Drug-interaction and Formulation Aspects of Taxanes in the Treatment of Cancer

AJ ten Tije

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Geneesmiddel-interacties en Formuleringsaspecten van Taxanen bij de Behandeling van Kanker

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"Passion makes the world go round.
Love just makes it a safer place"
Ice-T (1958-): The Ice Opinion

Voor mijn ouders

Chapter 1

Introduction to the thesis

Introduction to the thesis

General info development taxanes

Over forty years ago, samples of the *Taxus brevifolia*, the pacific yew tree, were screened by the National Cancer Institute (NCI) for anticancer activity. Screening indicated that an extract from the tree possessed activity against tumour cell lines. Paclitaxel the active compound of the extract, was isolated in its pure form in 1969.[1] The mechanism of action was not described until 1979 when Schiff and Horwitz discovered its unique mechanism of cytotoxicity.[2] In contrast to other mitotic agents, paclitaxel and docetaxel promoted the assembly of tubulin and stabilised the resulting microtubules. Clinical studies with paclitaxel started in 1983. At the same time French researchers produced semisynthetic derivatives of baccatin III, an extract from the needles of the European yew *Taxus baccata*, and modified it with a chemically synthesised side chain. Docetaxel emerged from these efforts and entered clinical trials in 1990.[3]

Drug vehicle, drug-interactions, tissue penetration and age are all factors affecting drug pharmacokinetics, and are the focus of this thesis.

Vehicle selection

A major difficulty in the development of both paclitaxel and docetaxel was their insolubility in water. Paclitaxel as currently formulated (Taxol®), is dissolved in a vehicle containing Cremophor EL (CrEL) and alcohol. CrEL, a non-ionic surfactant, is a polyoxyethylated castor oil. Docetaxel (Taxotere®) is currently formulated in polysorbate 80 (Tween 80), an oleate ester of sorbitol. Though the vital role that pharmaceutical excipients have in drug formulation has been neglected, it is now well recognised that excipients can result in adverse effects[4] and have the potential to cause drug interactions.[5] This is reviewed in the second chapter of this thesis. Investigations in the disposition of Tween 80 are outlined in the third chapter.

Interaction

In this thesis the influence of other chemical compounds on the pharmacokinetics (PK) of paclitaxel and docetaxel is investigated. The overexpression of the transmembrane drug transporter P-glycoprotein (P-gp) plays an important role in pharmacokinetics and clinical drug resistance.[6,7] Numerous clinical trials have been performed to develop inhibitors of P-gp with the aim to overcome drug resistance.[8] Unfortunately, the combination

of anticancer agents and P-gp inhibitors necessitated significant dose reduction of the anticancer drugs due to a substantial rise in serious side effects.[9] Intended modulation of paclitaxel by co-administration of the potent P-glycoprotein inhibitor valspodar was studied with specific focus on the PK of the unbound fraction of paclitaxel to explore the interaction between these drugs that are both substrates of the cytochrome P450 isozyme 3A (CYP3A).

Docetaxel is also primarily metabolised by CYP3A. Due to significant interindividual differences in CYP3A activity docetaxel PK is subject to large interindividual differences.[10] In patients with prostate cancer this anticancer drug is combined with ketoconazole because both drugs are known to have anti-prostate cancer properties.[11] Unfortunately, ketoconazole is a potent CYP3A inhibitor. Therefore, this combination is likely to have undesirable clinical consequences due to a much slower metabolising rate of docetaxel in the presence of this inhibitor. A more desirable side-effect of this drug combination is possibly the reduction of interindividual variation in docetaxel PK, leading to a more predictable toxicity profile and allowing optimal dosing strategy whilst maintaining cytotoxic efficacy.

CSF Penetration

Although the brain is among the best perfused organs in the body, most drugs do not accumulate into the brain due to the blood brain barrier (BBB). In patients treated for MBC with docetaxel a high incidence of isolated central nervous system (CNS) metastasis was noted, suggesting that the CNS might be a sanctuary site for malignant cells during chemotherapeutic treatment. On the other hand, several studies suggested that the BBB might be disrupted in the presence of metastasis, suggesting the possibility of penetration of the cytotoxic agent. We performed a PK study on penetration of docetaxel in the cerebrospinal fluid (CSF).

Elderly

Elderly patients with cancer are not only less likely to receive chemotherapy, they are similarly underrepresented in clinical trials, despite the fact that more then 50% of all new patients with breast and lung cancer are older than 65 years.[12,13] This is leading to an important treatment bias against older cancer patients. In an effort to assess the PK, toxicity and responses of older patients with cancer to taxane treatment we conducted

several studies to investigate the effects of both paclitaxel and docetaxel in these patients.

