circles) in plasma of 5 patients with cancer (docetaxel dose, 75 mg/m^2).

liquid-chromatographic assay with tandem mass-spectrometric detection [9]. The mean docetaxel fu increased from 3.93% to 5.35% at the end of infusion, and then gradually declined to values similar to those in samples obtained immediately before drug administration (Table 1). Semilogarithmic concentration-time profiles of total and unbound docetaxel in plasma in the 5 patients are shown in Figure 1.

In conclusion, the presented data indicate that docetaxel fu in plasma samples of patients treated with the drug can be reliably determined using this assay. The procedure is more cost-efficient and less time consuming than other currently available assays, and likely introduces less bias and random error. This is because the use of standardized equilibrium dialysis plates such as those used here will improve reproducibility of the method, along with minimal volume shift and minor occurrence of leakage. The developed procedure is currently used to prospectively evaluate relationships between pharmacokinetic characteristics of unbound drug- and docetaxel-mediated toxicity in a large cohort of patients with cancer.

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Chapter 9

Comparative Pharmacokinetics of Weekly and Every 3-Week Docetaxel

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Abstract

Purpose: Weekly administration of docetaxel has demonstrated comparable efficacy together with a distinct toxicity profile with reduced myelosuppression, although pharmacokinetic data with weekly regimens are lacking. The comparative pharmacokinetics of docetaxel during weekly and once every 3 weeks (3-weekly) administration schedules was evaluated.

Experimental Design: Forty-six patients received weekly docetaxel 35 mg/m^2 as a 30 min infusion alone (n=8) or in combination with irinotecan (n=12), or in 3-weekly regimens, as a 1 h infusion at 60 mg/m^2 with doxorubicin (n=10), 75 mg/m^2 alone (n=9) or 100 mg/m^2 alone (n=7). Serial blood samples were obtained immediately before and up to 21 days after the infusion. Plasma concentrations were measured by liquid chromatography-mass spectrometry and analyzed using compartmental modeling.

Results: Mean±SD docetaxel clearance values were similar with weekly and 3-weekly schedules (25.2±7.7 versus 23.7 ± 7.9 L/h/m^2); values for half-life were also similar with both schedules of administration (16.5±11.2 and 17.6±7.4 h). With extended plasma sampling beyond 24h post-infusion, docetaxel clearance is 18% lower and the terminal half-life is 5-fold longer. At 35 mg/m^2, the mean±SD docetaxel concentration on day 8 was 0.00088±0.00041 μg/mL (1.08±0.51 nM); at 75 mg/m^2, concentrations on day 8, 15, and 22 were 0.0014±0.00043 μg/mL (1.79±0.53), 0.00067±0.00025 μg/mL (0.83±0.31 nM), and 0.00047±0.00008 μg/mL (0.58±0.099 nM), respectively.

Conclusion: Docetaxel pharmacokinetics are similar with weekly and 3-weekly regimens. Prolonged circulation of low nanomolar concentrations of docetaxel may contribute to the mechanism of action of docetaxel through suppression of microtubule dynamics and tumor angiogenesis and enhanced cell radiosensitivity in combined modality therapy.

Introduction

The antineoplastic agent docetaxel acts by disrupting the microtubular network, and is one of the most active agents in the treatment of locally advanced or metastatic breast, non-small cell lung, and ovarian cancer [1-4]. The docetaxel dose used for treating cancer patients has ranged from 60 to 100 mg/m^2 as a 1-hour intravenous infusion given once every 3 weeks (hereafter referred to as "3-weekly"). In this

regimen, neutropenia occurs in virtually all patients, and grade 4 neutropenia occurs in 75% of patients given 100 mg/m^2 (N = 2045; febrile neutropenia incidence, 12%); grade 3/4 neutropenia occurs in 65% of patients given 75 mg/m^2 (N = 176; febrile neutropenia incidence, 6.3%), and grade 4 neutropenia occurs in 75% of patients given 60 mg/m^2 (N = 174; febrile neutropenia incidence, 0%) (see: http://www.taxotere.com/ - last accessed july 13, 2004). Other side effects include alopecia, asthenia, dermatologic reactions, fluid retention, hypersensitivity reactions, and stomatitis. Drug exposure-toxicity relationships are clearly defined for docetaxel administered as monotherapy at doses of 75 to 100 mg/m^2 in 3-weekly regimens where the AUC of total plasma concentrations during the first cycle of treatment is related to incidence of grade 4 neutropenia and febrile neutropenia [5].

Recent clinical trials have examined single-agent docetaxel administered at doses of 35 to 40 mg/m^2 given weekly for six consecutive weeks followed by two weeks without treatment [6-14], or on other weekly schedules such as 3 consecutive weeks with one week of rest [15-17] (hereafter "weekly"). Administration of weekly schedules significantly changed the toxicity profile of docetaxel with a reduction in acute toxicity and only mild myelosuppression. Fatigue and asthenia appeared as the dose-limiting side effects and nail changes and excessive lacrimation became more common. The response rates observed with weekly administration of single-agent docetaxel in phase II studies in metastatic breast cancer and advanced non-small cell lung cancer are within the range reported in other studies of 3-weekly docetaxel [18-21], and in general, the planned dose intensity is equivalent to that used in 3-weekly regimens.

At present, the pharmacokinetic profile of docetaxel administered in weekly treatment regimens has not been previously reported. The objectives of the study were to compare the pharmacokinetics of docetaxel during weekly and 3-weekly administrations and to describe plasma drug concentrations during extended periods with both schedules.

Patients and Methods

Chemicals and Reagents

Docetaxel powder (batch, #990720; purity, 99.5%) was supplied by Aventis Pharma (Vitry-sur-Seine Cedex, France). The internal standard, paclitaxel (Lot, #061K1158; purity, 100%) was obtained from Sigma Chemical Co. (St. Louis, MO). Methanol and acetonitrile were purchased from EM Science (Gibbstown, NJ), *n*-butyl

chloride from AlliedSignal, Inc. (Muskegon, MI), and formic acid (88%, v/v in water) from J.T. Baker (Phillipsburg, NJ). All chemicals were of HPLC grade or better. Purified water was obtained by filtration and deionization using a Milli-Q-UF system (Millipore, Milford, MA), and was used throughout. Drug-free plasma originated from Pittsburgh Blood Plasma, Inc (Pittsburgh, PA).

Patients and Treatment

Docetaxel was administered as part of several clinical study protocols and pharmacokinetic data were gathered into this study. The clinical docetaxel preparation (Taxotere; Aventis) contained 20 or 80 mg of the drug formulated in 0.5 mL and 2.0 mL, respectively, of polysorbate 80, and was diluted in ethanol – water (13:87, v/v) to a 10-mg/mL concentrate. This solution was diluted in 250-mL infusion bags with 0.9% (w/v) sodium chloride in water to a concentration of 0.30 – 0.74 mg/mL. Individual drug doses were normalized to body-surface area and administered as part of a clinical study protocol once every week at a dose of 35 mg/m^2 alone (n=8) or 30 minutes before irinotecan 50 mg/m^2 (n=12), or once every-3-weeks (3-weekly) at a dose of 60 mg/m^2 1 hour after doxorubicin 60 mg/m^2 (n=10), 75 mg/m^2 alone (n=9), or 100 mg/m^2 alone (n=7). The drug was given as a 0.5-hour (35 mg/m^2) or 1-hour (60, 75, and 100 mg/m^2) continuous intravenous infusion using an infusion system with an in-line 0.22-μm filter. The clinical protocols were approved by the local institutional review boards (Baltimore, MD, and Rotterdam, the Netherlands), and all patients provided written informed consent before enrollment. Patients had adequate renal and hepatic function defined as: (i) serum creatinine ≤ 2.0 × the institutional upper limit of normal (ULN); (ii) total bilirubin ≤ ULN; and (iii) if alkaline phosphatase was ≤ ULN, any elevations in AST/ALT; or if AST/ALT were ≤ ULN, any elevation in alkaline phosphatase; patients with ALT and/or AST > 1.5 × ULN with concomitant alkaline phosphate > 2.5 × ULN were not eligible for treatment with docetaxel on the administrations schedules described in the present manuscript.

Pharmacokinetic Sampling

Pharmacokinetic studies were part of each study protocol and were performed during the first week of therapy for the weekly regimens, and during the first cycle of treatment for the 3-weekly regimens; pharmacokinetic studies were performed during the second cycle of treatment in three of seven patients receiving docetaxel 100 mg/m^2. Blood samples were collected in vacutainer tubes containing heparin as anticoagulant from a peripheral site contralateral to the infusion site. Blood samples

were immediately placed in an ice-water bath, centrifuged within 30 minutes of collection at 1000 × *g* for 10 minutes at 4°C, and were stored at or below –20 °C until analysis. The following sampling schemes were used: (i) docetaxel 35 mg/m^2 given alone or followed by irinotecan: pre-treatment, 29 minutes (immediately before the end of the infusion), and post-infusion at 10 and 30 minutes and at 1, 3, 7.5, 24, and 48 hours and pre-treatment on day 8; (ii) docetaxel 60 mg/m^2 with doxorubicin: pre-treatment, 30 minutes during the infusion, 59 minutes (immediately before the end of infusion), and post-infusion at 10 and 30 minutes, and at 1, 2.5, 5, 22, and 46 hours, and prior to cycle 2 on day 22; (iii) docetaxel 75 mg/m^2 alone: pre-treatment, 30 minutes during the infusion, 59 minutes (immediately before the end of infusion), and post-infusion at 10 and 30 minutes, and at 1, 3, 7, 24, and 48 hours and on days 8, 15, and 22; and (iv) docetaxel 100 mg/m^2 alone: pre-treatment, 30 and 55 minutes during the infusion, end of infusion, and post-infusion at 10, 20, and 30 minutes, and at 1, 1.3, 2, 4, 8.5, 24, 48, and 72 h and on days 8, 15, and 22.

Analytical Assay

Docetaxel was quantitated in plasma using high-performance liquid chromatography (HPLC) with tandem mass-spectrometric detection. The method was validated according to the recommendations provided by the FDA (http://www.fda.gov/cvm/guidance/published.htm - last accessed July 13, 2004). Briefly, drug was extracted from 1.0 mL of plasma by liquid-liquid extraction with a mixture of acetonitrile – *n*-butyl chloride (1:4, v/v). The eluent was evaporated under a stream of nitrogen and reconstituted with 200 µL of methanol – water (50:50, v/v). The analytical system consisted of a Model 2690 chromatograph (Waters, Milford, MA) equipped with a model 996 photodiode-array detector. Chromatographic separations were achieved on a Waters X-Terra MS column (50 × 2.1 mm internal diameter) packed with a ODS stationary phase with a 3.5-µm particle size, protected by a Phenomenex C18 (4.0 × 3.0 mm internal diameter) guard column (Torrance, CA). The mobile phase was composed of a mixture of acetonitrile – water (80:20, v/v) containing 0.1% (w/v) formic acid, and was delivered isocratically at a flow rate of 0.2 mL/min. Detection was performed with a MicroMass Quattro LC triple-quadrupole mass spectrometer (Cary, NC) in the positive ion mode. The electrospray ionization operated at 3.6 kV and the cone voltage of 20 V. The detector was programmed to allow the $[MH]^+$ ions of docetaxel (*m/z* 808.49) and that of the internal standard, paclitaxel (*m/z* 854.99), to pass through the first quadrupole and into the collision cell. The collision energy for collision-induced dissociation was set at 8.0 eV, with argon

used as collision gas at a pressure of 0.0027 mbar. The daughter ions of docetaxel (*m/z* 527.52) and paclitaxel (*m/z* 509.44) were monitored through the third quadrupole. The dwell time per channel for data collection was set at 0.5 seconds.

Docetaxel plasma concentrations were quantitated over the range of 0.50 to 100 nM. The accuracy and precision of quality control (QC) samples, which included docetaxel concentrations of 2.0, 20.0, 80.0 nM, and an 80-nM QC that was diluted 100-fold prior to processing, were < 15%. At the assay lower limit of quantitation (LLOQ) of 0.50 nM (400 pg/mL), accuracy was 103% and between-run precision was 17.5%. This represents a 25 to 50-fold increase in sensitivity as compared to analytical assays based on HPLC with UV detection [22-28], although an analytical assay based on HPLC with mass spectrometric detection with an LLOQ of 0.30 nM has recently been described [29]. For quantitation of docetaxel in unknown samples, QC samples at low, medium, and high concentrations were assayed in duplicate and were distributed among the calibrators and unknown samples in the analytical run; no more than 33% of the quality assurance samples were greater than ± 15% of the nominal concentration. Samples with docetaxel concentrations greater than the assay upper limit of quantitation of 100 nM were diluted with analyte free human plasma prior to extraction and quantitation. Depending on the docetaxel dose level, plasma samples were pre-diluted at volume ratios of 1:10, 1:50, or 1:100.

Pharmacokinetic Data Analysis

Individual docetaxel pharmacokinetic parameters were estimated using model-dependent methods as implemented in Adapt II release 4 (Biomedical Simulations Resource, Los Angeles, CA) [30]. Pharmacokinetic parameters were estimated twice for each patients using: (i) data from time zero to 24 hours post-treatment (conventional plasma sampling scheme) for comparison to previously published pharmacokinetic data; and (ii) from time zero to the last measurable concentration on 8, 15, or 22 (extended plasma sampling scheme). This latter analysis was performed only if patients had measurable docetaxel concentrations on day 8 or later. Data were fit with either a two- or three-compartment model using weighted least-squares as the estimation procedure, and inverse variance of the output error (linear) as the weighting option. Model discrimination was guided by inspection of the weighted sum of squares and the coefficient of variation of the fitted pharmacokinetic parameters and by the Akaike information criterion [31]. Maximum plasma concentration (C_{max}) values were obtained from the model-estimated plasma concentration at the end of the docetaxel infusion. Calculated secondary pharmacokinetic parameters included half-life during

the terminal phase of the disposition curve ($t_{1/2,\lambda z}$) and systemic clearance (CL). The area under the plasma concentration-time curve (AUC) was calculated as dose divided by CL. For weekly regimens, the cumulative AUC during a 3-week treatment period was calculated by multiplying the AUC during week 1 of treatment by 3 with the assumption that docetaxel clearance did not change during weeks 2 and 3 of treatment.

Statistical Considerations

Pharmacokinetic parameters are presented as mean values ± SD, and for all tests the a priori cut-off for statistical significance was taken at $P < .05$. Analysis of variance was used to compare C_{max} values and cumulative AUC during a 3-week treatment period at the different dose levels. The method of Tukey-Kramer HSD was used to adjust for multiple comparisons of mean values. Differences between pharmacokinetic parameter values, which were calculated using data from sampling to 24 h or extended sampling, were compared by a paired Student's *t*-test. Statistical calculations were performed using the software package JMP version 3.2.6 (SAS Institute, Carey, NC).

Group sample sizes of 20 were calculated to achieve approximately 70% power to detect a ratio of 1.50 between the clearance of docetaxel in the respective treatment groups, using a double-sided test with a significance level (α) of 0.05 and assuming equal variances for both groups. This statistical calculation was performed in the SISA Binomial program (D.G. Uitenbroek, Hilversum, the Netherland, 1997; available at hhtp://home.clara.net/sisa/samsize.htm [accessed July 13, 2004]).

Results

Data from twenty-three female and 23 male patients were included this pharmacokinetic study and patient demographic data are summarized in Table 1. Docetaxel pharmacokinetic parameters determined from plasma concentrations measured from time zero to 24 hours post-treatment are displayed in Table 2. Docetaxel pharmacokinetic parameters were similar when docetaxel 35 mg/m^2 was given alone or with irinotecan ($P > 0.50$), similar to earlier findings of this combination given in a 3-weekly regimen [32, 33]; therefore, data from both schedules were combined and summary statistics are presented in Table 2. Mean ± SD docetaxel clearance values were similar with weekly and 3-weekly schedules (overall means, 25.2 ± 7.7 versus 23.7 ± 7.9 L/h/m^2; $P < 0.5467$); values for half-life were also similar with both schedules of administration (overall means, 16.5 ± 11.2 and 17.6 ± 7.4 h, P =

0.6990). Docetaxel C_{max} and AUC values at the different dose levels are shown in Figure 1. Mean ± SD C_{max} values with docetaxel administered at a dose of 35 mg/m^2 over 30 min (1.93 ± 0.60 μg/mL) were similar to those observed at doses of 60 mg/m^2 (1.55 ± 0.41 μg/mL) and 75 mg/m^2 (2.18 ± 0.71 μg/mL) administered over 1 h, but significantly lower ($P < 0.0001$) than at 100 mg/m^2 (4.15 ± 1.35 μg/mL) over 1 h. The difference in C_{max} observed between the weekly and 3-weekly schedules is not related to a pharmacokinetic difference but to the shorter infusion duration in the weekly schedule (30 min versus 1 h) and the different doses administered in the 3-weekly regimens (60, 75, and 100 mg/m^2).

Table 1. Patient demographic data[a]

	Schedule of administration	
Characteristic	Weekly	Every-3-week
Number of patients	20	26
Sex, female/male	5/15	18/8
Age, years	65 (47 – 77)	47 (26 – 71)
BSA, m^2	1.96 (1.57 – 2.55)	1.83 (1.54 – 2.29)
Tumor Type		
Breast		12
Melanoma		4
NSCLC	12	
Prostate	8	1
Unknown primary		4
Other[2]		5
Docetaxel dose, mg/m^2		
35	20	
60		10
75		9
100		7

[a]Continuous data are given as median with range in parenthesis, and categorical data as number of patients; [b]Other tumor types include one each of cervix, head and neck, melanoma, rectum, sarcoma, unknown primary.