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Chapter 2

Pharmacological effects of formulation vehicles: implications for cancer chemotherapy

A.J. ten Tije,[1] J. Verweij,[1] W.J. Loos,[1] A. Sparreboom[1,2]

[1]Department of Medical Oncology, Erasmus MC - Daniel den Hoed Cancer Center, Rotterdam, the Netherlands; [2]National Cancer Institute, Bethesda, Maryland, USA

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Abstract

The non-ionic surfactants Cremophor® EL (CrEL; polyoxyethyleneglycerol triricinoleate 35) and polysorbate 80 (Tween® 80; polyoxyethylene-sorbitan-20-monooleate) are widely used as drug formulation vehicles, including for the taxane anticancer agents paclitaxel and docetaxel. A wealth of recent experimental data has indicated that both solubilisers are biologically and pharmacologically active compounds, and their use as drug formulation vehicles has been implicated in clinically important adverse effects, including acute hypersensitivity reactions and peripheral neuropathy. CrEL and Tween® 80 have also been demonstrated to influence the disposition of solubilised drugs that are administered intravenously. The overall resulting effect is a highly increased systemic drug exposure and a simultaneously decreased clearance, leading to alteration in the pharmacodynamic characteristics of the solubilised drug. Kinetic experiments revealed that this effect is primarily caused by reduced cellular uptake of the drug by large spherical micellar-like structures with a highly hydrophobic interior, which act as the principal carrier of circulating drug. Within the central blood compartment, this results in a profound alteration of drug accumulation in erythrocytes, thereby reducing the free drug fraction available for cellular partitioning and influencing drug distribution as well as elimination routes. The existence of CrEL and Tween® 80 in blood as large polar micelles has also raised additional complexities in the case of combination chemotherapy regimens with taxanes, such that the disposition of several coadministered drugs, including anthracyclines and epipodophyllotoxins, is significantly altered. In contrast to the enhancing effects of Tween® 80, addition of CrEL to the formulation of oral drug preparations seems to result in significantly diminished drug uptake and reduced circulating concentrations.

The drawbacks presented by the presence of CrEL or Tween® 80 in drug formulations have instigated extensive research to develop alternative delivery forms. Currently, several strategies are in progress to develop Tween® 80- and CrEL-free formulations of docetaxel and paclitaxel, which are based on pharmaceutical (e.g. albumin nanoparticles, emulsions and liposomes), chemical (e.g. polyglutamates, analogues and prodrugs), or biological (e.g. oral drug administration) strategies. These continued investigations should eventually lead to more rational and selective chemotherapeutic treatment.

Table 1. Examples of clinical drug preparations using Cremophor® EL or Tween® 80

Agent	Therapeutic class	Amount administered (mL)[a]
Cremophor EL		
Kahalalide F	Antineoplastic	~0.5[b]
Diazepam	Sedative	1.5
Aplidine	Antineoplastic	~1.5[b]
Teniposide	Antineoplastic	1.5
Didemnin B	Antineoplastic	2.0
Cyclosporin	Immunosuppressive	3.5
C8KC	Photosensitiser	5.5
Propofol	Anaesthetic	7.0
Clanfenur	Antineoplastic	10.3
BMS-247550	Antineoplastic	~10[b]
DHA-paclitaxel	Antineoplastic	19.9
Paclitaxel	Antineoplastic	25.8
Tween® 80		
Carzelesin	Antineoplastic	0.1
Docetaxel	Antineoplastic	2.0
Etoposide	Antineoplastic	2.0

[a] For an average patient with a body-surface area of 1.77 m^2.

[b] Investigational agent for which recommended dose has not yet been established.

Paclitaxel and docetaxel are hydrophobic antineoplastic agents demonstrating significant antitumour activity against a broad spectrum of human malignancies. After the identification of paclitaxel as the active ingredient in crude ethanolic extracts of the bark of the Pacific yew tree, *Taxus brevifolia L*, the development of this drug was suspended for over a decade because of problems in drug formulation.(1) After investigation of a large variety of excipients to enable parenteral administration of paclitaxel, the formulation approach using the polyoxyethylated castor oil derivative, Cremophor® EL (CrEL; polyoxyethyleneglycerol triricinoleate 35), represented the most viable option.(2) Currently, paclitaxel is commercially available as vials containing 30 mg of drug dissolved in 5 mL of CrEL/dehydrated ethanol USP (1:1 by volume). CrEL is widely used as a vehicle for the solubilisation of a number of other hydrophobic drugs, including

anaesthetics, vitamins, sedatives, photosensitisers, immunosuppresives, and (experimental) anticancer drugs (Table 1). The amount of CrEL per administration of paclitaxel is relatively high, and therefore its toxicological and pharmacological behaviour in the context of chemotherapeutic treatment with paclitaxel is of major importance.[3]

The structurally related taxane docetaxel is prepared by chemical manipulation of 10-deacetyl-baccatin III, an inactive precursor isolated from the needles of the European yew tree, *Taxus baccata L.*[4] Like paclitaxel, it is a potent inhibitor of cell replication by stabilisation of the microtubule cytoskeleton. For clinical use, this slightly less hydrophobic agent is formulated in another polyoxyethylated surfactant, polysorbate 80 (Tween® 80). The clinically used formulation consists of 80 mg of docetaxel in 2 mL of undiluted Tween® 80. This non-ionic surfactant is also used to solubilise several other anticancer drugs, including etoposide and minor-groove-binding cyclopropylpyrroloindole analogues such as carzelesin (Table 1).

In recent years, substantial evidence has been generated suggesting that CrEL and Tween® 80 are biologically and pharmacologically active compounds. In this report, we will review the physicochemical and biological properties of both non-ionic surfactants, with a focus on their effects on the disposition characteristics of the carried drugs and that of other agents administered concomitantly.

1. Physicochemical properties of surfactants

CrEL is a white to off-white viscous liquid with an approximate molecular weight of 3000 Da and a specific gravity 1.05-1.06. It is produced by the reaction of castor oil with ethylene oxide at a molar ratio of 1:35. Castor oil is a colourless or pale yellow fixed oil obtained from the seeds of *Ricinus communis*, with an extremely high viscosity, and consists mainly of the glycerides of ricinoleic, isoricinoleic, stearic, dihydroxystearic, and oleic acids. The non-ionic surfactant produced from castor oil is usually of highly variable composition, with the major component (about 87%) identified as oxyethylated triglycerides of ricinoleic acid (Figure 1). As a result of the heterogeneous nature of castor oil and its variable composition, the polyoxyethylated components of CrEL have been poorly characterised. Using fractionation by cyclodextrin-modified micellar electrokinetic capillary chromatography (CD-MEKC) and UV detection, in combination with delayed extraction matrix-assisted laser desorption/ionisation time of flight mass spectrometry (DE-MALDITOF-MS), a more detailed structural elucidation

and a semiquantitative analysis of CrEL components was achieved recently.[5] These investigations indicated that the elimination of water from ricinoleic acid during the synthesis of CrEL leads to various previously unidentified species, including (glycerol-)polyoxyethylene-$\Delta^{9,11}$-didehydrostearate. It is noteworthy that equipment used for intravenous administration of CrEL should be free of polyvinylchloride, since CrEL is capable of leaching phtalate-type plasticisers from polyvinylchloride infusion bags and polyethylene-lined tubing sets, which can cause severe hepatic toxicity.[6,7]

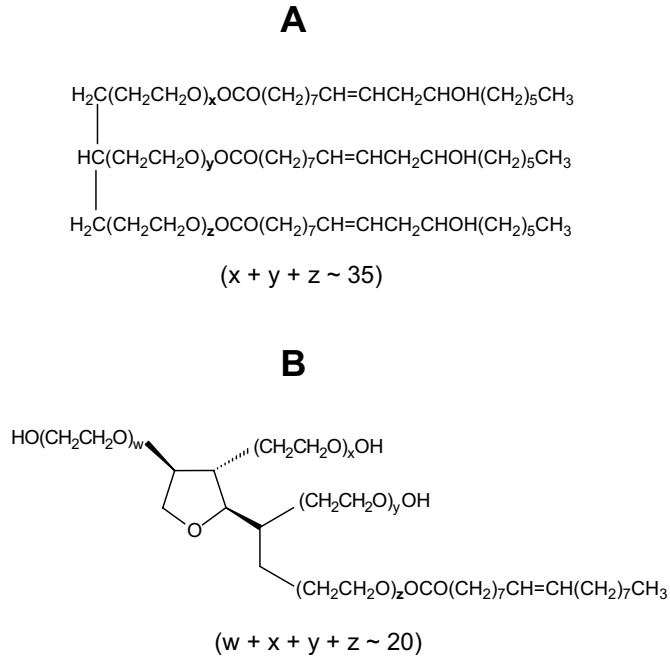

Figure 1. Chemical structures of the primary constituents of CrEL (polyoxyethyleneglycerol triricinoleate 35; A) and Tween 80 (polyoxyethylene-20-monooleate; B).