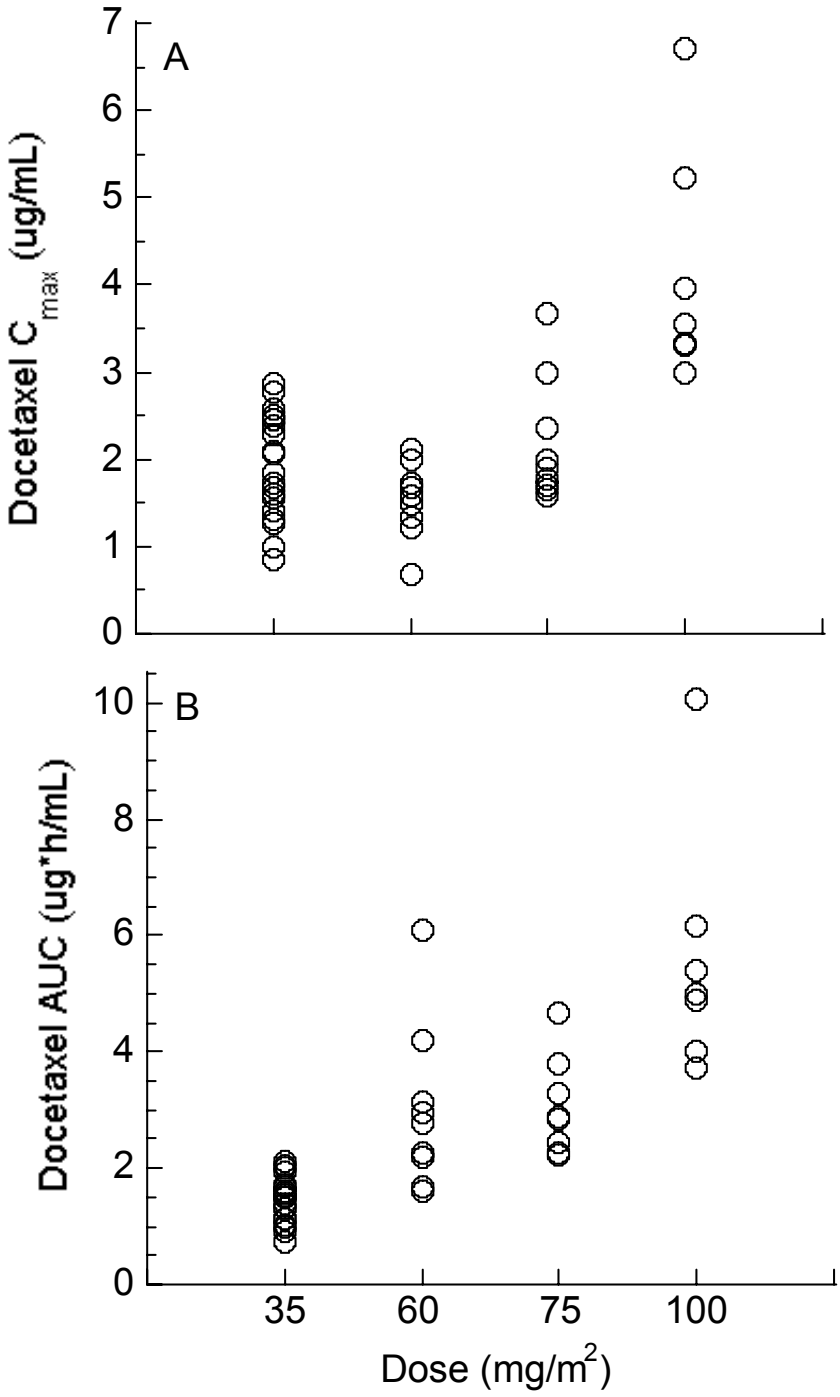

Figure 1. Docetaxel peak plasma concentration [C_{max} (panel A)] and area under the plasma concentration-time curve [AUC (panel B)] values as a function of dose (mg/m^2). The difference in C_{max} observed between the weekly and 3-weekly schedules is not related to a pharmacokinetic difference but to the shorter infusion duration in the weekly schedule (30 min versus 1 h) and the different doses administered in the 3-weekly regimens (60, 75, and 100 mg/m^2).

Table 2. Docetaxel pharmacokinetic parameters: sampling to 24 hours post-treatment[a]

				Parameter			
Schedule	Concurrent Drug	No. of Patients	Infusion Time (h)	C_{max} (μg/mL)	AUC (μg×h/mL)	CL (L/h/m^2)	$t_{1/2,\lambda z}$ (h)
Weekly							
35 mg/m^2	None	8	0.50 ± 0.035	1.85 ± 0.73	1.32 ± 0.42	29.1 ± 10.2	15.6 ± 12.0
35 mg/m^2	Irinotecan	12	0.54 ± 0.053	1.99 ± 0.52	1.59 ± 0.40	22.5 ± 4.2	17.1 ± 12.7
	ALL DATA	20	0.52 ± 0.044	1.93 ± 0.60	1.48 ± 0.41	25.2 ± 7.7	16.5 ± 11.2
3-Weekly							
60 mg/m^2	Doxorubicin	10	1.01 ± 0.031	1.55 ± 0.41	2.85 ± 1.40	25.0 ± 9.7	18.0 ± 9.29
75 mg/m^2	None	9	1.04 ± 0.036	2.18 ± 0.71	3.05 ± 0.85	25.8 ± 6.3	17.5 ± 7.3
100 mg/m^2	None	7	1.03 ± 0.047	4.15 ± 1.35	5.62 ± 2.12	19.6 ± 5.60	17.2 ± 6.2

[a]Data represent mean ± SD. Docetaxel plasma concentration-time data from time zero to 24 hours post infusion were used for calculation of pharmacokinetic parameters.

Since docetaxel clearance is not schedule dependent, exposure-intensity (AUC) comparisons between weekly and 3-weekly schedules are equivalent to dose-intensity comparisons. The AUC during 3 weeks of treatment is larger following the 35 mg/m^2 weekly dose (4.44 ± 1.24 μg/mL) (cumulative 3-week dose of 105 mg/m^2) compared to 60 mg/m^2 (2.85 ± 1.40 μg/mL) and 75 mg/m^2 (3.05 ± 0.85 μg/mL) given 3-weekly but is similar to 100 mg/m^2 (5.62 ± 2.12 μg/mL) given 3-weekly.

Docetaxel pharmacokinetic parameters calculated from data that included extended plasma sampling to days 8 to 22 post-treatment are listed in Table 3. Observed and model-simulated docetaxel concentration-time profiles from representative patients receiving docetaxel 35 mg/m^2 as a 30 min infusion and 75

mg/m^2 as a 1 h infusion are shown in Figure 2 and mean observed docetaxel concentration-time profiles following single agent administration at 35 mg/m^2 (30 min infusion) (n=9), 75 mg/m^2 (1 h infusion) (n=9), and 100 mg/m^2 (1 h infusion) (n=7) are illustrated in Figure 3.

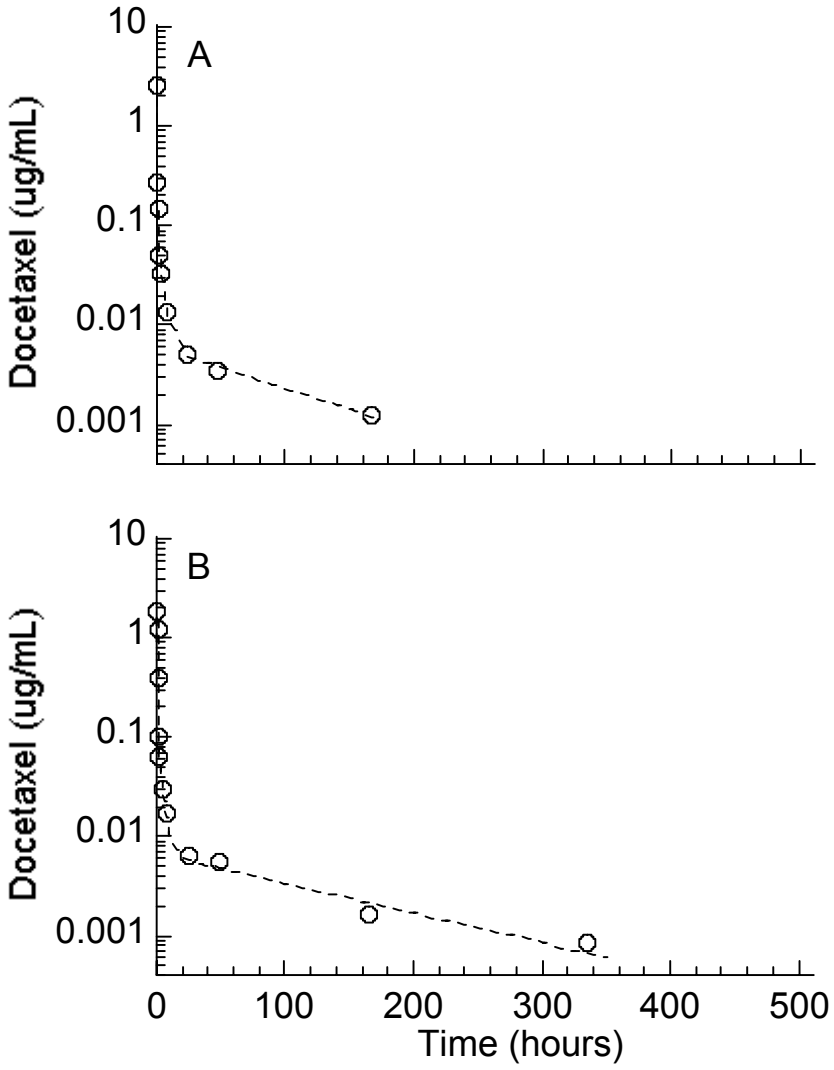

Figure 2. Plasma concentration-time profiles from extended plasma sampling following administration of docetaxel 35 mg/m^2 administered as a 30 min infusion (panel A) and 75 mg/m^2 administered as a 1 h infusion (panel B). Dashed lines are simulated concentrations from fitting a 3-compartment model to the observed concentration-time data. The following equation converts docetaxel concen-trations in units of μg/mL to nM: concentration (nM) = concentration (μg/mL) × 1237.79.

Table 3. Docetaxel pharmacokinetic parameters: extended sampling to days 8 to 22 post- treatment[a]

Schedule	Concurrent Drug	No. of Patients[b]	Parameter AUC (µg×h/mL)	CL (L/h/m^2)	$t_{1/2,\lambda z}$ (h)	Concentration (µg/mL)[c] C_{day8}	C_{day15}	C_{day22}
Weekly								
35	None	6	1.63 ± 0.30	22.0 ± 3.8	61.3 ± 12.5	0.00086 ± 0.00025	NA	NA
35	Irinotecan	10	1.87 ± 0.37	19.4 ± 3.69	60.4 ± 24.6	0.00089 ± 0.00050	NA	NA
	ALL DATA	16	1.78 ± 0.35	20.3 ± 3.81	60.7 ± 20.3	0.00088 ± 0.00041		
3-Weekly								
60	Doxorubicin	5	3.74 ± 0.77	16.6 ± 3.64	135 ± 21.9	ND	ND	0.00077 ± 0.00044
75	None	9	3.41 ± 0.98	23.2 ± 5.66	91.7 ± 32.1	0.0014 ± 0.00043	0.00067 ± 0.00025[d]	0.00047 ± 0.00008[d]
100	None	4	7.87 ± 2.90	14.4 ± 6.37	120 ± 80.5	0.0036 ± 0.0022	0.0022 ± 0.0019	0.0073[e]

[a]Data represent mean ± SD. Docetaxel plasma concentration-time data from time zero to the last measurable concentration on day 8, 15, or 22 were used for calculation of pharmacokinetic parameters; [b]The number of patients represents those with measurable docetaxel concentrations above the analytical assay lower limit of quantitation on day 8 or later; [c]The following equation converts docetaxel concentration in units of µg/mL to nM: concentration (nM) = concentration (µg/mL) * 1237.79; [d]Concentrations on day 15 and 22 were BLQ in 1 of 9 and 5 of 9 patients, respectively; [e]0.5 mL of sample was available for analytical analysis, which precluded measurement of docetaxel concentrations < 1.0 nM; concentrations on day 22 were below the lower limit of quantitation of the analytical method in 3 of 4 patients.

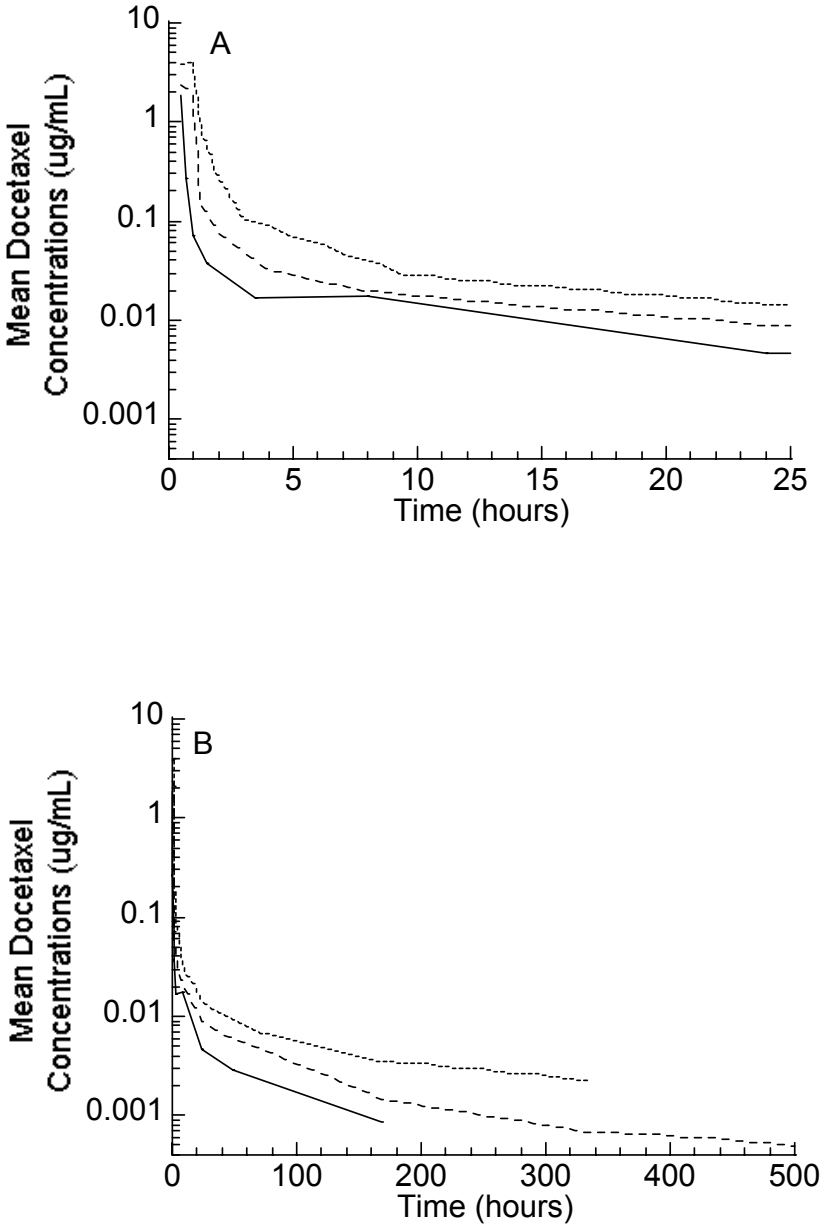

Figure 3. Observed mean docetaxel plasma concentrations following administration of docetaxel 35 mg/m^2 administered as a 30 min infusion (solid line), 75 mg/m^2 administered as a 1 h infusion (dashed line), and 100 mg/m^2 administered as a 1 h infusion (dotted line). Panel A includes concentrations to 24 hours post infusion and panel B includes concentrations to day 22 (approximately 500 hours). The following equation converts docetaxel concentrations in units of μg/mL to nM: concentration (nM) = concentration (μg/mL) × 1237.79.

With the weekly schedules, clearance values were 19% lower when calculated from concentration-time data from extended sampling to day 8 than versus 24-hour data; with the 3-weekly schedules, docetaxel clearance values were 10 to 34% lower

with extended sampling to day 22 (overall paired means, 19.7 ± 5.1 L/h/m^2 versus 24.0 ± 6.6; $P < 0.0001$). These differences reflect the fact that samples were obtained for longer time periods thus allowing for a more accurate estimate of the terminal disposition half-life. By measuring docetaxel concentrations over an extended sampling time period of 3 weeks, the calculated terminal disposition half-life of docetaxel was found to be about 5-fold longer than that estimated on the basis of the standard 24-hour sampling interval (overall paired means, 86.4 ± 44.9 versus 16.6 ± 8.2 h; $P < 0.0001$). At 35 mg/m^2, the mean ± SD docetaxel concentration on day 8 was 0.00088 ± 0.00041 μg/mL (1.08 ± 0.51 nM); at 75 mg/m^2, concentrations on day 8, 15, and 22 were 0.0014 ± 0.00043 μg/mL (1.79 ± 0.53 nM), 0.00067 ± 0.00025 μg/mL (0.83 ± 0.31 nM), and 0.00047 ± 0.00008 μg/mL (0.58 ± 0.099 nM), respectively.

Discussion

In the present study, we describe the comparative pharmacokinetics of docetaxel in plasma following weekly and 3-weekly administration schedules. The data complement previous knowledge on the clinical pharmacology of docetaxel, and may have practical implications for its clinical use. Previous published studies of docetaxel pharmacokinetics have only focused on 3-weekly schedules in which the drug is administered as a 1-hour intravenous infusion [34,35]. There is great current interest in evaluating the administration of docetaxel on weekly schedules. Weekly regimens appear to have comparable antitumor efficacy as 3-weekly schedules, together with a distinct toxicity profile with reduced myelosuppression [18-21]. Given the known exposure-toxicity relationship that has been defined for 3-weekly regimens with docetaxel monotherapy (AUC during cycle 1 and neutropenia) [5], we attempted to understand the differences in the toxicity profile between weekly and 3-weekly docetaxel by evaluating the comparative pharmacokinetics for both schedules. The use of a sensitive analytical method based on liquid chromatography with tandem mass-spectrometric detection allowed the determination of docetaxel exposure during the entire week or cycle of therapy.

At doses of 35 mg/m^2 given weekly and 100 mg/m^2 given 3-weekly, the predicted AUC over a 3-week period for weekly administration (4.44 μg×h/mL) was similar to that during cycle 1 of the 3-weekly regimen (5.62 μg×h/mL in the present study; 4.81μg×h/mL in reference 5). Given the difference in the incidence of severe myelosuppression between the two schedules, the pharmacokinetic data suggests that the same exposure-toxicity relationship defined previously for 3-weekly regimens with

docetaxel monotherapy [5], may not apply to weekly regimens. It is possible, however, that measurement of unbound drug concentrations is required to understand exposure-toxicity relationships that apply to both regimens. Indeed, it has recently been shown that the plasma protein binding of docetaxel is decreased in the presence of the docetaxel vehicle polysorbate 80 at concentrations that may be achieved at the end of the docetaxel infusion when given at doses used in 3-weekly regimens [36,37]. The influence of polysorbate 80 on docetaxel protein binding is presumably the result of a complex formation of polysorbate 80 with serum proteins and/or a displacement interaction on the main docetaxel binding protein, alpha-1-acid glycoprotein [38], caused by a polysorbate 80 degradation product(s) [39]. Regardless of the exact mechanistic basis for this phenomenon, this finding indicates that exposure to the (pharmacologically active) fraction of unbound docetaxel may increase with an increase in dose (from 35 mg/m^2 to 75 or 100 mg/m^2), which would be expected to result in more severe hematological toxicity. However, docetaxel is often administered as a 30-minute infusion with weekly regimens and as a 1-hour infusion with 3-weekly regimens, which may achieve similar polysorbate 80 concentrations at the end of the docetaxel infusion. Measurement of polysorbate 80 plasma concentrations with weekly and 3-weekly regimens is in progress.

The similar exposure-intensity and dose-intensity [6] for docetaxel is consistent with observations of comparable efficacy of weekly and 3-weekly regimens in phase II trials in patients with metastatic breast cancer and advanced non-small cell lung cancer [18-21], and with preclinical studies suggesting that the antitumor activity of docetaxel is independent of the dose/schedule of administration [40]. Weekly and 3-weekly docetaxel regimens are being directly compared in breast cancer in the adjuvant setting. Similar to docetaxel, paclitaxel appears to have comparable efficacy when administered in high-dose or low-dose regimens in patients with metastatic breast cancer [41-43].

By measuring docetaxel concentrations over an extended sampling time period of 3 weeks, docetaxel clearance values were on average 10 to 35% lower than those determined from the 24-hour data. Because a 25% decrease in docetaxel clearance has been shown to be associated with a significant increase (150%) in the odds of developing febrile neutropenia [5], when combining data from different studies for pharmacokinetic, pharmacodynamic, and/or pharmacogenetic analysis, it will be important to include data utilizing similar sampling schemes and equally sensitive analytical methods if extended sampling strategies are used.

With the use of prolonged plasma sampling schemes, the calculated terminal disposition half life of docetaxel was approximately 86 h, which is about 5-fold longer than that estimated on the basis of conventional 24-hour sampling intervals, and almost 9-fold longer than published values [35]. Consequently, docetaxel concentrations are maintained above 0.0008 μg/mL (1.0 nM) for 7 days with weekly schedules and above 0.0004 μg/mL (0.5 nM) for 21 days with 3-weekly regimens (Table 3, Figure 3). This observation is of particular relevance with regard to potential mechanisms of action of the taxanes where low nanomolar concentrations have been shown to inhibit cell proliferation without arresting cells at mitosis [44,45], suppress microtubule dynamics [46], inhibit tumor angiogenesis [47-49], and enhance cell radiosensitization [50].

There is current preclinical and clinical interest in the potential antiangiogenic properties of the taxanes. Indeed, docetaxel and paclitaxel have recently been shown to be potent and specific inhibitors of endothelial cell migration in vitro [51], vascular endothelial cell growth factor secretion [52], and angiogenesis in vitro and in vivo at IC_{50} values of approximately ≤ 1 nM (47-49). It has been suggested that weekly schedules of taxanes possess antiangiogenic properties relative to 3-weekly schedules because a weekly schedule of paclitaxel has been shown to induce responses in some patients with tumors refractory to paclitaxel administered every 21 days [53,54]. However, this has not been demonstrated unequivocally in *in vivo* preclinical models. Moreover, if maintaining low nanomolar concentrations for prolonged periods contributes to the antiangiogenic properties of docetaxel, than this mechanism of action should apply to both schedules of administration given the similarity in circulating concentrations.

Collectively, these data show that the altered toxicity profiles observed with weekly docetaxel administrations may not be explained by a change in plasma pharmacokinetics of total drug, and that previously defined exposure-toxicity relationships for 3-weekly regimens do not apply to weekly regimens. In addition, we have shown by applying an extended sampling time period of 21 days that until now the circulation time of docetaxel in cancer patients has been greatly underestimated. The presently observed prolonged terminal disposition phase of docetaxel should be taken into consideration when designing future clinical trials of docetaxel administered in novel drug combinations and combined modality therapy, and when evaluating alternative schedules of administration.

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Chapter 10

Prospective Evaluation of the Pharmacokinetics, CYP3A Phenotype, and Toxicity Profile of Docetaxel in the Elderly

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Submitted

Abstract

Purpose: To prospectively study the pharmacokinetics and toxicity profile of docetaxel as well as cytochrome P450 3A (CYP3A) phenotype in elderly patients with cancer.

Patients and Methods: Docetaxel was administered at a dose 75 mg/m^2 once every 3 weeks to 25 elderly cancer patients aged ≥ 65 years and 26 cancer patients aged < 65 years. CYP3A was phenotyped using the erythromycin breath test (ERMBT) prior to drug administration. Pharmacokinetic studies were performed during the first cycle of therapy.

Results: Of 51 patients treated, 20 aged ≥ 65 years (median [range], 71 years [65-80]) and 20 aged < 65 years (53 years [26-64]) were evaluable for pharmacokinetic and CYP3A studies, and 39 were evaluable for toxicity. Patient characteristics were similar (P ≥ .15) between the 2 cohorts. The ERMBT parameter, percentage ^{14}C-exhaled/h, was not altered in elderly patients (mean, 2.38% vs 2.74%; P = .23), suggesting similar CYP3A4 activity. Mean (SD) docetaxel clearance was also similar between the 2 cohorts: 30.1 (18.3) L/h versus 30.0 (14.8) L/h (P = .98). The development of febrile neutropenia was associated with higher AUC values (P = .02). The percentage of patients with grade 4/febrile neutropenia was 63%/16% versus 30%/0% (P ≥ .06) in the older and younger cohort, respectively; febrile neutropenia in the elderly cohort may be related to drug exposure and not age.

Conclusion: CYP3A activity and docetaxel pharmacokinetics are unaltered in elderly patients. It is concluded that docetaxel 75 mg/m^2 in a 3-weekly regimen is feasible in the elderly.

Introduction

Docetaxel is a semi-synthetic taxane derived from an extract of the needles of the European yew tree (*Taxus baccata*), and acts by disrupting the microtubule network [1]. The drug has significant antitumor activity against numerous tumors and is approved for treatment of locally advanced or metastatic breast and non-small cell lung cancers. In patients with advanced breast cancer receiving docetaxel 100 mg/m^2 as a 1 hour infusion every 3 weeks (3-weekly), grade 4 and febrile neutropenia occur in 84% and 11.8% of patients, respectively (see: http://www.taxotere.com/ - last accessed July 13, 2004); in patients with non-small cell lung cancer receiving 75 mg/m^2, grade 3/4 and febrile neutropenia occur in 65% and 6.3% of patients,

respectively. Other side effects include alopecia, asthenia, dermatologic reactions, fluid retention, hypersensitivity reactions, and stomatitis. Drug exposure-toxicity relationships have been extensively studied for docetaxel monotherapy administered 3-weekly and indicate that the area under the curve (AUC) of total plasma concentrations during the first cycle of treatment is related to incidence of grade 4 neutropenia and febrile neutropenia [2].

As the population in Western countries ages and life expectancy increases [3], there is an increasing number of cancer patients 65 years of age or older that might benefit from chemotherapeutic treatment. There is often hesitation to treat elderly patients with chemotherapy due, in part, to the older patient being more susceptible to therapy-related toxicity [4-6]. However, studies have demonstrated that elderly patients with good performance status and lacking comorbidities are not at increased risk for treatment-related toxicities [4-7]. Studies also indicate that undertreatment is associated with inferior outcome in older patients [8-10]. Little is known about the clinical pharmacokinetics and pharmacodynamics of anticancer agents, including docetaxel, and their relation to drug tolerance and outcome in the elderly [4-6]. Docetaxel administered in weekly schedules at lower doses has been found to be both efficacious and generally well tolerated in elderly patients [11-13], and a study evaluating the population pharmacokinetics of weekly docetaxel showed no effect of age on drug clearance [14]. There is general reluctance to administer docetaxel 3-weekly to elderly patients due to the prevalence of neutropenia with docetaxel therapy [15], although this has not been adequately evaluated in a clinical trial.

The objective of the present study was to prospectively characterize the pharmacokinetic and toxicity profile of docetaxel during one cycle of treatment when administered at a dose of 75 mg/m^2 once every 3 weeks to patients aged less or older than 65 years. Because docetaxel undergoes extensive metabolism by cytochrome P450 3A (CYP3A) [16], CYP3A activity was assessed prior to treatment to determine if the function and/or expression of enzyme is altered with increasing age.

Patients and methods

Patient eligibility

Patients were eligible when they had histologically or cytologically confirmed solid tumor malignancies, for which docetaxel was a viable treatment option. Other criteria for patient enrollment were: 1) age ≥ 18 years; 2) performance score (PS) < 3 according to the Eastern Cooperative Oncology Group criteria; 3) adequate bone

marrow function as defined by pre-therapy values of hemoglobin ≥ 8.0 g/dL, ANC ≥1,500/μL, and platelet count ≥ 100,000/μL; 4) creatinine ≤ 2.0 × the institutional upper limit of normal (ULN); 5) total bilirubin < 1.5 × ULN; 6) if alkaline phosphatase was <2.5 × ULN, any elevations in AST/ALT; or if AST/ALT were <1.5 × ULN, any elevation in alkaline phosphatase; patients with ALT and/or AST ≥ 1.5 × ULN with concomitant alkaline phosphate ≥ 2.5 × ULN were not eligible for treatment; 7) peripheral neuropathy ≤ grade 1 and no symptomatic brain metastasis; 8) no previous treatment with docetaxel; and 9) no concomitant use of phenytoin, carbamazepene, barbiturates, rifampicin, phenobarbital, St. John's wort, and ketoconazole. All concomitant drugs and the use of herbal medicines were recorded. The clinical protocols were approved by the local institutional review boards (Baltimore, MD, Rotterdam, the Netherlands, and Washington, DC), and all patients provided written informed consent before enrollment. Before treatment, a complete registration form was received by the coordinating center (Baltimore, MD), and a study number was assigned. Patients who did not have complete pharmacokinetic and CYP3A phenotyping studies during cycle 1 were replaced.

Drug Treatment

Two groups of patients were studied based on age. The control group consisted of patients aged 18 to 64 years, and the elderly group consisted of patients aged 65 years or older. The clinical docetaxel preparation (Taxotere; Aventis Pharmaceuticals) containing 20 or 80 mg of the drug formulated in 0.5 mL and 2.0 mL of polysorbate 80, respectively, was diluted with a solution of 13% ethanol in water to a 10 mg docetaxel/mL concentration. This solution was diluted further in a 250-mL infusion bag or bottle of either 0.9% sodium chloride solution or 5% dextrose solution to produce a final concentration of 0.30 – 0.74 mg/mL. Individual drug doses were normalized to body-surface area and administered intravenously over 1 h at a dose of 75 mg/m^2 every 3 weeks in both treatment groups. Dexamethasone, 8 mg orally every 12 hours for 5 doses (3 days), was administered starting 24 h before drug treatment. Patients did not routinely receive anti-emetic prophylaxis. After 1 cycle of therapy, treatment continued at the discretion of the treating physician until tumor progression, development of unacceptable toxicity, or patient withdrawal.

Patient Evaluation

The extent of prior treatment was assessed two-fold: 1) the number of prior treatment regimens; and 2) patients were considered heavily pretreated if they received ≥ 2 cycles of mitomycin C, ≥ 4 cycles of carboplatin, ≥ 6 cycles with cisplatin or an alkylating cytostatic drug. Pretreatment evaluations included assessment of PS, height, weight, toxicity assessment, a complete blood count with differential (CBC), and the following serum chemistries: creatinine, alkaline phosphatase, AST, ALT, total bilirubin, α1-acid glycoprotein (AAG), and albumin.

Toxicity assessment and a CBC with differential were performed weekly for a total of 3 weeks (1 cycle). Toxicity assessments were performed according the National Cancer Institute Common Toxicity Criteria version 2.0. Management of toxicity was at the discretion of the treating physician per institutional guidelines.

Erythromycin Breath Test (ERMBT)

Within one week prior to docetaxel administration during cycle 1, CYP3A activity was determined using the ERMBT. The ERMBT dose consisted of 0.04 mg [^{14}C-N-methyl]-erythromycin, containing 3 μCi of radioactivity, dissolved in 4.5 mL of 5% dextrose solution. The dose was administered as an intravenous bolus injection over approximately 1 min. Breath samples were collected in balloons post-injection at 5, 10, 15, 20, 25, 30 and 40 minutes. Samples were shipped to Metabolic Solutions (Nashua, NH) for measurement of breath carbon dioxide. The data was reported as percentage ^{14}C metabolized per min (% ^{14}C exhaled/min) at each time point. The conventional ERMBT parameter, percentage ^{14}C metabolized per hour (% ^{14}C exhaled/h), was calculated using the equation $y = -65.988 \cdot x^2 + 54.645 \cdot x + 0.0377$, where x is the value for % ^{14}C exhaled/min at the 20 min time point [17]. The area under the % ^{14}C exhaled/min-time curve from time zero to 40 min (AUC_{0-40}) was determined using the linear trapezoidal method. The ERMBT parameter, $1/T_{max}$, was determined as described previously [18]. A mono-exponential equation was also fitted to the % ^{14}C exhaled/min-time data and the time of the maximum % ^{14}C/min (T_{max}) was the estimated value.

Pharmacokinetic Sampling and Assay

Blood samples were collected for docetaxel pharmacokinetic studies during the first cycle of treatment cycle at the following time points: pre-treatment, 30 min during the infusion, 59 min (immediately before the end of the infusion), and post-infusion at 10 and 30 min, 1, 3, 7, 24, and 48 h, and on day 8. Samples were collected in a 10 mL

heparinized tube and placed on ice until further processing within 30 minutes of collection. Plasma was isolated by centrifugation at 4 ^{o}C, at 1000 g for 10 minutes and frozen at or below –20 °C until the time of analysis.

Docetaxel was quantitated in plasma over the range of 0.50 nM to 100 nM using a validated liquid chromatographic method with tandem mass-spectrometric detection, as previously described [19]. The bias and precision of quality control (QC) samples, which included docetaxel concentrations of 2.0, 20.0, 80.0 nM, and an 80-nM QC that was diluted 100-fold prior to processing, were < 15%. At the assay lower limit of quantitation of 0.50 nM (~400 pg/mL), bias and precision were < 20%.

Individual docetaxel pharmacokinetic parameters were estimated using model-dependent methods as implemented in Adapt II release 4 (Biomedical Simulations Resource, Los Angeles, CA) [20]. Concentration-time data were fit with a three-compartment model using weighted least-squares as the estimation procedure, and inverse variance of the output error (linear) as the weighting option. Calculated secondary pharmacokinetic parameters included half-life during the terminal phase of the disposition curve ($t_{1/2,\lambda z}$) and systemic clearance (CL). The AUC was calculated as dose divided by CL. Maximum plasma concentration (C_{max}) values were the observed values.

Statistical Analysis

Group sample sizes of 20 in both age groups (< 65 years and ≥ 65 years) were calculated to achieve 88% power to detect a ratio of 1.50 between clearance variances in the respective groups, using a two-sided F test with a significance level (α) of .05. Sample size calculations were performed using the computer program SISA-binomial (Uitenbroek DG, 1997, Available http://home.clara.net/sisa/binomial.htm, accessed july 13, 2004).

Docetaxel and ERMBT pharmacokinetic parameters were summarized as the mean, standard deviation, and range. For continuous variables, nonparametric tests were used to compare mean values between the two age groups. The method of Tukey-Kramer was used to adjust for multiple comparisons of mean values. Categorical variables were compared using 2-tailed Fisher's Exact Test for 2-by-2 tables. Statistical calculations were performed using the software package JMP version 3.2.6 (SAS Institute, Carey, NC).

Table 1. Patient demographics

	Age < 65 years (n = 20)		Age ≥ 65 years (n = 20)	
	Median	(Range)	Median	Range
Age (years)	53	(26 – 64)	71	(65 – 80)
Body surface area (m^2)	1.93	(1.49 – 2.45)	1.85	(1.45 – 2.25)
Sex[a]				
Female	10		9	
Male	10		11	
AAG (mg/dL)[a]	159	(86 – 257)	126	(60 – 201)
Liver Function Tests				
AST (× ULN)	0.95	(0.30 – 3.9)	0.80	(0.40 – 4.7)
ALT (× ULN)	0.70	(0.20 – 6.6)	0.50	(0.10 – 1.5)
Alkphos (×ULN)	0.85	(0.50 – 2.0)	0.80	(0.40 – 6.2)
Total bilirubin	0.50	(0.30 – 1.1)	0.40	(0.20 – 0.60)
CYP3A Activity (% ^{14}C exhaled/h)[a]	2.38	(0.83 – 4.35)	2.74	(0.78 – 5.79)
ECOG performance status[a,b]				
0	4		4	
1	15		12	
2	1		3	
Primary tumor type[a]				
Breast	5		3	
Head and neck	3		1	
Lung	5		3	
Melanoma	3		0	
Prostate	0		5	
Angiosarcoma	0		3	
Unknown	1		4	
Other	3		1	
Prior treatment[a]				
None	1		8	
1-2 cytotoxic regimens	14		11	
≥ 3 cytotoxic regimens	5		1	
Light	12		13	
Heavy	8		7	

[a]Data are mean (range) values; [b]Data is number of patients; [c]Baseline performance status was not performed in one patient aged ≥ 65 years.