In contrast to CrEL, Tween® 80 is a relative homogenous and reproducible, amber-coloured, viscous liquid (270-430 centistokes) with a molecular weight of 1309.7 Da, and a density of 1.064 g/mL. The base

chemical name of the major component of Tween® 80 is polyoxyethylene-20-sorbitan monooleate (Figure 1), which is structurally similar to the polyethyleneglycols. Like most non-ionic surfactants, CrEL and Tween® 80 are capable of forming micelles in aqueous solution, with critical micellar concentrations of 0.009% (weight/volume) and 0.01% (weight/volume), respectively, in protein-free aqueous solution.[8]

2. Biological properties of surfactants

2.1 Acute hypersensitivity reactions

The most extensively described biological effect of drugs formulated with CrEL is an acute hypersensitivity reaction characterised by dyspnoea, flushing, rash, chest pain, tachycardia, hypotension, angioedema and generalised urticaria, and this reaction has been attributed to CrEL.[9-12] Nevertheless, allergic reactions to taxanes formulated without CrEL have been reported as well,[13] suggesting that some functionality of the taxane molecule contributes, in part, to the observed effect. Already in the 1970s it was demonstrated that CrEL-containing drug preparations (e.g. rectal diazepam) can cause complement activation.[14,15] The mechanistic basis for this effect has not been fully elucidated, but a number of seminal studies indicate that CrEL-mediated complement activation plays a significant role. It has been postulated that due to binding of naturally occurring anticholesterol antibodies to the hydroxyl-rich surface of CrEL micelles, complement C3 is activated, leading to the clinical signs of hypersensitivity reactions.[16] The CrEL-induced complement activation is clearly concentration dependent, with a minimum CrEL concentration of approximately 2 µL/mL being required, a concentration readily achieved in plasma of cancer patients following standard doses of paclitaxel.[17] This explains why slowing down the infusion rate of paclitaxel formulated with CrEL can alleviate hypersensitivity symptoms, and also explains the need for proper dissolution of CrEL-containing drugs to prevent large variations in CrEL infusion rate leading to unpredictable reactions.[18] A recent investigation into the structure-activity relationships of surfactant-mediated complement activation has shown that several analogues of CrEL have reduced ability to induce complement activation as measured by a decrease in serum concentrations of the SC5b-9 marker (Figure 2). Additional clinical studies will be required to evaluate the clinical utility of some of these substitute vehicles for CrEL-containing drugs.

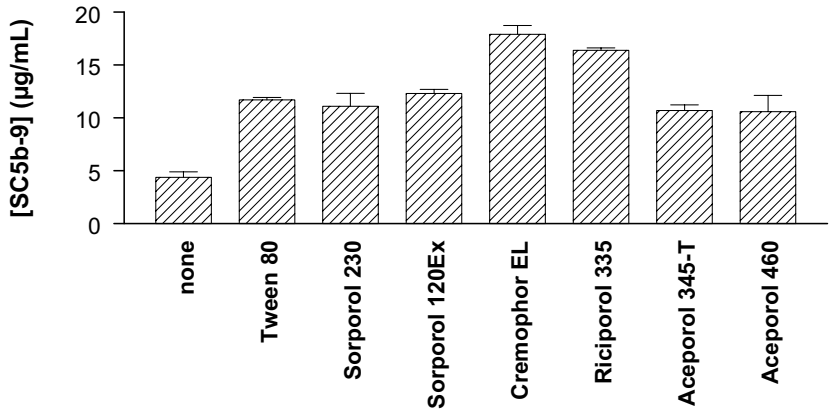

Figure 2. Vehicle-mediated complement activation in human serum by CrEL, Tween® 80 and some structurally related analogues. Experiments were based on 50 µL human serum incubations (45 minutes at 37°C) in the presence of each respective vehicle at a concentration of 10 µL/mL. The complement activation marker SC5b-9 was measured by enzyme-linked immunoassay. Data are presented as mean values (bars) ± SD (error bars) of triplicate observations and were obtained from Loos et al.[19]

In studies with dogs it was demonstrated that CrEL, mainly its minor free-fatty acid constituents such as oleic acid, can cause histamine release.[20] Despite premedication with corticosteroids, and histamine H_1 and H_2 blockers, minor reactions (e.g. flushing and rash) still occur in approximately 40% of all patients,[21-24] with major potentially life-threatening reactions observed in 1.5 to 3% of treated patients.[9]

Oleic acid is also present in Tween® 80, and thus may be a cause of hypersensitivity reactions to docetaxel therapy or other therapies using drugs with Tween® 80 as a solvent. Patients allergic to intravenously administered etoposide tolerated the oral formulation, which is devoid of Tween® 80, very well.[25-28] The early clinical studies with docetaxel revealed an incidence of hypersensitivity reactions ranging from 5-40%, with only a minority of more than grade 2 on the 4-point scale of the National Cancer Institute common toxicity criteria.[29-31] Hypersensitivity reactions to docetaxel therapy can be effectively ameliorated by premedication with

corticosteroids and antihistamines,[32] consistent with a role of histamine in its aetiology. A comparative evaluation of paclitaxel- and docetaxel-mediated non-haematological toxicities, with the drugs given in an every 21-day schedule, is provided in Table 2.