Results

Between August 2002 and September 2003, 51 patients (26 were aged < 65 and 25 were ≥ 65 years) were enrolled on this study. Of these patients, 40 (20 in each age group) were evaluable for pharmacokinetic and ERMBT studies. Patients were not evaluable for pharmacokinetic studies for the following reasons: 1) severe hypersensitivity reaction with discontinuation of drug treatment (1 patient); 2) inability

to perform pharmacokinetic studies due to poor venous access (2 patients); 3) plasma samples became thawed during shipment for analytical analysis (7 patients); and 4) erroneous administration of a lower docetaxel dose of 50 mg/m^2 (1 patient). Patient characteristics for the 40 evaluable patients are listed in Table 1. Body surface area, liver function, performance status, and prior treatment were similar between the 2 cohorts (P ≥ .15), although pre-treatment serum α_1-acid glycoprotein concentrations were 20% lower in the elderly (mean, 126 mg/dL [≥ 65 years] vs 159mg/dL [< 65 years]; P = .04).

Table 2. Docetaxel pharmacokinetic parameters

	< 65 years			≥ 65 years		
Parameter	Mean	SD	Range	Mean	SD	Range
Cmax (μg/mL)	4.06	1.38	1.65 – 6.36	3.44	1.58	0.88 – 6.52
AUC (μg/mL×h)	5.69	2.27	2.47 – 10.2	6.01	3.23	1.54 – 13.7
Cl (L/h)	30.0	14.8	13.7 – 68.8	30.1	18.3	9.5 – 91.6
Cl (L/h/m^2)	15.4	6.94	7.30 – 30.1	16.6	10.0	5.20 – 49.2
V_c (L)	5.24	2.63	2.16 – 10.1	6.24	3.45	2.76 – 16.3
V_c (L/m^2)	2.70	1.28	1.29 – 5.25	3.45	1.94	1.44 – 8.78
V_{ss} (L)	803	370	399 – 1479	923	435	382 – 2408
V_{ss} (L/m^2)	413	170	185 – 788	513	249	193 – 1301
$t_{1/2,\alpha}$ (h)	0.078	0.031	0.046 – 0.15	0.087	0.024	0.051 – 0.013
$T_{1/2,\beta}$ (h)	1.78	1.34	0.84 – 6.91	1.65	0.51	0.66 – 2.60
$T_{1/2,\gamma}$ (h)	64.6	19.2	45.9 – 117	72.8	32.8	32.2 – 164

Plasma Pharmacokinetics

Docetaxel pharmacokinetic parameters were similar in the elderly and younger patient cohorts (P ≥ .15; Table 2). Mean (SD) docetaxel clearance was 30.1 (18.3) L/h in patients aged ≥ 65 years and 30.0 (14.8) L/h in patients < 65 years (P = .98). Interpatient variability in clearance was larger in the elderly (9.6-fold) versus the younger patients (5.0-fold) (Figure 1B). One patient aged 70 years had the highest clearance of 91.6 L/h. Removal of this outlier clearance value (> 3 standard deviations) from the elderly group resulted in a mean (range) clearance of 27 (9.5 to

48.3) L/h and interpatient variation (5.1-fold) similar to the younger patients. It is possible that the patient with an outlier value for clearance was in the elderly group by chance, and hence, there appears to be no age-related interpatient variation in docetaxel clearance.

CYP3A Phenotyping

The ERMBT was performed 24 hours before docetaxel treatment in 82% of patients and immediately before the docetaxel infusion in 18% of patients. The ERMBT parameter, percentage ^{14}C-exhaled/h, was not altered in elderly patients (mean, 2.74 %; range, 0.78 to 5.79) compared to patients < 65 years (mean, 2.38%; range, 0.83 to 4.35; P = .23) suggesting similar CYP3A4 activity between the 2 age groups. The other ERMBT parameters (% ^{14}C exhaled/min, $AUC_{0\text{-}40}$, and $1/T_{max}$) were also similar between the 2 groups (P ≥ .42). Interpatient variation in CYP3A activity was 7.4-fold and 5.2-fold in patients ≥ 65 years and < 65 years, respectively (Figure 1A).

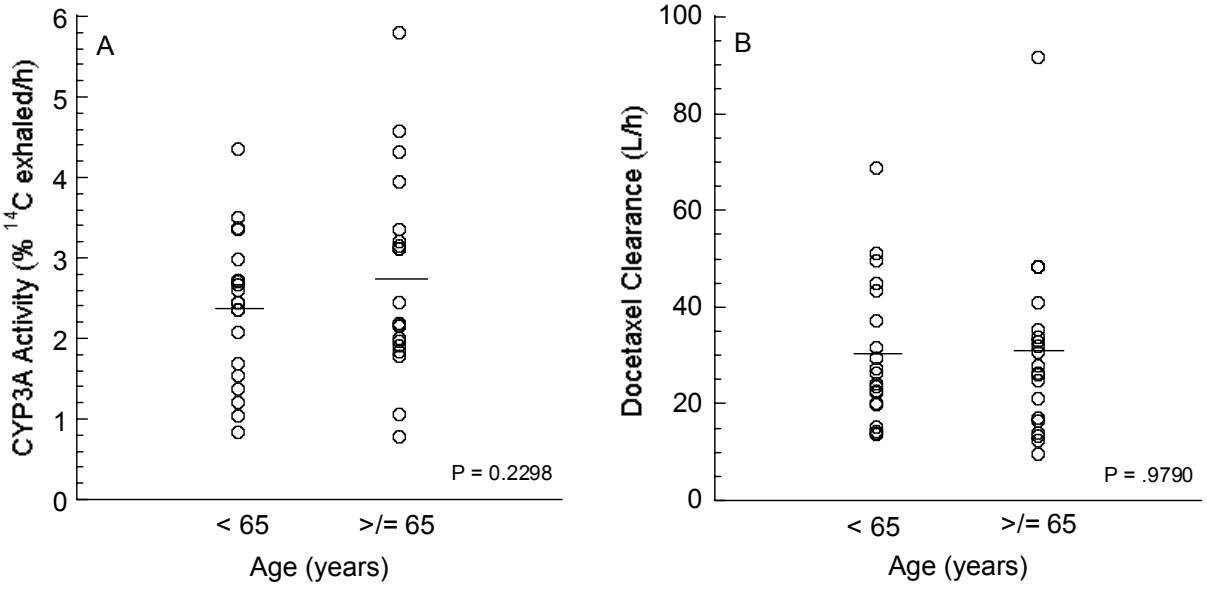

*Figure 1. [**A**] CYP3A activity (%^{14}Cexhaled/h) and [**B**] docetaxel clearance as a function of age group. Lines represent the mean values.*

Toxicity

Twenty patients aged < 65 years and 19 patients aged ≥ 65 years, respectively, were evaluable for hematological toxicity. The incidence of grade 3 and 4 neutropenia and febrile neutropenia, the ANC nadir, and percentage decrements in ANC are summarized in Table 3. The absolute neutrophil count nadir occurred on day 8 in 85% and 80% of patients in the younger and elderly groups, respectively, and no patient had grade 4 neutropenia for > 7 days. Grade 4 neutropenia occurred more frequently in the elderly (63% versus 30%), but the difference was not statistically significant (P = .06); however, because the sample size was not calculated to detect statistical differences in docetaxel-mediated neutropenia between the 2 groups, the possibility of such a difference cannot be fully excluded. Three elderly patients developed febrile neutropenia. One patient had metastatic pancreatic cancer with a performance status of 2, and her disease progressed rapidly 3 weeks after docetaxel treatment at cycle 1. One patient had metastatic prostate cancer, having received prior treatment with bicalutamide, and one patient had adenocarcinoma of unkown primary without any prior chemotherapy. All three patients were treated with broad-spectrum antibiotic therapy without administration of growth factors, and ANC values returned to pretreatment values on day 15.

Table 3. Hematological toxicity

Treatment Group	Neutropenia[a]			ANC nadir (× 109/L)[b]	% Decrease in ANC[b]
	Grade 3	Grade 4	Febrile		
< 65 years	7 (35%)	6 (30%)	0 (0)	1.1 (0.08 – 5.5)	83 (42 – 98)
≥ 65 years	1 (5%)	12 (63%)	3 (16%)	0.61 (0.05 – 1.8)	92 (46 – 99)

[a]Data is number of patients (% of patients); [b]Data is mean (range)

The association between docetaxel AUC and neutropenia was assessed (Figure 2). Patients with febrile neutropenia had significantly higher AUC values (mean, 10.2 μg/mL×h) than patients with grade 0 to 3 (mean AUC, 5.6 μg/mL×h) or uncomplicated grade 4 neutropenia (mean, 5.6 μg/mL×h; P = .02) (Figure 2A). It is likely that development of febrile neutropenia in the 3 elderly patients versus no patients in the younger cohort was related to higher drug exposure in these individual patients rather than age.

Table 4. Maximum grade of non-hematological toxicity

Toxicity	< 65 years								≥ 65 years							
	1		2		3		4		1		2		3		4	
Alopecia	3	(16%)	5	(26%)					10	(53%)						
Asthenia	4	(21%)	5	(26%)	1	(5%)			3	(16%)	4	(21%)	1	(5%)		
Fluid retention									2	(11%)	1	(5%)				
Nausea	4	(21%)	3	(16%)					5	(25%)	3	(16%)				
Oral mucositis	1	(5%)	3	(16%)					4	(21%)	1	(5%)				
Cutaneous toxicity	1	(5%)	1	(5%)					1	(5%)						
Neuropathy	2	(11%)	1	(5%)					4	(21%)	2	(11%)	1	(5%)		
Vomiting	3	(16%)	1	(5%)					3	(16%)	2	(11%)				

Data is number of patients (% of patients)

Percentage decrements in ANC were greater in those patients with AUC values in the upper quartile (mean decrement, 93%) compared to those with AUC values in the interquartile range (mean, 77%; P = .02) (Figure 2B).

Nineteen patients in both age groups were evaluable for non-hematological toxicity. Non-hematological toxicities that were monitored are listed in Table 4. The most frequent toxicities occurring in > 20% of patients were grade 1 or 2 alopecia, asthenia, nausea, oral mucositis, cutaneous toxicity, and neuropathy. The frequency of non-hematological toxicities appeared similar between the 2 age groups, although the small number of patients and low incidence precluded statistical evaluation.

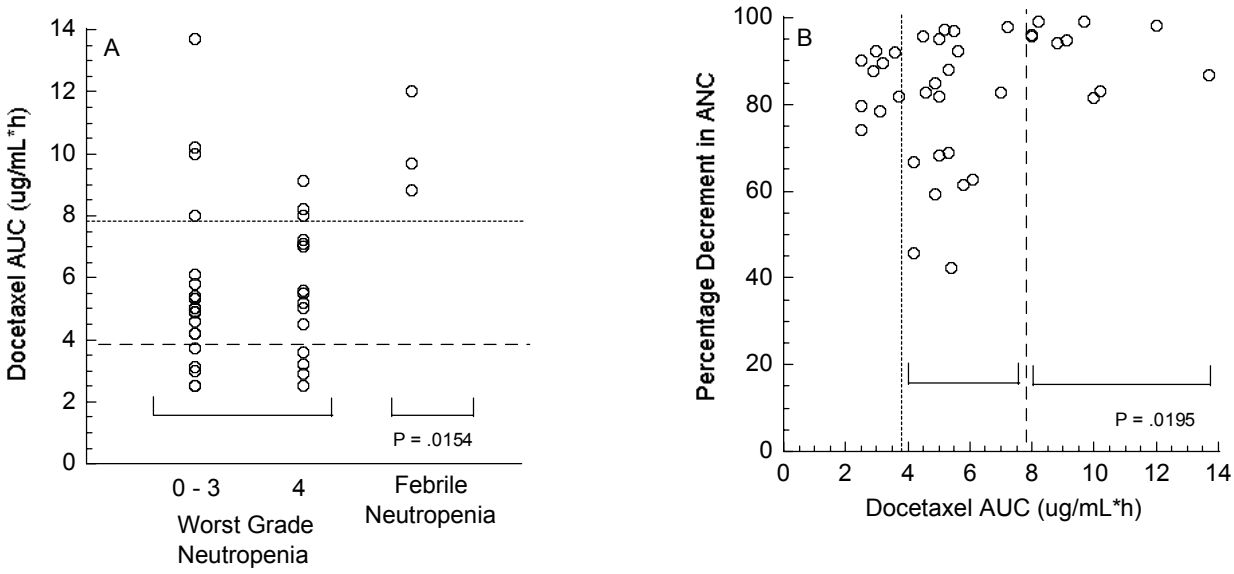

*Figure 2. [**A**] Worst grade of neutropenia (grade 0–3 versus grade 4 versus febrile neutropenia) and [**B**] percentage decrease in ANC as a function of docetaxel AUC. Dotted lines are the 25% quantile and dashed lines are the 75% quantile.*

Discussion

Despite the widespread clinical use of docetaxel, only few data are available on the effect of aging on the pharmacokinetic and pharmacodynamic behavior of this drug. Recent investigations have emphasized the disappointingly low participation of elderly patients in cancer treatment trials and the barriers associated with patient accrual [3]. Several of the factors identified include the lack of information on age-related changes in organ function and on the pharmacokinetics and pharmacodynamics

of anticancer agents. Indeed, while some studies have examined the efficacy and feasibility of chemotherapy in elderly patients, including several studies with weekly docetaxel in breast and nonsmall cell lung cancers [11-13], little is known about the pharmacokinetic behavior of the anticancer agents under evaluation. A few exceptions include studies that evaluated the pharmacokinetics of anthracyclines, cisplatin, ifosfamide, methotrexate and paclitaxel in elderly patients, although most of these studies provide data for a limited number of patients (< 10 patients aged greater than 65 years) and did not include a comparative cohort of younger patients [4-21]. In an attempt to fill this gap of knowledge, we have prospectively evaluated the pharmacokinetics of docetaxel administered once every 3 weeks as well as the phenotypic activity of the major enzyme involved in its elimination, CYP3A, in elderly cancer patients in comparison to younger patients. Overall, the results indicate that there is no statistically significant change in the pharmacokinetics of docetaxel or in CYP3A activity, as measured by the ERMBT, between the two studied age groups. These data complement previous knowledge on the clinical pharmacology of docetaxel, and may have important practical implications for its optimal use in the elderly.

The influence of age on the expression and activity of drug-metabolizing enzymes remains controversial with reports describing either a decline in activity or no change in activity in elderly patients [22-24]. In the current study, docetaxel clearance and the associated interpatient variability (approximately 5-fold) were found to be similar in both treatment groups. Likewise, CYP3A activity and its interpatient variation was not significantly altered with age in this study. Prior *in vitro* studies have suggested an age related decline in CYP3A activity [25]. However, our results are consistent with *in vivo* studies applying the ERMBT as a phenotyping probe of CYP3A-mediated drug clearance where no decrease in CYP3A activity was observed as a function of age [22,24].

The incidence of grade 3/4 neutropenia in the elderly group (68%) was consistent with other studies evaluating docetaxel monotherapy at 75 mg/m^2 once every 3 weeks (65%). Neutropenia resolved within 7 days in all patients without administration of growth factors. It is noteworthy that incidences of neutropenic fever were observed in 3 patients (16%) in the elderly group, which might seem more prevalent than that observed in other studies (6.3%). These 3 patients, however, all had docetaxel clearance values in the lower quartile, which was shown to be associated with the severity of neutropenia. The apparent inconsistencies between unaltered docetaxel clearance in both age groups and a slightly increased incidence of neutropenic fever in

the elderly needs to be interpreted with caution, as our trial was not designed to detect statistical differences in variability in docetaxel-mediated neutropenia between the tested groups with sufficient power. Therefore, the provided information on neutropenia, which was based on a sparse set of hematological toxicity data (e.g., blood cells measured on a once a week basis), should not be taken as evidence for a meaningful clinical difference in toxicity between the two age groups and/or as an argument for the use of standard reductions in docetaxel dose administered to the elderly. In line with this contemplation, previous studies with weekly docetaxel schedules in heavily pretreated elderly patients indeed appeared to be both effective and very well tolerated [11,12].

The incidence of non-hematological toxicities was also similar between both age groups. It is important to note, however, that docetaxel-mediated non-hematological toxicity was not assessed over multiple cycles of treatment as has been done with weekly docetaxel schedules [11-13], where the development of non-hematological toxicities often occur at later cycles. Further investigation is clearly required to shed light on this aspect as well as on efficacy of the once every 3 weeks treatment schedule in elderly cancer patients.

The current pharmacokinetic findings with docetaxel are in contrast with recent data obtained for the related drug, paclitaxel, where drug clearance was found to be inversely correlated with patient age. In addition, exposure to the pharmacologically active fraction unbound paclitaxel was approximately 25% increased in the elderly as compared to younger patients [21]. The mechanisms underlying the discrepant findings observed with paclitaxel and docetaxel are not clear, but may involve age-dependent differences in elimination pathways involved with each agent as well as a differential influence of pharmacokinetic interference by their respective formulation vehicles (ie, polysorbate 80 vs Cremophor EL). Regardless, it further underscores the importance of conducting appropriately designed prospective clinical trials to recognize potential alterations in the pharmacokinetic profile of anticancer drugs with advancing age.

In conclusion, this study indicates that docetaxel pharmacokinetics are not altered in the elderly and that age appears to be an unimportant consideration in drug dosing when considering the potential for age-related changes in drug clearance. The overall incidence of grade 3/4 neutropenia in the elderly cohort was similar to historical data with single-agent docetaxel 75 mg/m^2, and the incidence of febrile neutropenia in the cohort of elderly patients studied may likely be related to drug exposure and not to age. Therefore, on the basis of these results it is concluded that the administration of

docetaxel in a 3-weekly regimen at a dose of 75 mg/m^2 is feasible in the elderly. In view of the wide degree of interindividual variability in drug clearance in both age groups, further evaluations of alternative dosing strategies for individual patients to decrease this variability and improve therapy are still urgently needed.

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Chapter 11

Relation of Systemic Exposure to Unbound Docetaxel and Neutropenia

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Submitted

Abstract

Objective: To evaluate the association between exposure to unbound docetaxel and neutropenia in cancer patients and to identify factors influencing unbound docetaxel clearance.