2.2 Peripheral neurotoxicity

A well-known adverse effect of agents formulated in CrEL is peripheral neurotoxicity,[35] but it is less well acknowledged that CrEL may play an important causative role. In a study performed with radiolabelled paclitaxel in rats, no detectable paclitaxel could be demonstrated in the peripheral nerve fibers,[36] but electrophysiological studies in patients with neuropathy after treatment with paclitaxel have shown evidence of both axonal degeneration and demyelinisation.[37] In approximately 25% of patients treated with cyclosporin, neurotoxicity is noted.[38] This adverse effect is never induced by oral formulations of cyclosporin, which is consistent with observations that CrEL is not absorbed intact when given orally. Moreover, CrEL plasma concentrations achieved with therapeutic doses of intravenous paclitaxel or cyclosporin have been shown to produce axonal swelling, vesicular degeneration and demyelinisation in rat dorsal root ganglion neurons.[39,40] The precise mechanism of this CrEL-induced neurotoxicity remains unclear, but recent work has indicated that unsaturated fatty acids may cause neurotoxicity, possibly due to the appearance of peroxidation products[39,40]. This suggests that the ethoxylated derivatives of castor oil probably account for most of the neuronal damage in addition to the presence of residual ethylene oxide residues.[41]

A detailed investigation into neurological adverse effects associated with docetaxel chemotherapy was recently performed in a group of 186 patients.[42] Twenty-one patients developed mild to moderate sensory neuropathy on treatment at a wide range of cumulative doses (50-750 mg/m^2) and dose levels (10-115 mg/m^2). Ten of these patients also developed weakness in proximal and distal extremities of varying degree.

Nine of the 21 patients had received neurotoxic chemotherapy before, and 16 were treated with docetaxel at a dose level of 100-115 mg/m^2. This suggests that docetaxel produces a mild and predominantly sensory neuropathy in a high proportion of treated patients. This adverse effect appears to be dose-dependent and may be severe and disabling at higher dose levels.[42-44] Corticosteroid co-medication does not prevent docetaxel-induced neuropathy.[45]

Table 2. Comparative nonhaematological toxicity of paclitaxel and docetaxel[a]

Adverse effect	Incidence (%)	
	paclitaxel (n=812)	docetaxel (n=2045)
Hypersensitivity reactions[b]		
All	41	15
Severe (at least grade 3)	2	2
Fluid retention[b,c]		
All	0	64
Severe	0	6.5
Nail changes[d]		
All	2	31
Severe (at least grade 3)	0	2.5
Peripheral neuropathy[e]		
All	60	49
Severe (at least grade 3)	3	4
Skin toxicity[f]		
All	2	48
Severe (at least grade 3)	0	5

a Data represent overall incidence as percentage of patients with solid tumours treated with single-agent regimens containing either paclitaxel formulated in a mixture of Cremophor® EL and ethanol at doses of 135–300 mg/m^2 or docetaxel formulated in Tween® 80 at a dose of 100 mg/m^2, given every 21-days.[33,34]

b All patients received a 3-day dexamethasone premedication (docetaxel, n = 92).

c Characterised by one or more of the following events: poorly tolerated peripheral oedema, generalised oedema, pleural effusion requiring urgent drainage, dyspnoea at rest, cardiac tamponade, or pronounced abdominal distension (due to ascites).

d Mostly changes in pigmentation or discoloration of the nail bed.

e Mostly peripheral sensory (numbness, paraesthesias, loss of proprioception), axonal degeneration and secondary demyelination.

f Primarily involves pressure or trauma sites (e.g. hands, feet, and elbows).

Tween® 80 is capable of producing vesicular degeneration. This property depends on the polyethylene substitutions produced by reaction of the polyol compound with ethylene oxide. However, the incidence of neurotoxicity during treatment with docetaxel is much lower as compared to that of paclitaxel (Table 2).[46,47] Furthermore, the Tween® 80-containing epipodophyllotoxin etoposide is not known to be neurotoxic. This suggests that the aetiology of taxane-induced neuropathy is different for paclitaxel and docetaxel, with formulation vehicles contributing to the overall picture to a different extent.

2.3 Dyslipidaemia

In the mid-1970s, lipoprotein alterations caused by CrEL were mentioned for the first time.[48] Later, CrEL was found to alter the buoyant density of high-density lipoprotein (HDL) and shift the electrophoretic and density gradient HDL to low-density lipoprotein (LDL).[49-52] These authors demonstrated the strong affinity of paclitaxel for serum lipoprotein degradation products, potentially affecting the pharmacokinetics of the drug by altering protein binding characteristics. High concentrations of CrEL may also cause dyslipidaemia, possibly resulting in rouleaux formation of erythrocytes.[53] Although cyclosporin is known for its atherosclerosis-inducing capacities, it remains unclear if the observed hyperlipidaemia after CrEL administration is contributing to this risk for vascular accidents. *In vivo* studies of the effects of cyclosporin on the de-endothelialised carotid artery of New Zealand White rabbits treated with therapeutic doses of cyclosporin (15 mg/kg/day) or with a vehicle control (CrEL) revealed intimal proliferation in both groups.[54] Mean plasma cholesterol levels were moderately increased in both groups. Although this may have contributed to foam cell formation in the cyclosporin-treated animals, it was not the sole determinant, as foam-cell-rich lesions were not observed in animals receiving only CrEL. In contrast, Tatou et al observed significant adverse effects of CrEL on endothelial function and vascular muscle on isolated and perfused rat hearts, leading to a reduction of coronary flow and aortic output.[55] The potential clinical implications with respect to these CrEL-related phenomena remain unknown.

2.4 Inhibition of P-glycoprotein activity

P-glycoprotein is a drug transporting membrane protein, and its expression is increased in tumour cells having a multidrug resistance phenotype.[56,57] Several *in vitro* studies in the early 1990s observed

modulation of the activity of P-glycoprotein by CrEL.[58-60] Later, similar phenomena were observed for various other non-ionic surfactants, including Tween® 80,[61,62] Solutol HS 15,[63] and Triton X-100.[64] However, *in vivo* studies never demonstrated reversal of multidrug resistance by any non-ionic surfactant, including CrEL and Tween® 80.[65-67] The extremely low volume of distribution of CrEL and the rapid degradation of Tween® 80 *in vivo* are the likely explanations for this lack of *in vivo* efficacy (see section 3.2). Indeed, the volume of distribution of CrEL is approximately equal to the volume of the blood compartment, suggesting that concentrations necessary to affect reversal of multidrug resistance *in vitro* are not reached *in vivo* in solid tumours.[68] However, it should be noted that the pharmacokinetic selectivity of CrEL for the central blood and bone marrow compartment can provide an advantage to treatment of haematological malignancies with resistance to chemotherapy caused by elevated P-glycoprotein expression.[69]

2.5 Intrinsic antitumor effects

Cell-growth inhibitory properties of CrEL were first observed by Fjällskog et al in doxorubicin-resistant human breast-cancer cell lines,[70,71] and were later confirmed in other malignant cell types.[72,73] The formation of free radicals by peroxidation of polyunsaturated fatty acids and/or a direct pertubing effect on the cell membrane are possible mechanisms responsible for this type of cell growth inhibition.[74-76] Using *in vitro* clonogenic assays, however, it has been demonstrated that CrEL, at clinically-achievable concentrations, can antagonise the cytotoxicity of paclitaxel by a cell-cycle block.[77] Several reports also suggest that Tween® 80 has intrinsic antitumour activity in animal models,[78-80] which might be linked to the release of oleic acid, a fatty acid known to interfere with malignant cell proliferation due to formation of peroxides[81] and inhibition of angiogenesis.[82] The exact contribution of Tween® 80 to antitumour activity observed in patients treated with chemotherapeutic drugs formulated in this vehicle substance has not been clarified.

3. Pharmacological properties of surfactants

3.1 Analytical methods

At present, a large variety of analytical procedures are available for clinical pharmacokinetic studies with CrEL and Tween® 80. The first assay developed for measurement of CrEL concentrations in patient material was

based on the ability of this vehicle to modulate daunorubicin efflux in multidrug resistant T-cell leukaemia VLB_{100} cells.[83] Alternatively, a more sensitive and reliable method was developed that required sample volumes of only 20 µL.[84] This method is based on measurement of ricinoleic acid after base-induced hydrolysis (saponification) of CrEL followed by an acylchloride formation, precolumn derivatisation with naphthylamine, and reversed-phase high-performance liquid chromatography (HPLC) to detect N-ricinoleoyl-1-naphthylamine at 280 nm. Because of the high costs and the time-consuming nature of both assays, a new method, based on a selective binding of CrEL to the Coomassie Brilliant Blue G-250 dye in protein-free extracts was developed for human plasma samples.[85,86] This method has also been used to measure Tween® 80 concentrations in murine and human plasma.[87] More recently, a potentiometric titration method for CrEL was developed for quantitative analysis in urine and plasma based on coated wire electrode as an end-point indicator with sodium tetraphenylborate at 20°C and pH 10.[88] Each of these methods has its drawbacks and limitations, and the methodological differences between them probably contribute to the variations in measured CrEL concentrations.