Methods: Docetaxel was administered once every 3 weeks at a dose of 75 mg/m^2 to 49 patients with normal liver function (n=40) or mild elevations in liver function tests (n=9), or at 50 mg/m^2 to patients with moderate elevations in liver function tests (n=6). Pharmacokinetic studies and toxicity assessments were performed during the first cycle of therapy. Total docetaxel concentrations were determined by HPLC and tandem mass spectrometry and unbound docetaxel fraction was determined by equilibrium dialysis.

Results: In patients with normal liver function, unbound docetaxel disposition was characterized by mean (± SD) Cmax, AUC, and clearance values of 233 ± 101 ng/mL, 321 ± 143 ng/mL×h, and 565 ± 329 L/h, respectively, and unbound clearance varied 8.5-fold; polysorbate 80 exhibited mean (± SD) Cmax, AUC, and clearance values of 451 ± 221 μg/mL, 528 ± 217 μg/mL×h, and 8.18 ± 3.66 L/h, respectively, and clearance varied 6.7-fold. Unbound docetaxel clearance (*P*=.020) and polysorbate 80 clearance (*P*=.092) were reduced in patients with liver impairment. From multiple linear regression analysis, only polysorbate 80 AUC and liver impairment were significantly associated with unbound docetaxel clearance. Unbound docetaxel AUC was better correlated than total AUC with both percentage decrements in absolute neutrophil count (*P*=.002 *versus P*=.029) and worst grade of neutropenia (*P*=.013 *versus P*=.220), where higher exposure was associated with worse hematological toxicity.

Conclusions: Exposure to unbound docetaxel is more closely related to drug-induced hematologic toxicity than total drug and should be considered for future pharmacologic investigations.

Introduction

Docetaxel is a semi-synthetic taxane derived from an extract of the needles of the European yew tree (*Taxus baccata*), and acts by disrupting the microtubule network [1]. The drug has significant antitumor activity against numerous tumors and is approved for treatment of locally advanced or metastatic breast and non-small cell lung cancers at doses ranging from 60 to 100 mg/m^2 administered as a 1 hour infusion

every 3 weeks (3-weekly). Neutropenia is the dose-limiting toxicity of docetaxel administered on 3-weekly schedules. In patients with normal liver function receiving docetaxel at 75 mg/m^2, grade 3/4 and febrile neutropenia occur in 65% and 6.3% of patients, respectively [2]. Other side effects include alopecia, asthenia, dermatologic reactions, fluid retention, hypersensitivity reactions, and stomatitis.

Docetaxel's pharmacokinetic profile is characterized by substantial interpatient variability in total clearance (>10-fold), which has important clinical consequences. In a population pharmacokinetic-pharmacodynamic study of more than 600 patients receiving docetaxel monotherapy at doses ranging from 75 to 100 mg/m^2 administered 3-weekly, a reduction in total docetaxel clearance by 50% was associated with an increase in the odds of developing grade 4 neutropenia and febrile neutropenia by 430% and 300%, respectively [3]. Even a 25% decrease in docetaxel clearance is associated with a 150% increase in the odds of developing febrile neutropenia [3]. In addition, docetaxel exposure has also been related to treatment efficacy. The area under the curve (AUC) was a significant predictor of time to tumor progression in non-small cell lung cancer [3], and underdosing (e.g., lower docetaxel AUC due to increased clearance) was associated with a worse time to progression and time to death [4].

Elimination routes of docetaxel are mediated principally by the hepatic cytochrome P450 3A (CYP) isoforms CYP3A4 and CYP3A5 [5]. The major docetaxel metabolites and less than 10% of the parent drug are excreted into the feces, whereas total urinary excretion is less than 10% [1]. The metabolites demonstrate substantially reduced cytotoxic activity as compared to the parent drug, making biotransformation by CYP3A a major route of inactivation [6]. Liver impairment presenting with concurrent elevations in serum transaminases in the presence of normal total bilirubin is associated with reduced total clearance by 25% [3] and elevated total bilirubin greater or equal to 1.5 times the upper limit of institutional normal is associated reduced total docetaxel clearance by greater than 50% [7,8].

Docetaxel is extensively bound to albumin and alpha1-acid glycoprotein (AAG), and the latter is the main determinant of variability in docetaxel serum binding [9]. In cancer patients, AAG concentrations vary approximately 5-fold between patients [10], which may contribute to differences in protein binding and systemic drug clearance. From a population pharmacokinetic modeling analyses, AAG has been identified as a significant predictor of total docetaxel clearance, with high AAG levels being associated with reduced docetaxel clearance [11]. Recently, it has been shown that the plasma binding of docetaxel is further influenced by the presence of its formulation

vehicle polysorbate 80, such that the unbound fraction of docetaxel increases during the docetaxel infusion [12]. This time-dependent change in unbound fraction resulted, on average, in a 12% higher unbound fraction on the basis of the AUC ratio of unbound to total drug, as compared with that obtained from pretreatment plasma concentrations.

The clinical significance of increasing unbound fraction of docetaxel during the infusion is unknown. The purpose of this study was to investigate the association between unbound docetaxel exposure and the principal toxicity, neutropenia. In addition, an analysis was undertaken to identify factors influencing unbound docetaxel clearance.

Patients and methods

Patient eligibility

Patients were eligible when they had histologically or cytologically confirmed solid tumors, for which docetaxel was a viable treatment option. Other criteria for patient enrollment were: 1) age ≥ 18 years; 2) performance score (PS) < 3 according to the Eastern Cooperative Oncology Group criteria; 3) adequate bone marrow function as defined by pre-therapy values of hemoglobin ≥ 8.0 g/dL, ANC ≥ 1,500/μL, and platelet count ≥ 100,000/μL; 4) creatinine ≤ 2.0 × the institutional upper limit of normal (ULN); 5) total bilirubin < 1.5 × ULN; 6) if alkaline phosphatase was < 2.5 × ULN, any elevations in AST/ALT; or if AST/ALT were < 1.5 × ULN, any elevation in alkaline phosphatase; patients with ALT and/or AST ≥ 1.5 × ULN with concomitant alkaline phosphate ≥ 2.5 × ULN, considered mild liver impairment, were eligible for treatment and received a reduced dose of docetaxel; 7) peripheral neuropathy ≤ grade 1 and no symptomatic brain metastasis; 8) no previous treatment with docetaxel; and 9) no concomitant use of phenytoin, carbamazepine, barbiturates, rifampin, phenobarbital, St. John's wort, and ketoconazole. All concomitant drugs and the use of herbal medicines were recorded. The clinical protocols were approved by the local institutional review boards (Rotterdam, The Netherlands, Baltimore, MD, and Washington, DC), and all patients provided written informed consent before enrollment. Before treatment, a complete registration form was received by the coordinating center (Baltimore, MD), and a study number was assigned.

Retrospectively, patients were grouped according to baseline liver function to determine the association between elevations in liver transaminases and/or alkaline phosphatase and unbound docetaxel clearance. Patients in liver function group 1 had

no elevations in AST/ALT or alkaline phosphatase as described for liver function groups 2 and 3. Patients in liver function group 2 had concurrent elevations in transaminases and alkaline phosphatase as follows: AST/ALT >1 .0 × ULN concurrent with alkaline phosphatase ≥ 2.5 × ULN, or AST/ALT ≥ 1.5 × ULN concurrent with alkaline phosphatiase > 1.0 × ULN, or isolated elevations of AST/ALT or alkaline phosphatase ≥ 5.0 × ULN. Patients in liver function group 3 had concurrent elevations in transaminases as previously described [3]: AST/ALT ≥ 1.5 × ULN concurrent with alkaline phosphatase ≥ 2.5 × ULN.

Drug treatment

The clinical docetaxel preparation (Taxotere; Aventis Pharmaceuticals, Bridgewater, NJ) containing 20 or 80 mg of the drug formulated in 0.5 mL and 2.0 mL of polysorbate 80, respectively, was diluted with a solution of 13% ethanol in water to a 10 mg docetaxel/mL concentration. This solution was diluted further in a 250-mL infusion bag or bottle of either 0.9% sodium chloride solution or 5% dextrose solution to produce a final concentration of 0.30 – 0.74 mg/mL. Individual drug doses were normalized to body-surface area and administered intravenously over 1 h at a dose of 75 mg/m^2 every 3 weeks. Patients with ALT and/or AST ≥ 1.5 × ULN with concomitant alkaline phosphate ≥ 2.5 × ULN received a reduced docetaxel dose of 50 mg/m^2, which has been shown to be tolerated in patients with similar degrees of liver impairment [7,8]. Dexamethasone, 8 mg orally every 12 hours for 5 doses (3 days), was administered starting 24 h before drug treatment. Patients did not routinely receive anti-emetic prophylaxis. After 1 cycle of therapy, treatment continued at the discretion of the treating physician until tumor progression, development of unacceptable toxicity, or patient withdrawal.

Patient evaluation

The extent of prior cytotoxic treatment was assessed two-fold: 1) the number of prior treatment regimens; and 2) patients were considered heavily pretreated if they received ≥ 2 cycles of mitomycin C, ≥ 4 cycles of carboplatin, or ≥ 6 cycles with cisplatin or an alkylating agent. Pretreatment evaluations included assessment of PS, height, weight, toxicity assessment, a complete blood count with differential (CBC), and the following serum chemistries: creatinine, alkaline phosphatase, AST, ALT, total bilirubin, AAG, and albumin.

Toxicity assessment and a CBC were performed weekly for a total of 3 weeks (1 cycle). Toxicity assessments were performed according the National Cancer Institute

Common Toxicity Criteria version 2.0. Management of toxicity was at the discretion of the treating physician per institutional guidelines.

Pharmacokinetic sampling and assay

Blood samples were collected for docetaxel and polysorbate 80 pharmacokinetic studies during the first cycle of treatment cycle at the following time points: pre-treatment, 30 min during the infusion, 59 min (immediately before the end of the infusion), and post-infusion at 10 and 30 min, 1, 3, 7, 24, and 48 h, and on day 8. Samples were collected in a 10 mL heparinized tube and placed on ice until further processing within 30 minutes of collection. Plasma was isolated by centrifugation at 4 ^{o}C, at 1000 g for 10 minutes and frozen at or below –20 °C until the time of analysis.

Total docetaxel concentrations and polysorbate 80 concentrations were quantitated in plasma over the range of 0.50 nM to 100 nM and 1 to 100 μg/mL, respectively, using a validated liquid chromatographic method with tandem mass-spectrometric detection, as previously described [13]. The bias and precision of quality control (QC) samples were < 15%. At the assay lower limit of quantitation, bias and precision were < 20%.

The fraction unbound docetaxel in plasma (fu) was determined using a validated method based on equilibrium dialysis as previously described [14]. Briefly, equilibrium dialysis was conducted on a rotator at 37°C in a humidified atmosphere of 5% CO_2 using 96-wells microdialysis plates (Harvard Apparatus, Holliston, Mass). The dialysis compartments in each well were separated by a regenerated cellulose membrane with a 5-kDa molecular weight cut-off (Harvard Apparatus). Experiments were carried out with 250-μl aliquots of plasma containing a tracer amount of [*G*-^{3}H]docetaxel (Moravek Biochemicals, Brea, Calif) against an equal volume of 0.01 M phosphate buffer (pH 7.4). Following a 4-hour reaction time, measurement of total radioactivity in both compartments was measured by liquid scintillation counting for 1 minute. The tritium-label is relatively stable and the volume shift during dialysis is negligible, hence the results were used directly without applying a correction factor. The concentration of unbound docetaxel (Cu) was calculated from the concentration of total docetaxel in plasma (Cp) as: $Cu = Cp \times fu$. The within-run and between-run variability (reproducibility) and bias (accuracy) of the method were less than 15% on the basis of repeat analyses of quadruplicate samples with differing docetaxel fu values (depending on the spiked polysorbate 80 concentration) on 6 consecutive days. The mean relative standard deviation was less than 10%, assuring high discriminatory

power in the detection of changes in the fraction unbound docetaxel in patient samples with different AAG and polysorbate 80 concentrations.

With the pharmacokinetic sampling scheme used, total docetaxel concentrations are best described by a 3-compartment model [15]. Because docetaxel unbound fraction increased during the infusion, which contributed to increasing docetaxel concentrations unrelated to the dosing rate over a 1 hour infusion, standard noncompartmental methods were used for calculation of pharmacokinetic parameters, including those for total docetaxel and polysorbate 80. To determine the contribution of increasing unbound fraction to overall unbound docetaxel exposure, unbound docetaxel concentrations were calculated for estimation of pharmacokinetic parameters twice as follows: 1) unbound fraction measured at each sampling time point was multiplied by the corresponding total concentration ($Cu = Cp \times fu$), or 2) unbound fraction measured at pre-treatment was calculated by the total concentration at each time point after the start of the docetaxel infusion ($Cu = Cp \times fu_{pre\text{-}treatment}$). Values for Cmax and $Cmax_{pre\text{-}treatment\ fu}$ and AUC and $AUC_{pre\text{-}treatment\ fu}$ values were then compared. Noncompartmental analysis was performed using the software program Winnonlin version 3.0 (Pharsight Inc., Mountain View, Calif).

Statistical analysis

Docetaxel and polysorbate 80 pharmacokinetic parameters were summarized as the mean, standard deviation, and range. For continuous variables, nonparametric tests were used to compare mean values between different groups. When 3 or more groups were compaired, a trend test was used [16]. Categorical variables were compared using 2-tailed Fisher's Exact Test for 2-by-2 tables. Multiple linear regression models were used to assess the influence of polysorbate 80 exposure (C_{max}, AUC), AAG, age and liver function group (2 or 3) (predictor variables) on unbound docetaxel clearance (outcome variable). Predictor variables were evaluated for colinearity; if several variables were correlated, only one was maintained in the model. Regression coefficients, standard errors of the coefficients, and the associated *P*-values were determined from the multiple linear regression modeling. Stepwise backward elimination was performed to systematically exclude the least significant factors until the *P*-value was $< .05$. Multiple linear regression modeling was performed using the software program Stata version 8.2 (Stata Corp., College Station, TX). The a priori level of significance was set at $P < .05$.

Table 1. Patient demographics

	Liver function group		
	Group 1 (n = 40) Median (Range)	Group 2 (n = 9) Median (Range)	Group 3 (n = 6) Median (Range)
Docetaxel dose (mg/m^2)	75	75	50
Age (years)	62 (27 – 80)	60 (26 – 70)	53 (36 – 64)
Body surface area (m^2)	1.88 (1.45 – 2.50)	1.80 (1.50 – 2.12)	1.73 (1.63 – 2.08)
Sex[a]			
Female	19	6	4
Male	21	3	2
AAG (mg/dL)	129 (60 – 257)	176 (81 – 254)	159 (116 – 271)
Liver function tests			
AST (× ULN)	0.75 (0.30 – 3.2)	2.6 (0.80 – 4.7)	2.9 (1.7 – 10.6)
ALT (× ULN)	0.50 (0.10 – 2.2)	1.0 (0.40 – 6.6)	2.1 (0.6 – 3.1)
Alkphos (× ULN)	0.80 (0.40 – 2.0)	1.4 (1.1 – 6.2)	3.5 (2.5 – 15.4)
Total bilirubin (× ULN)	0.40 (0.20 –1.1)	0.40 (0.20 – 0.70)	0.80 (0.40 – 1.0)
ECOG performance status [a,b]			
0	7	1	1
1	28	5	5
2	4	3	
Primary tumor type[a]			
Breast	8	5	
Head and neck	4		4
Lung	7	2	2
Melanoma	5		
Prostate	3	2	
Angiosarcoma	3		
Unknown	5		
Other	5		
Prior treatment[a]			
None	8	1	2
1-2 cytotoxic regimens	30	4	2
≥ 3 cytotoxic regimens	2	4	2
Light	26	4	3
Heavy	14	5	3

[a]Values are number of patients; [b]Baseline performance status was not performed in one patient in liver function group 1.

Results

Pharmacokinetic studies were performed in 55 cancer patients receiving docetaxel therapy. Forty-eight patients received docetaxel 75 mg/m^2 and 7 patients received 50 mg/m^2. One patient receiving 50 mg/m^2 erroneously received a lower docetaxel dose,

where the intended dose was 75 mg/m^2. Patient characteristics as a function of liver function group are summarized in Table 1.

Docetaxel pharmacokinetics

Concentration-time curves for unbound and total docetaxel from a representative patient are shown in Figures 1A and 1B; this patient, representing liver function group 1, had average values for unbound and total docetaxel pharmacokinetic parameters and change in fraction unbound during the docetaxel infusion. Unbound docetaxel pharmacokinetic parameters are summarized in Table 2. In 40 patients receiving docetaxel 75 mg/m^2 with normal liver function tests (liver function group 1), unbound docetaxel disposition was characterized by mean (± SD) Cmax, AUC, and clearance values of 233 ± 101 ng/mL, 321 ± 143 ng/mL×h, and 565 ± 329 L/h, respectively, and unbound clearance varied 8.5-fold. Patients in liver function group 2 did not have reduced unbound clearance as compared to patients with normal liver function parameters (Table 2; Figure 3A); unbound clearance varied 13.0-fold, which was more variable than those in liver function group 2, most likely due to the inclusion of 1 patient with an outlier value for unbound clearance (2770 L/h). A trend for reduced unbound docetaxel clearance in patients with moderate elevations in liver function tests (liver function group 3) was observed (P = .020)

In 40 patients with normal liver function parameters, the mean (± SD) pre-treatment fu expressed as a percentage was 4.72 ± 1.79% and protein binding as determined by the ratio of $AUC_{unbound}/AUC_{total}$ expressed as a percentage was 5.66 ± 1.40% (Table 2); overall, protein binding varied 2.5-fold and was independent of liver function. Although statistically associated, a strong linear correlation was not observed between pre-treatment fu and $AUC_{unbound}/AUC_{total}$ (R^2=0.4593, P=.459), demonstrating that accurate assessment of unbound docetaxel exposure can not be determined from a pre-treatment fu only. The discordance between pre-treatment fu and $AUC_{unbound}/AUC_{total}$ is most likely due to increasing unbound fraction after the start of the infusion. Compared to pretreatment values, the unbound fraction was significantly increased at 30 min during the infusion and at the end of the infusion (P = .0012), with a decline in fu after the end of the infusion and return to values close to baseline fu at 3 to 7 hours post-infusion (data not shown). The maximum increase in fraction unbound docetaxel occurred at the end of the infusion, where the mean (± SD) fu value was 5.68 ± 1.56% (range, 2.78 to 9.56%) (N = 55); this represents an average increase in the fu of 24% compared to the pretreatment fu (mean ± SD, 4.60 ± 1.63%; range, 1.19 to 8.63%; N = 55).

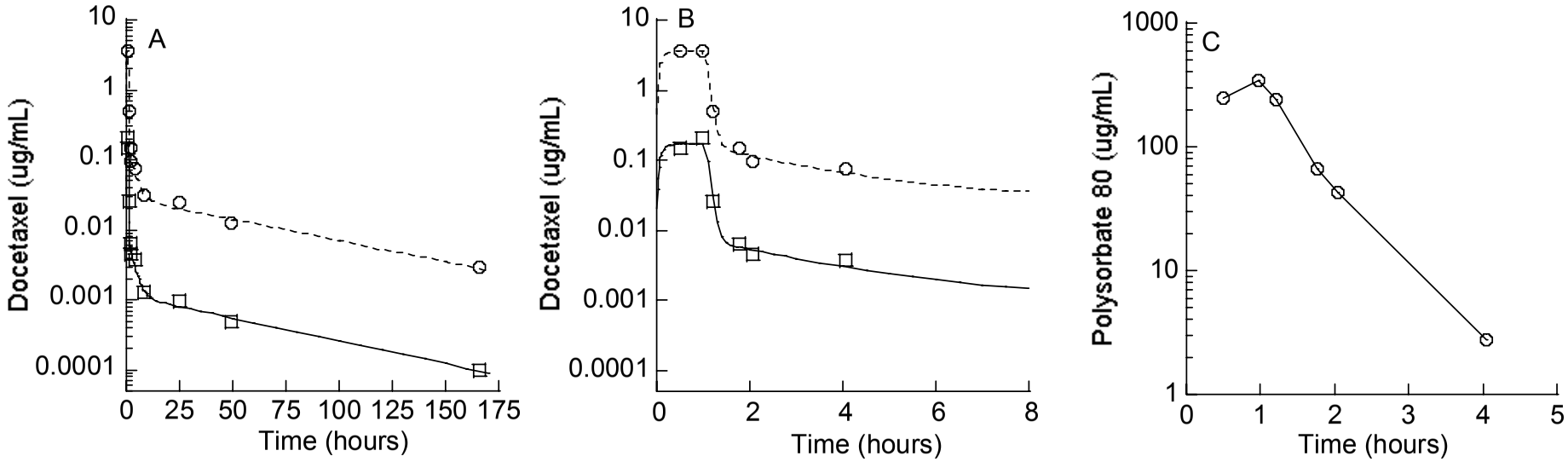

Figure 1. (A): representative concentration-time profiles for unbound and total docetaxel, where the squares and circles represent the observed values for unbound and total docetaxel, respectivelthe linesy; the lines represent simulated concentrations from the fit of a 3 compartment model to the data. (B): illustrates the same data in panel A, but shows concentrations from time zero to 8 hours. (C): representative concentration-time profile for polysorbate 80 from the same patient shown in panels A and B.

Table 2. Pharmacokinetic parameters

Parameter	Liver Function Group		
	Group 1 75 mg/m^2* n = 40	Group 2 75 mg/m^2 n = 9	Group 3 50 mg/m^2 nN = 6
Unbound docetaxel			
Cmax (ng/mL)	233 ± 101 (57.4 – 489)	213 ± 92.4 (43.8 – 329)	190 ± 40.3 (137 – 228)
AUC (ng/mL*h)	321 ± 143 (96.4 – 584)	296 ± 176 (50.9 – 654)	361 ± 132 (217 – 533)
Clearance (L/h)	565 ± 329 (207 – 1763)	715 ± 790 (199 – 2770)	269 ± 94.9 (161 – 414)
Pre-treatment fu (%)	4.72 ± 1.79 (1.19 – 8.63)	4.42 ± 1.32 (2.71 – 6.58)	4.00 ± 0.56 (3.34 – 4.75)
$AUC_{unbound}/AUC_{total}$ (%)	5.66 ± 1.40 (3.41 – 8.59)	4.92 ± 0.98 (3.24 – 6.33)	4.93 ± 0.84 (3.68 – 5.80)
$Cmax_{pre\text{-}treatment\ fu}$ (ng/mL)	181 ± 92.1 (42.4 – 390)	164.8 ± 71.3 (40.8 – 247)	151 ± 55.3 (83.5 – 241)
$AUC_{pre\text{-}treatment\ fu}$ (ng/mL*h)	268 ± 147 (59.1 – 667)	233 ± 140 (42.5 – 496)	323 ± 141 (178 – 565)
Total docetaxel			
Cmax (µg/mL)	3.89 ± 1.49 (0.88 – 6.44)	4.14 ± 1.60 (1.51 ± 6.52)	3.56 ± 1.27 (1.82 – 5.35)
AUC (µg/mL×h)	5.75 ± 2.51 (2.30 – 12.1)	5.95 ± 3.55 (1.57 – 13.4)	7.54 ± 3.06 (3.75 – 12.6)
Clearance (L/h)	30.0 ± 14.2 (12.4 – 74.0)	30.7 ± 23.8 (9.69 – 89.8)	13.4 ± 6.02 (7.17 – 24.0)
Polysorbate 80			
Cmax (µg/mL)	451 ± 221 (210 – 1199)	379 ± 163 (28.9 – 593)	344 ± 88.5 (244 – 458)
AUC (µg/mL×h)	528 ± 217 (244 – 1212)	466 ± 190 (56.2 – 725)	484 ± 173 (208 – 708)
Clearance (L/h)	8.18 ± 3.66 (3.00 – 20.2)	13.1 ± 19.6 (4.30 – 65.2)	5.62 ± 2.50 (3.11 – 9.95)

Data are presented as mean ± SD (range)

* One patient erroneously received 50 mg/m^2 and Cmax and AUC values for this patient were not included in the summary statistics.

To determine the contribution of time-dependent changes in fu on the exposure to unbound docetaxel, Cmax and AUC values were calculated using the pre-treatment fu and the observed fu at each time point (Table 2). On average, unbound Cmax and AUC values were 28% and 19% higher, respectively, when the observed fu was used *versus* the pre-treatment unbound fraction.

Total docetaxel pharmacokinetic parameters (Table 2) are similar to those reported previously following administration of docetaxel 75 mg/m^2 as a 1-hour infusion [15].

*Table 3. Multiple regression model for docetaxel unbound clearance**

	Coefficient	Standard error	P value
Intercept	5.94	.0998	< .0001
1/polysorbate 80 AUC	112	29.3	<.0001
Liver function (group 3)	-.663	.209	.003
Overall model	R^2 = .3224, P < .0001		

*Unbound clearance is lower with a negative coefficient value.

Polysorbate 80 pharmacokinetics

A concentration-time curve for polysorbate 80 from a representative patient is shown in Fig 1C, and polysorbate 80 pharmacokinetic parameters are summarized in Table 2. As described previously [13,17], polysorbate 80 concentrations were undetectable after 4 hours (3 hours post infusion) in all but a few patients. In patients receiving docetaxel 75 mg/m^2 in liver function group 1, polysorbate 80 exhibited mean (± SD) Cmax, AUC, and clearance values of 451 ± 221 ug/mL, 528 ± 217 ug/mL×h, and 8.18 ± 3.66 L/h, respectively; in this population, polysorbate 80 clearance varied 6.7-fold. Similar to unbound docetaxel, polysorbate 80 clearance was not reduced in patients in liver function group 2, but a trend for reduced clearance was observed in patients with moderate elevations in liver function tests (liver function group 3), although this did not reach a level of statistical significance (P = .092).

Predictors of unbound docetaxel clearance

AAG concentration was significantly correlated with pre-treatment unbound fraction (R^2=0.3863; P<.0001) (Fig 2A). However, no association was noted between AAG concentration and unbound docetaxel clearance (Fig 2B).

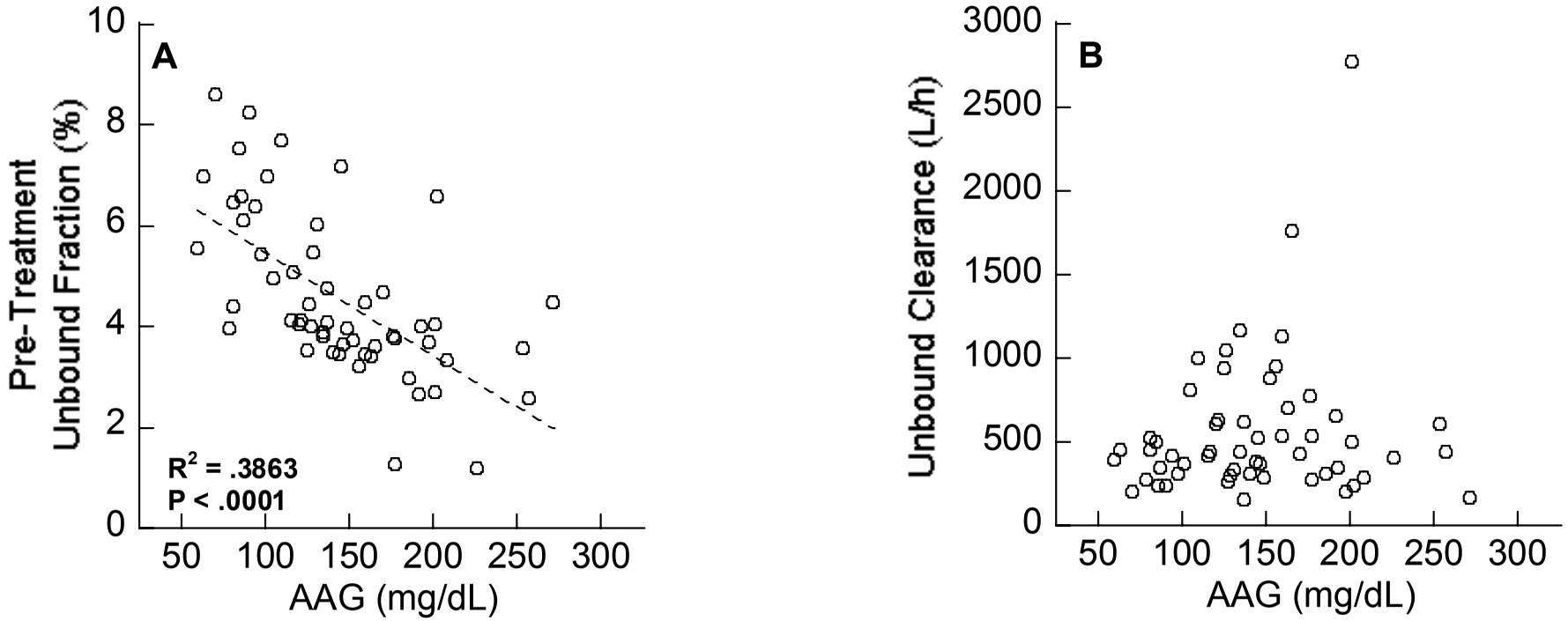

Figure 2. Relationship between pre-treatment AAG concentration and pre-treatment unbound fraction (A) and unbound docetaxel clearance (B). The dashed line is from linear regression analysis.

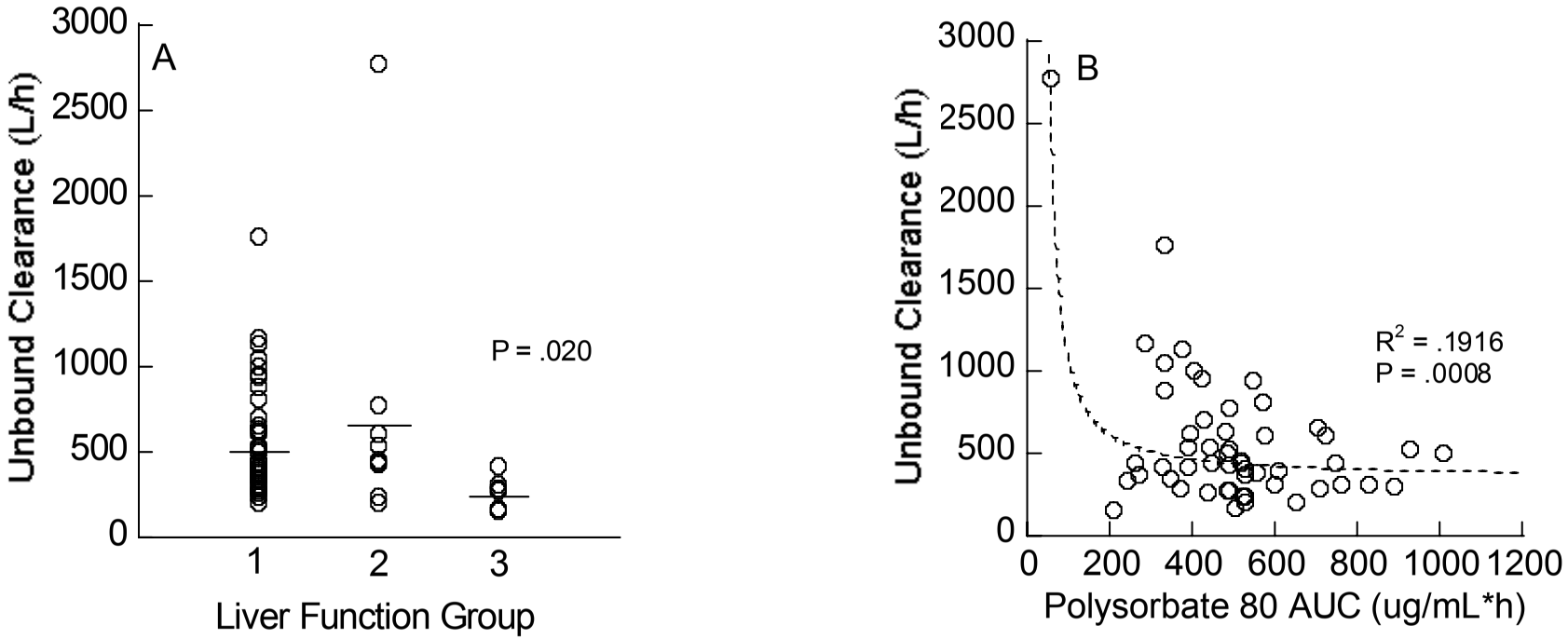

Figure 3. (A): unbound docetaxel clearance as a function of liver function group; solid lines are mean values and the P value is from a trend analysis. (B): association between polysorbate 80 AUC and unbound docetaxel clearance; the solid line is from linear regression analysis of reciprocal polysorbate 80 AUC and log-transformed unbound clearance.

This is presumed to be due to the effect of polysorbate 80 exposure on unbound docetaxel clearance [12]. Considering what is known regarding predictors of total docetaxel clearance, specifically AAG concentration, age and liver function, a multiple linear regression analysis was performed to determine if these variables in addition to polysorbate 80 exposure were associated with unbound docetaxel clearance. Although polysorbate Cmax and AUC were associated with unbound docetaxel clearance, polysorbate 80 AUC was a better predictor. And since Cmax and AUC were highly correlated, only polysorbate AUC was included in the multivariate model. However, the reciprocal of polysorbate 80 AUC gave a better prediction than un-transformed AUC, and thus 1/polysorbate AUC was included in the model. Furthermore, because unbound clearance had a skewed distribution, unbound clearance was log-transformed. After stepwise backward deletion, only 1/polysorbate AUC and liver function group 3 were retained in the final model (R^2 = .3402; P < .00001) (Table III), which explained 32% of the total variability. Fig 3B illustrates the relationship between polysorbate 80 AUC and unbound docetaxel clearance, where higher AUC values are associated with lower unbound clearance values; polysorbate 80 alone accounted for 19% of the variability in unbound docetaxel clearance.

Relation between unbound docetaxel exposure and neutropenia

Fifty-two of 55 patients were evaluable for hematological toxicity. The incidence of grade 3 and 4 neutropenia and febrile neutropenia, and percentage decrements in ANC are summarized in Table 4. Overall, 26 of 52 patients experienced grade 4 neutropenia and four of 26 patients with grade 4 neutropenia experienced febrile neutropenia. The development of grade 0 – 3 *versus* grade 4 neutropenia was not related to extent of prior cytoxic therapy, which was categorized as lightly- or heavily-pretreated (P = .776).