In addition to the Coomassie Brilliant Blue G-250 colourimetric dye-binding assay, various other analytical procedures are available for Tween® 80. Initially measurement of the polyoxyethylated portion of the molecule was used for quantification of Tween® 80 concentrations. The so-called polyol moiety is detectable by a wide variety of methods, including a resorcinol-glucose precipitation, a colourimetric method using ammonium cobaltothiocyanate, turbidimetric or gravimetric procedures, and complex formation with barium phosphomolybdic reagent.[89,90] The ammonium cobaltothiocyanate complexation has also been used in combination with HPLC and UV detection for analysis of Tween® 80 in urine and ascites fluid, using either post-column or on-line complexation.[91-94] A less complex procedure that does not require complexation involves a one-step hydrolysis with sulphuric acid followed by HPLC with UV detection at 210 nm.[95] Most recently, Tween® 80 concentration in human plasma samples have been analysed by a liquid chromatographic assay with tandem mass-spectrometric detection, with a 60-fold increased sensitivity as compared with previous published assays.[96]

3.2 Pharmacokinetics

The various analytical methods described above have been used in different pharmacokinetic studies of CrEL, sometimes leading to conflicting results and conclusions. There have been no studies thus far comparing the different analytical methods. Initial pharmacokinetic analyses have indicated that CrEL shows linear pharmacokinetic behaviour.[97] However, with prolongation of infusion duration from 1-3 and 24 hours, CrEL clearance increased from about 160 to 300 and 400 mL/h/m^2, respectively (Figure 3).[17] A recently developed population pharmacokinetic model revealed that the plasma concentration-time data of CrEL were best fitted to a three compartment model with Michaelis-Menten elimination (Table 3).[98,99]

It thus appears that CrEL shows schedule-dependent pharmacokinetics, possibly related to saturated elimination due to capacity-limited CrEL metabolism within the systemic circulation. This schedule dependency leads to an increase in systemic exposure, and thus an increase in CrEL related biological effects, with shortening of the infusion duration. An example of this phenomenon is the apparent increase of allergic reactions in 1-hour versus 3- or 24-hour infusions of paclitaxel,[9,100] as well as increased incidence of peripheral neuropathy with shorter paclitaxel infusions.[101,102] The observed changes in adverse effects as a function of paclitaxel infusion duration will need to be confirmed in larger comparative trials in order to provide recommendations for treating clinicians.

The terminal half-life of CrEL amounts to approximately 80 hours with reported values ranging between 10 and 140 hours, depending on the sampling time period and the method used for CrEL analysis. Therefore, studies using sparse-sampling strategies with application of the bioassay method may lead to underestimation of the terminal half-life.[103] With the more sensitive colourimetric assay, detectable concentrations of CrEL were demonstrated even 1 week after initial treatment.[68] Despite this relatively long terminal disposition phase of CrEL, long-term weekly administration of paclitaxel does not cause significant accumulation of CrEL although the vehicle is always detectable in pre-dose samples.[104] In all studies, the observed volume of distribution of CrEL was extremely small and almost equal to the volume of the central blood compartment. As outlined, this implies that tissue and tumour delivery of CrEL is insignificant.[68]

Little is known about elimination routes of CrEL. Pharmacokinetic studies in patients with hepatic dysfunction treated with paclitaxel suggested that hepatobiliary elimination of CrEL is not of major importance.[105]

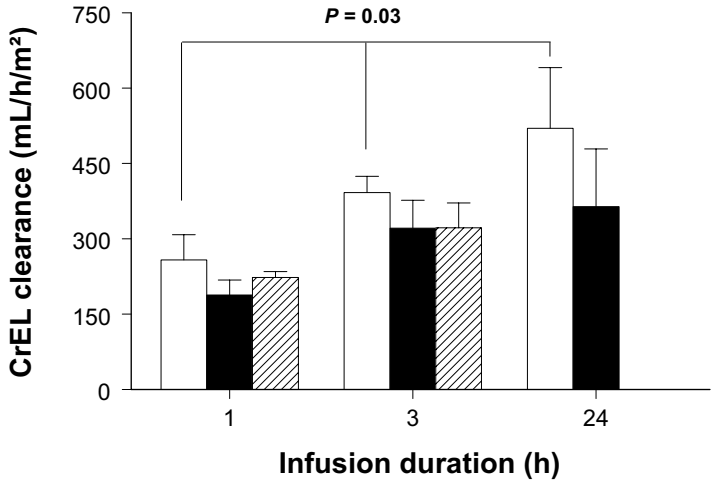

Figure 3. Effect of infusion duration on the clearance of CrEL. Data are expressed as mean values (bars) ± SD (error bars) and were obtained from patients treated with paclitaxel formulated in CrEL at dose levels of 135 mg/m² (white bars; CrEL dose, 11.3 mL/m²), 175 mg/m² (black bars; CrEL dose, 14.6 mL/m²), or 225 mg/m² (hatched bars; CrEL dose, 18.8 mL/m²).[17]

Despite its highly hydrophilic nature, the renal elimination of CrEL accounts for less than 0.1% of the administered dose and CrEL pharmacokinetics in a patient with severely impaired renal function was not different from those in historic controls.[10] It is possible that elimination pathways for CrEL are mainly dictated by serum carboxylesterase-induced degradation, leading to the release of free fatty acids such as ricinoleic acid. This metabolic route occurs apparently at a low rate and the involved enzymes may be easily saturated, which explains the peculiar time-dependent non-linear pharmacokinetics of this vehicle.