Table 4. Hematological toxicity

Liver function group	No. of patients	Neutropenia[a]			% Decrease ANC[b]
		Grade 3	Grade 4	Febrile	
1	40	6	23	4	90 (46 – 100)
2	7	3	1	0	87 (42 – 96)
3	5	0	2	0	81 (0 – 94)

[a]Data are presented as number of patients (% of patients); [b]Data are presented as median (range)

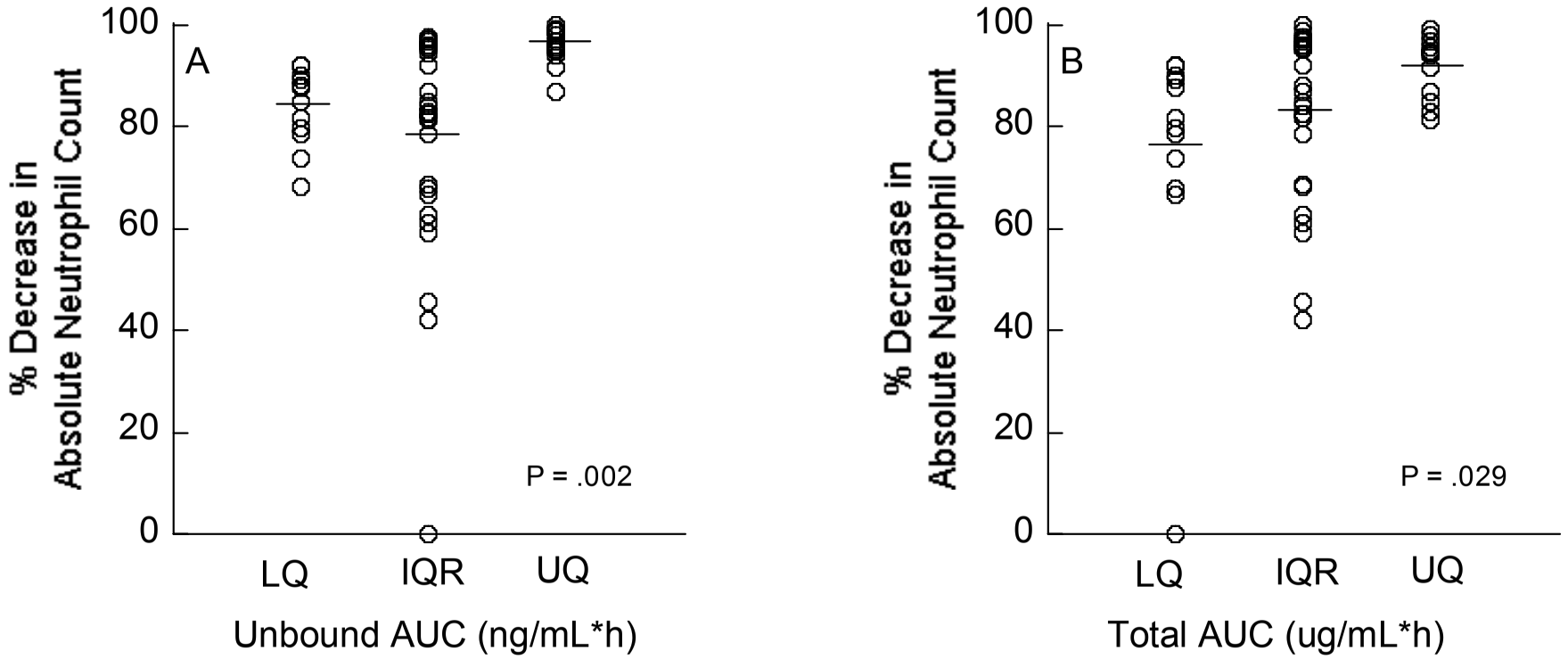

Figure 4. Percentage decrements in absolute neutrophil cound (ANC) as function of unbound (A) and total (B) AUC quartile: LQ, lower quartile, IQR, interquartile range; UQ, upper quartile. Solid lines are mean values and P values are from a trend test.

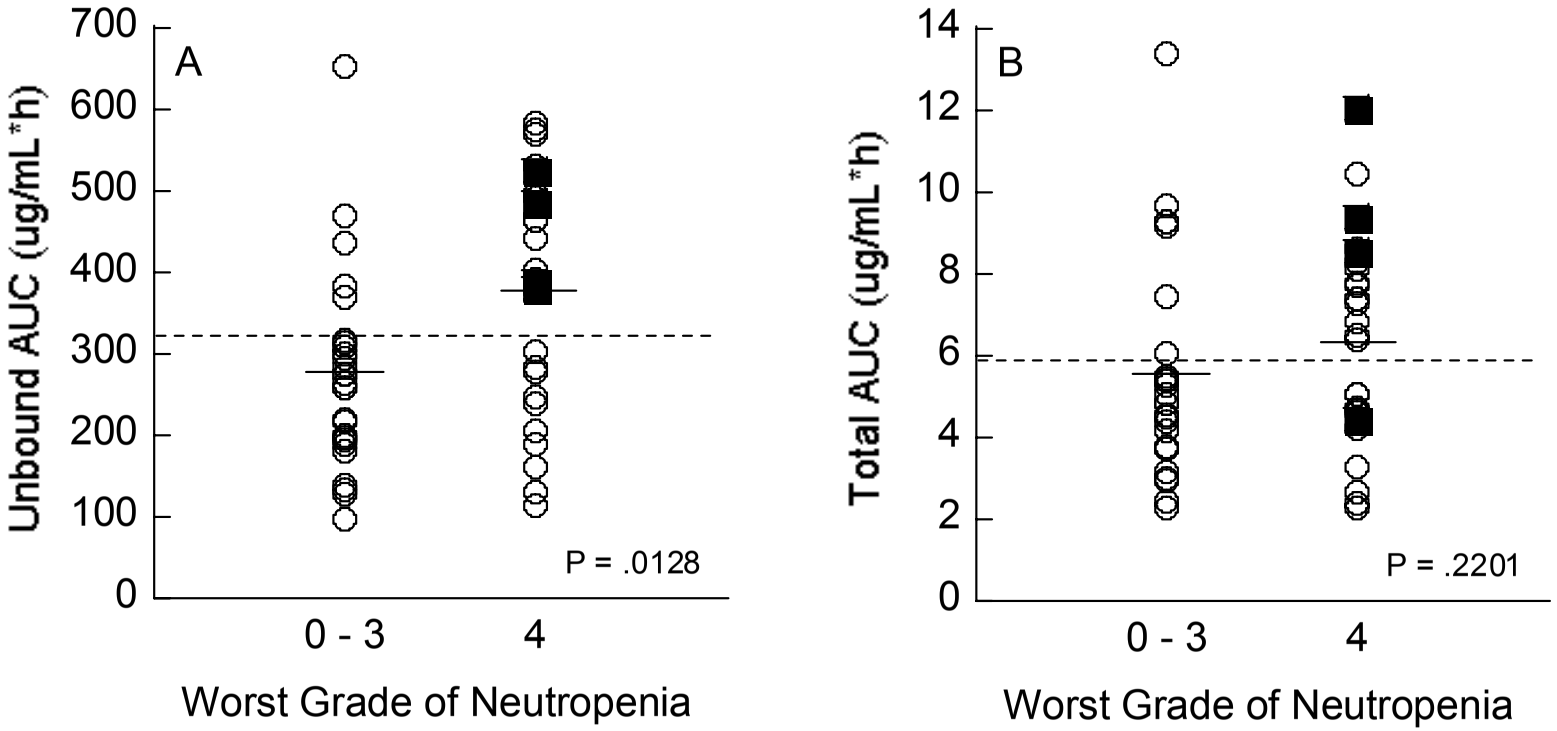

Figure 5. Grade of neutropenia as a function of unbound (A) and total (B) AUC. The solid symbols represent patients with febrile neutropenia. Solid lines are mean values and the dashed lines are the overall mean value.

The association between unbound docetaxel AUC and neutropenia was assessed, as was the relation between total docetaxel AUC and neutropenia for the purpose of comparison. Patients in liver function group 3 received a reduced dose of docetaxel to achieve AUC values similar to those in liver function groups 1 and 2 [3,7]. Unbound and total AUC values were similar in the former and latter groups (P = .395); therefore, AUC values at the different dose levels were combined to assess the relation between docetaxel exposure and neutropenia. Unbound docetaxel AUC was better correlated with both percentage decrements in absolute neutrophil count (ANC) (Fig 4; P = .002 *versus* P = .029) and worst grade of neutropenia (Fig 5; P = .013 *versus* P = .220). The percentage decrements in ANC was greater in those patients with unbound AUC values in the upper quartile (mean decrement, 95.4%) compared to those with AUC values in the interquartile range (mean decrement, 76.9%) (P = .020) (Fig 4A). Likewise, patients who developed grade 4 neutropenia had significantly higher unbound AUC values (mean, 374 ng/mL×h) than patients with grade 0 to 3 neutropenia (mean, 275 ng/mL×h) (P = .013) (Fig 5A). In addition, all patients who developed neutropenic fever had unbound AUC values above the overall mean value, whereas only 3 of 4 patients had total AUC values above the overall mean value.

Discussion

Previous studies examining relationships between the pharmacokinetics of the anticancer drug docetaxel and treatment outcome have consistently focused on measurement of total docetaxel concentrations in plasma [3,18], disregarding effects of variable levels of systemic binding on the fraction unbound drug. The present study demonstrates that the hematologic toxicity induced by docetaxel is significantly better correlated with the systemic exposure to unbound drug than total drug.

Variability in systemic drug binding has frequently been demonstrated in humans [19]. However, the clinical significance of this variability to drug disposition and pharmacodynamics depends largely upon intrinsic pharmacokinetic characteristics of the drug. For most anticancer drugs, the interindividual variation in plasma protein binding is quite small in patients with normal liver function and drug-metabolizing capability. Therefore, vascular binding is usually not an important consideration in therapeutic drug monitoring and in the evaluation of pharmacokinetic-pharmacodynamic relationships. However, in rare instances, the total concentration is not reflective of the unbound drug level [20]. For some anticancer agents, this situation arises if the drug demonstrates protein concentration-dependent binding (e.g., imatinib

[21] and 7-hydroxystaurosporine (UCN-01) [22]), or when irreversible or near-covalent binding occurs after therapeutic doses of an anticancer drug (e.g., platinum-containing agents). Indeed, cisplatin, carboplatin, and oxaliplatin are currently the only agents for which unbound concentrations are routinely measured and for which the relation between unbound drug and therapeutic effects has been demonstrated [23].

The significant relationship between exposure to total docetaxel and the percentage decrease in neutrophil count at nadir, as described previously in a study involving more than 600 patients receiving docetaxel in similar regimens [3], was not observed in the current investigation. It is likely that this apparent inconsistency is a direct consequence of the differences in sample size, which might become an important consideration when the pharmacodynamic evaluation is based on a sparse set of hematological toxicity data (i.e., with blood cells measured on a once a week basis). Experimental evidence has also suggested that information on the entire time course of changes in blood cell counts might be more physiologically-relevant endpoint than the nadir count [24]. In order to further resolve this issue with respect to the current data set, a population analysis for docetaxel-mediated neutropenia is currently being planned by taking into account the entire time course of neutrophils.

The current data on docetaxel describe a previously unrecognized type of time-dependence for the fraction unbound drug, which was recently shown *in vitro* to be associated with its formulation vehicle, polysorbate 80 [12]. In the present study, although the fraction unbound docetaxel in pretreatment samples was correlated with individual levels of AAG, only exposure to polysorbate 80 (and not AAG) was significantly associated with the clearance of unbound docetaxel in both univariate and multivariate analyses, thus demonstrating *in vivo* the influence of polysorbate 80 exposure on fraction unbound docetaxel. In this regard, it is of particular interest to note that for etoposide (VePesid; Bristol-Myers Squibb Co., Princeton, NJ), the only other registered anticancer drug formulated in polysorbate 80, the systemic exposure to unbound drug is also more precisely correlated with measures of hematologic toxicity than total drug levels [25]. Since the rate of administration and total dose of polysorbate 80 are similar between docetaxel and etoposide, this raises the possibility of a common mechanism being involved in pharmacokinetic-pharmacodynamic relationships for these drugs.

The mechanistic basis for the influence of polysorbate 80 on docetaxel fraction unbound during the infusion is as yet unknown. It is possible that polysorbate 80 or its metabolites (e.g., oleic acid) interfere with the binding of docetaxel to albumin and AAG(9) and lead to temporary increase in the fraction unbound drug. However, other

possible mechanisms, including inhibition of CYP3A [26,27] and/or P-glycoprotein [28] mediated elimination of docetaxel by polysorbate 80 early after drug administration, can not be excluded. These and several other possible mechanisms are currently under further investigation.

Collectively, this study demonstrates that the exposure to unbound docetaxel is more closely related to drug-induced hematologic toxicity than total drug. Since an increase in systemic exposure to unbound drug was associated with more severe neutropenia, these findings suggest that determination of unbound docetaxel concentrations is indicated for future pharmacologic investigations.

Acknowledgments

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Chapter 12

Summary, Conclusions and Future Perspectives

From the early nineties onward, the anticancer drug docetaxel has obtained a prominent position in the treatment of various human malignancies, including advanced breast cancer and non-small cell lung cancer. Many publications have since discussed the drug's clinical pharmacological properties, although many aspects still remain to be elucidated, in particular the factors contributing to the extensive degree of interindividual variability in the pharmacokinetic profile of docetaxel that is observed with commonly applied dosing regimens.

Chapter 2 provides a literature overview of recent developments into the understanding of factors involved in the pharmacokinetics of docetaxel.

In **chapter 3** an evaluation of the usefullness of body-surface area (BSA) is presented as an independent variable to explain interpatient variability in the exposure to docetaxel as well as 32 investigational agents, which were tested in phase I trials between 1991 and 2001, in 1650 adult cancer patients. It was noted that BSA-based dosing was statistically significantly associated with a reduction in interpatient variability in drug clearance for only five of the agents evaluated. This suggests that BSA should not be considered as the prime factor to be used in future studies, and evaluation of alternate dosing strategies for docetaxel are needed.

In **chapter 4** the influence of age, body size, concomitant drugs, dose, infusion duration, and sex on the clearance of docetaxel is explored in a retrospective analysis involving 152 individual patients. The upper extremes of body size were found to be associated with increased docetaxel clearance, whereas no consistent effect of age was discerned. Concomitant treatment of docetaxel with doxorubicin and capecitabine was associated with approximately 20% decreased and increased docetaxel clearance, respectively, in a multiple linear regression analysis. These findings further suggest that variables other than BSA should be incorporated in routine dosing strategies for docetaxel.

Variable response to cancer chemotherapy with docetaxel is presumed to be related, in part, to interindividual variation in expression of the enzyme cytochrome P-450 3A (CYP3A). In **chapter 5**, a study is described aimed at identifying the demographic, physiologic, and inheritable factors that influence CYP3A activity in 134 patients with cancer that underwent the erythromycin breath test as a CYP3A phenotyping probe. CYP3A activity varied up to 14-fold in this population, and was

not significantly influenced by age, sex, body size measures, and genetic polymorphisms in the CYP3A4 and CYP3A5 genes. However, in a multivariate analysis, liver function combined with the concentration of the acute-phase reactant, alpha-1 acid glycoprotein, explained approximately 18% of overall variation in CYP3A activity. This indicates that additional factors, including the inflammation marker C-reactive protein as well as concomitant use of other drugs, food constituents, and complementary and alternative medicine with inhibitory and inducible effects on CYP3A, are needed to reduce variation in CYP3A and the variable response to chemotherapeutic treatment with docetaxel.

In **chapter 6** the extent of docetaxel binding to plasma in the presence and absence of its excipient polysorbate 80 (Tween 80) was studied in relation to the pharmacokinetics of unbound docetaxel. Polysorbate 80, added at clinically relevant concentrations (up to 1.0 μL/mL), was found to increase the fraction unbound docetaxel (fu) by 12% (6.00 ± 1.03% versus 5.49 ± 1.01%, P = 0.038). Furthermore, the serum protein α_1-acid glycoprotein was significantly related to fu (P < 0.0018), with higher fu in the presence of lower protein levels, indicating that the plasma binding of docetaxel is influenced by both alpha$_1$-acid glycoprotein and its formulation vehicle. This study was made possible by the availability of validated analytical methods for the analysis of the fraction unbound docetaxel as determined using equilibrium dialysis (**chapter 8**).

Weekly administration of docetaxel has demonstrated comparable efficacy, together with a distinct toxicity profile with reduced myelosuppression, although pharmacokinetic data with weekly regimens are lacking. In **chapter 9**, the comparative pharmacokinetics of docetaxel during weekly and once every 3 weeks (3-weekly) administration schedules was evaluated in 46 cancer patients. The mean (± SD) docetaxel clearance values were similar with weekly and 3-weekly schedules (25.2 ± 7.7 versus 23.7 ± 7.9 L/h/m^2); values for half-life were also similar with both schedules of administration (16.5 ± 11.2 and 17.6 ± 7.4 hours). With the use of prolonged plasma sampling schemes, the calculated terminal disposition half life of docetaxel was found to be approximately 86 hours, which is about 5-fold longer than that estimated on the basis of conventional 24-hour sampling intervals, and almost 9-fold longer than published values. Consequently, docetaxel concentrations are maintained above 1.0 nM for 7 days with weekly schedules and above 0.5 nM for as long as 21 days with 3-weekly regimens. Measurement of low docetaxel

concentrations with extended sampling schemes was made possible by the availability of validated analytical methods for the determination of total docetaxel concentrations in human plasma based on liquid chromatography coupled with tandem mass spectrometric detection (**chapter 7**). This previously unrecognized prolonged circulation of low nanomolar concentrations of docetaxel may contribute to the mechanism of action of docetaxel through suppression of microtubule dynamics and tumor angiogenesis and enhanced cell radiosensitivity in combined modality therapy.

Despite the increasing numbers of elderly patients presenting with cancer, only few pharmacological studies have been conducted in this subgroup of patients. The pharmacokinetics of anticancer drugs may be altered with aging due to several factors, including differences in end organ function and body composition. In **chapter 10,** the pharmacokinetics of docetaxel in elderly patients (≥ 65 years) and patients < 65 years, revealed an unchanged mean (± SD) docetaxel clearance of 30.1 ± 18.3 vs 30.0 ± 14.8 L/h, respectively. In support of this lack of age-dependence, it was shown that phenotypic activity of CYP3A, as assessed using the erythromycin breath test, was not changing with advancing age. Although there was no significant difference observed in treatment-related side effects between the two age groups, the incidence of neutropenic fever seemed to be slightly increased in the elderly, but was attributed to drug exposure differences unrelated to age.

A multiple linear regression analysis, described in **chapter 11**, revealed that only exposure to polysorbate 80 and degree of liver impairment are significantly associated with the clearance of unbound docetaxel in a group of 55 patients treated with docetaxel. Furthermore, the unbound docetaxel AUC was better correlated than total AUC with both the percentage decrements in absolute neutrophil count (P=.002 *versus* P=.029) and worst grade of neutropenia (P=.013 *versus* P = .220), where higher exposure was associated with worse hematological toxicity. This suggests that exposure to unbound docetaxel is more closely related to drug-induced hematologic toxicity than total drug and should be considered for future pharmacologic investigations.

Future perspectives

The work presented in this thesis aimed at identifying factors involved in pharmacokinetic variability observed with docetaxel, and included an evaluation of common patient demographic characteristics, liver dysfunction, use of concomitant chemotherapy, protein binding, and interference by the vehicle polysorbate 80. However, a substantial degree of interpatient variation in the pharmacokinetics of docetaxel is still unaccounted for, and likely contributes to unpredictable treatment outcome (ie, toxicity and efficacy). The residual pharmacokinetic variability that cannot be explained by the factors evaluated in this thesis likely involve individual variation in hepatic metabolism by members of the cytochrome P-450 family and expression and function of transporters involved in docetaxel elimination such as P-glycoprotein.

Currently ongoing studies will focus on the role of inherited factors regulating the expression and function of these proteins, and will hopefully lead to the development of a more predictable pharmacokinetic behavior for docetaxel in individual patients through the use of dosing formulae on the basis on population pharmacokinetic-pharmacodynamic models. However, it is possible that in addition to genetic components, other environmental and physiological factors not studied here may influence the clinical pharmacology of this drug. Hence, it will be imperative to design additional prospective studies in the future employing both genotyping and phenotyping approaches to predict docetaxel elimination in order to eventually individualize and improve chemotherapeutic therapy.

Finally, the drawback presented by the presence of polysorbate 80 as an integral component of the pharmaceutical formulation of docetaxel has already instigated extensive research to develop alternative delivery systems. These alternative formulations might eventually enable a safer administration with less likelihood of interactions between the formulation vehicle and the active drug, and a reduced incidence and severity of vehicle-mediated side-effects. Such alternative formulations also should enable the drug to be administered without premedication and lead to a more predictable and sustained exposure of the tumor to the drugs, and to a more favorable treatment outcome.

Chapter 13

Samenvatting, Conclusies en Perspectieven

Sinds het begin van de jaren 1990 heeft het anti-kankermiddel docetaxel een belangrijke plaats verworven in de behandeling van diverse vormen van kanker, in het bijzonder van het mammacarcionoom en het niet-kleincellig bronchuscarcinoom. In tal van publicaties zijn de klinisch farmacologische eigenschappen van dit middel besproken; echter, vele aspecten zijn nog steeds onopgehelderd, met name de factoren die bijdragen aan de mate van variabiliteit tussen patiënten onderling in het farmacokinetisch gedrag van docetaxel dat wordt waargenomen bij de huidig toegepaste doseerstrategieën.

Hoofdstuk 2 geeft een literatuuroverzicht van recente ontwikkelingen in de diverse factoren die van invloed zijn op de farmacokinetiek van docetaxel.

In **hoofdstuk 3** wordt een studie beschreven waarin de mate waarin lichaamsoppervlak (BSA) bijdraagt aan het verklaren van de interpatiënt variabiliteit is onderzocht in de blootstelling aan docetaxel en 32 experimentele medicamenten die het onderwerp vormden van fase I onderzoek in de jaren 1991-2001, in een groep van 1650 volwassen kankerpatiënten. Er werd gevonden dat doseerstrategieën gebaseerd op BSA leiden tot een statistisch significante vermindering in de interpatiënt variabiliteit in de klaring van slechts 5 van de onderzochte medicamenten. Dit suggereert dat BSA niet gebruikt zou moeten worden als de primaire factor in toekomstig onderzoek, en dat vervolgonderzoek naar alternatieve doseerstrategieën voor docetaxel noodzakelijk is.

In **hoofdstuk 4** is de invloed van leeftijd, BSA, gelijktijdig toegediende medicatie, dosis, infusieduur, en geslacht op de klaring van docetaxel retrospectief onderzocht in een groep van 152 patiënten. De klaring van docetaxel bleek verhoogd te zijn in patiënten met extreme waarden voor BSA; echter, leeftijd had geen eenduidig effect op de klaring. Uit een multipele lineaire regressieanalyse bleek dat het gelijktijdig toedienen van docetaxel met doxorubicine of capecitabine was geassocieerd met respectievelijk een afname of toename van ongeveer 20% in de klaring van docetaxel. Deze bevindingen suggereren dat naast BSA andere variabelen geïncorporeerd zouden moeten worden in routinematige doseerstrategieën van docetaxel.

Eerder is verondersteld dat de variatie in mate waarin tumoren gunstig reageren op chemotherapeutische behandeling met docetaxel ten dele het gevolg is van interpatiënt variabiliteit in de expressie van het enzyme cytochroom P450 3A (CYP3A).

Hoofdstuk 5 beschrijft een studie waarin is gepoogd om demografische, fysiologische, en genetische factoren te identificeren die de activiteit van CYP3A beïnvloeden. Dit is onderzocht in 134 kankerpatiënten die voorafgaand aan behandeling een test ondergingen om de fenotypische activiteit van CYP3A vast te stellen, de zogenaamde erythromycine-ademtest. Er was een 14-voudige variatie in de activiteit van CYP3A in deze populatie, en de variatie was niet gerelateerd aan leeftijd, geslacht, lichaamsoppervlaktematen, en polymorfismen in de CYP3A4 en CYP3A5 genen. Met behulp van een multivariate-analyse werd echter gevonden dat leverfunctie in combinatie met de concentratie van het acute-fase eiwit, α_1-zure glycoproteïne, ongeveer 18% van de totale variatie in CYP3A activiteit kon verklaren. Dit suggereert dat additionele factoren noodzakelijk zijn om de variatie in CYP3A activiteit en de variatie in behandelingsuitkomst na behandeling met docetaxel verder te reduceren. Tot deze categorie van factoren behoren mogelijk de ontstekingsmarker C-reactive proteïne alsmede effecten van gelijktijdig toegediende andere medicamenten, voedselbestanddelen, en/of gebruik van alternatieve geneeswijzen die de functie van CYP3A kunnen remmen of stimuleren.

In **hoofdstuk 6** is in de aan- of afwezigheid van het oplosmiddel polysorbaat 80 (Tween 80) de mate van plasmabinding van docetaxel onderzocht in relatie tot de farmacokinetiek van het ongebonden docetaxel. Er werd gevonden dat polysorbaat 80, bij klinisch relevante concentraties (tot aan 1.0 μL/mL), de ongebonden fractie van docetaxel (fu) met 12% verhoogt (6.00 ± 1.03% versus 5.49 ± 1.01%, P = 0.038). Tevens bleek het serumeiwit α_1-zure glycoproteïne significant gerelateerd aan fu (P < 0.0018), zodanig dat fu toenam bij lagere eiwitspiegels, hetgeen suggereert dat de binding van docetaxel wordt beïnvloed door zowel α_1-zure glycoproteïne als het oplosmiddel. Tijdens dit onderzoek is gebruik gemaakt van een gevalideerde analytische methode waarin de fractie ongebonden docetaxel is bepaald met behulp van evenwichtsdialyse (**hoofdstuk 8**).

Eerder onderzoek heeft uitgewezen dat wekelijks toedienen van docetaxel niet ten koste gaat van de antitumoractiviteit terwijl het bijwerkingen profiel zodanig verandert dat de hematologische toxiciteit minder is in vergelijking met docetaxel toediening eenmaal per 3 weken (‘3-wekelijks’). Het farmacokinetisch profiel van docetaxel in de wekelijkse schema’s was niet eerder onderzocht. In **hoofdstuk 9** wordt een vergelijkend onderzoek beschreven naar de farmacokinetiek van docetaxel tijdens wekelijkse en 3-wekelijkse behandeling in 46 kankerpatiënten. De gemiddelde (± SD)

klaring van docetaxel bleek vergelijkbaar in de 2 onderzochte schema's (25.2 ± 7.7 versus 23.7 ± 7.9 L/h/m^2); de halfwaardetijd was ook niet verschillend tussen beide schema's (16.5 ± 11.2 and 17.6 ± 7.4 uur). Door toepassing van bloedbemonstering over een lange tijdsperiode werd een terminale halfwaardetijd voor docetaxel gevonden van ongeveer 86 uur, hetgeen 5-maal zo lang is als de waarde zoals geschat op basis van de gebruikelijke 24-uurs bemonstering, en circa 9-maal langer dan gepubliceerde waarden. Als gevolg hiervan konden docetaxel concentraties gedetecteerd worden van 1.0 nM gedurende 7 dagen in het wekelijkse schema, en van 0.5 nM gedurende maar liefst 21 dagen gedurende het 3-wekelijkse schema. De detectie van deze lage concentraties van docetaxel werd mogelijk gemaakt door de beschikbaarheid van een gevalideerde analytische methodiek voor het quantificeren van docetaxel concentraties in humaan plasma met behulp van vloeistofchromatografie gekoppeld aan massa-spectrometrische detectie (**hoofdstuk 7**). De eerder on-opgemerkte en langdurige aanwezigheid van docetaxel concentraties in het laag-nanomolaire gebied draagt mogelijk bij aan het werkingsmechanisme van docetaxel door onderdrukking van het microtubuline netwerk en tumorangiogenese alsmede door verbeterde cellulaire gevoeligheid bij combinatiebehandelingen.

Ondanks de toename in het aantal oudere kankerpatiënten is slechts een zeer beperkt aantal studies uitgevoerd in deze subgroep van patiënten. Het farmacokinetisch gedrag van antikankermiddelen kan veranderen met de leeftijd ten gevolge van verschillende factoren, zoals veranderingen in orgaanfunctie en lichaamssamenstelling. In de studie beschreven in **hoofdstuk 10** werd gevonden dat de farmacokinetiek van docetaxel in oudere (≥ 65 jaar) en jongere (< 65 jaar) patiënten niet verschillend is, met een gemiddelde (± SD) klaring van respectievelijk 30.1 ± 18.3 en 30.0 ± 14.8 L/h. Deze leeftijdsonafhankelijkheid is in overeenstemming met de bevinding dat de fenotypische activiteit van CYP3A, gemeten met behulp van de erythromycine-ademtest, niet veranderde met een toenemende leeftijd. Alhoewel er geen statistisch significant verschil was in docetaxel-gerelateerde bijwerkingen in beide leeftijdsgroepen bleek de incidentie van neutropene koorts enigszins toegenomen in de ouderen. Dit kon echter toegeschreven worden aan minieme verschillen in de blootstelling aan het middel die niet aan leeftijd zijn gerelateerd.

Met behulp van multipele lineaire regressieanalyse, als beschreven in **hoofdstuk 11**, kon aangetoond worden dat naast blootstelling aan polysorbaat 80 ook de mate van leverfunctiestoornis significant is geassocieerd met de klaring van het ongebonden

docetaxel in een groep van 55 patiënten behandeld met docetaxel. Dit onderzoek liet tevens zien dat de AUC van ongebonden docetaxel beter is gerelateerd aan het percentage daling in neutrofielen (*P*=.002 *versus P*=.029) en de neutropenie graad (*P*=.013 *versus P* = .220), zodanig dat grotere blootstelling resulteerde in meer hematologische toxiciteit. Dit suggereert dat de blootstelling aan ongebonden docetaxel beter voorspellend is voor behandelingsgerelateerde beenmergschade dan totaal docetaxel, en dat eerstgenoemde parameter prospectief geïmplementeerd zou moeten worden in vervolgonderzoek.

Toekomstige ontwikkelingen

Het beschreven werk in dit proefschrift had tot doel om factoren te identificeren die van invloed zijn op de farmacokinetische variabiliteit die wordt waargenomen na behandeling met docetaxel, inclusief demografische patiëntkarakteristieken, verminderde leverfunctie, gelijktijdig gebruik van andere chemotherapeutica, eiwitbinding in plasma, en beïnvloeding door het oplosmiddel polysorbaat 80. Echter, een substantiële mate van interpatiënt variabiliteit in de farmacokinetiek van docetaxel blijft onverklaard, en dit draagt vermoedelijk bij aan de onvoorspelbaarheid van de behandelingsuitkomst met betrekking tot toxiciteit en activiteit. De resterende farmacokinetische variabiliteit die niet verklaard kan worden aan de hand van de alhier bestudeerde factoren is mogelijk het gevolg van individuele variatie in levermetabolisme door cytochroom P-450 iso-vormen alsmede de expressie en functie van transporteiwitten die betrokken zijn bij de eliminatie van docetaxel, zoals het P-glycoproteïne.

Huidig en toekomstig onderzoek zal de rol van genetisch-bepaalde factoren, die de expressie en functie van deze eiwitten reguleren, verder bestuderen, en zal hopelijk leiden tot de ontwikkeling van een beter voorspelbaar farmacokinetisch gedrag van docetaxel in individuele patiënten door het gebruik van doseerformules afgeleid uit farmacologische populatiemodellen. Het is echter niet uitgesloten dat naast de genetische componenten ook niet-bestudeerde omgevingsfactoren en fysiologische elementen de klinische farmacologie van dit middel beïnvloeden. Derhalve zal het noodzakelijk zijn om in de nabije toekomst additioneel prospectief onderzoek uit te voeren waarin zowel genotypische als fenotypische testen worden uitgevoerd teneinde de blootstelling aan docetaxel te kunnen voorspellen en uiteindelijk daarmee chemotherapeutische behandeling te individualiseren en verbeteren.

Tot slot, de nadelen van de aanwezigheid van polysorbaat 80 als een essentieel ingrediënt van de huidige farmaceutische formulering van docetaxel heeft nu al geleid tot extensief onderzoek naar de ontwikkeling van alternatieve toedieningsvormen. Deze nieuwe formuleringen maken het uiteindelijk mogelijk om docetaxel toe te dienen met een geringer kans op ongewenste interacties tussen het oplosmiddel en het geneesmiddel, tezamen met een lagere incidentie van ernstige, oplosmiddel-gerelateerde bijwerkingen. Dergelijke alternatieve formuleringen kunnen theoretisch worden toegediend zonder premedicatie, en resulteren mogelijk in een beter voorspelbare en verlengde blootstelling van een tumor aan het middel, en in een gunstigere behandelingsuitkomst.

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Curriculum Vitae

The author of this thesis was born on July 17, 1964 in Santa Ana, California (USA). In 1982 she graduated from Mead High School in Spokane, Washington, and in 1986 she received the degree of Bachelor of Arts (Cum Laude) from Pacific Lutheran University, Tacoma, Washington. In 1991, she received the degree of Doctor of Pharmacy from the University of California at San Francisco, California. After completing a one-year Pharmacy Residency at the University of Nebraska Medical Center, Omaha, Nebraska, she completed a two-year Postdoctoral Fellowship in the Department of Pharmaceutical Sciences under the direction of Dr. Clinton Stewart at St. Jude Children's Hospital, Memphis, Tennessee. From 1994 to 1996, she was a Research Associate in Oncology, Johns Hopkins University, Baltimore Maryland and from 1996-1999, was an Assistant Member, Department of Clinical Research, Institute for Drug Development, Cancer Therapy and Research Center in San Antonio, Texas. In October of 1999, she returned to Johns Hopkins University as Assistant Professor of Oncology and Director of the Analytical Pharmacology Core Laboratory at the cancer center. In June 2004, she was promoted to Associate Professor in Oncology at Johns Hopkins University. The work presented in this thesis originated from collaborations with Prof.dr. Jaap Verweij at Erasmus University, Daniel den Hoed Cancer Center, Rotterdam, the Netherlands.

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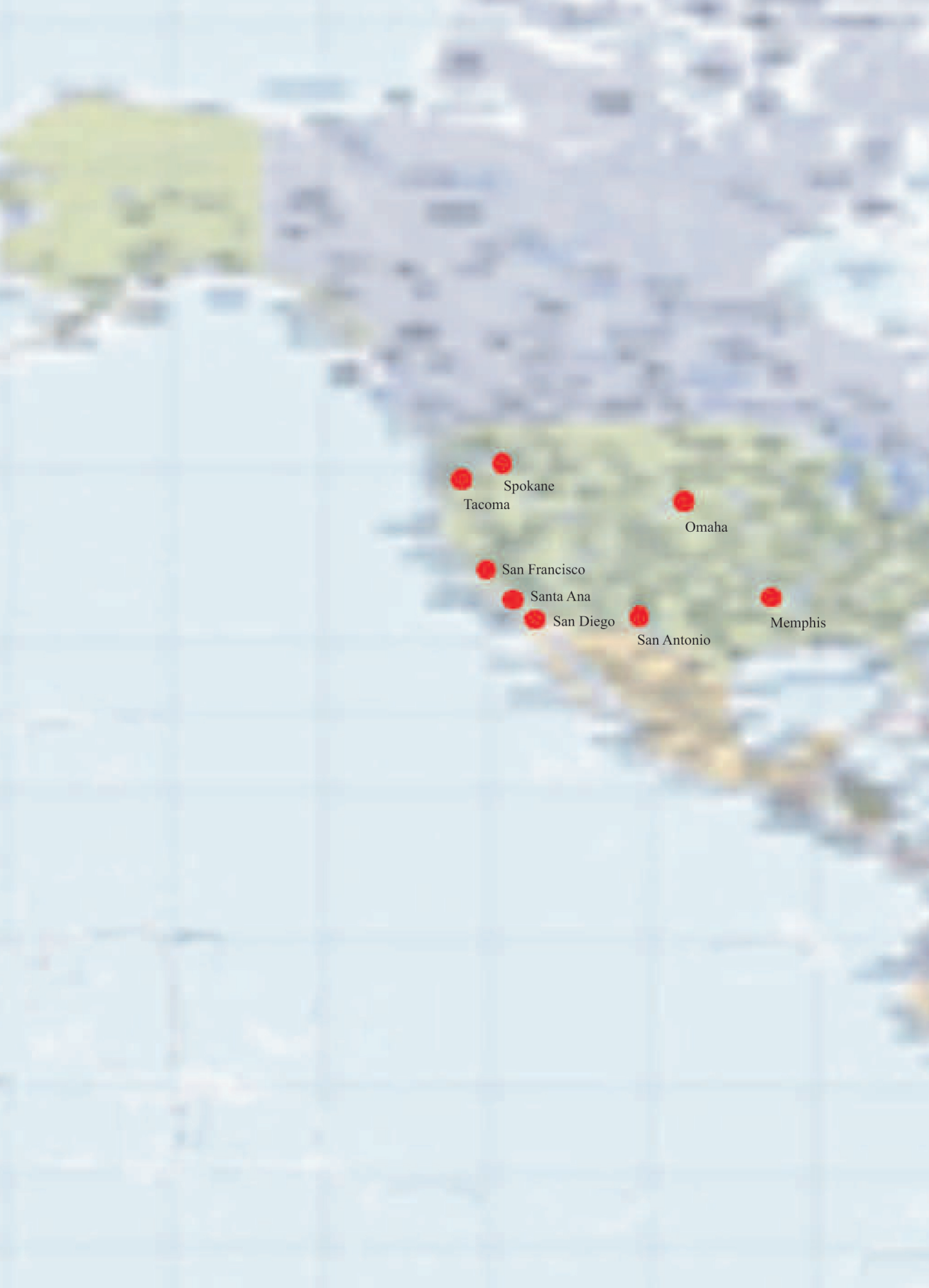
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