The pharmacokinetic behaviour of Tween® 80 is very different from that of CrEL. In animal studies a rapid decline of the concentration was shown after injection (Figure 4). Plasma concentration were below 0.05 µL/mL (i.e. the lower limit of quantification of the analytical method) within 15 minutes after the drug administration.[87] Observations in 5 patients treated with docetaxel as a 1-hour infusion at a dose of 100 mg/m² showed peak plasma

concentrations of Tween® 80 of 0.16 ± 0.05 μL/mL, consistent with more recent observations.[96,107] *In vitro* experiments have shown that this rapid elimination is caused by a rapid carboxylesterase-mediated hydrolysis in the systemic circulation, cleaving the oleic acid side chain from the molecule.[87]

Table 3. Population pharmacokinetic parameters of Cremophor® EL following paclitaxel administration[a]

Parameter	Estimate	RSE (%)
V_1 (L)	2.59	7
Q_2 (L/h)	1.44	24
V_2 (L)	1.81	9
Q_3 (L/h)	0.155	22
V_3 (L)	1.61	7
Km (mL/L)	0.122	61
V_{max} (mL/h)	0.193	9
Residual error		
Additional (mL/L)	0.0951	34
Proportional (%)	6.94	8

[a] Data are from patients treated with paclitaxel formulated in a mixture of Cremophor® EL and ethanol, and were from Van den Bongard et al.[99] Determination of Cremophor® EL in plasma samples was performed by pre-column derivatisation and reversed-phase high-performance liquid chromatography, as described elsewhere.[84]

Km = plasma concentration at half V_{max}; Q_2 and Q_3 = intercompartmental clearances from the central to the first or second peripheral compartments; RSE = relative standard error; V_{max} = maximum elimination rate; V_1, V_2 and V_3 = volumes of the central, first peripheral and second peripheral compartments.

Earlier studies performed in rats and humans with the structurally related surfactants polysorbate 20 and polysorbate 40 have shown similar metabolic pathways, with ester bond cleavage and subsequent oxidation of the fatty acid moiety (reviewed in Van Zuylen et al[108]).

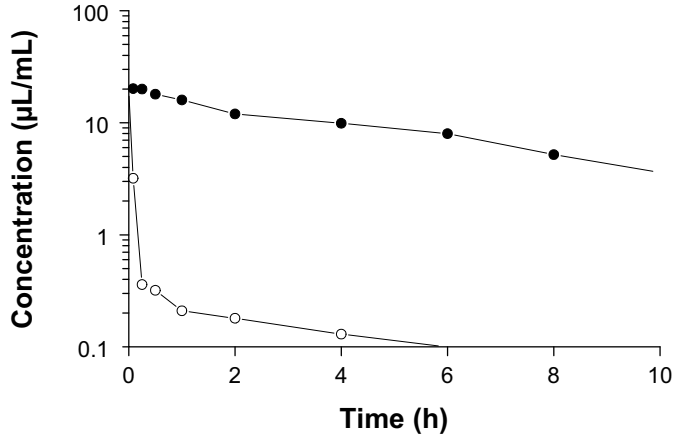

Figure 4. Comparative plasma concentration-time profiles of CrEL (closed symbols) and Tween® 80 (open symbols) in mice receiving 0.83 mL/kg of each vehicle by bolus injection. Data show mean values of 4 observations per time point and were obtained from Van Tellingen et al.[87]

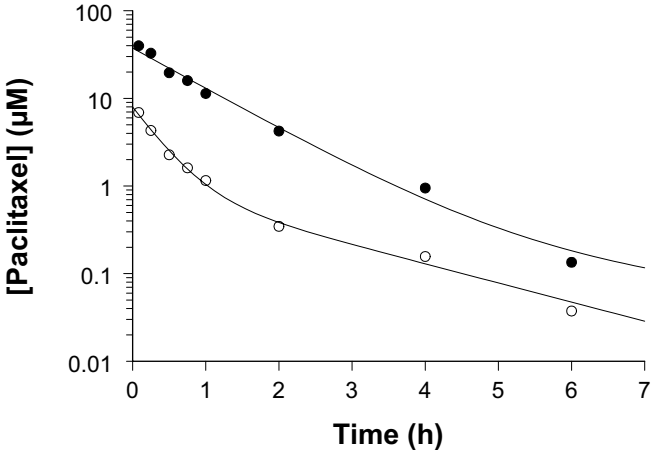

Figure 5. Effect of CrEL on the plasma concentration-time profiles of paclitaxel in mice treated at a paclitaxel dose of 10 mg/kg formulated with CrEL (closed symbols) or with Tween® 80 (open symbols). Data were obtained from Sparreboom et al.[122]

Table 4. Pharmakinetic effects of Cremophor® EL and Tween® 80 on intravenously administered drugs

Agent	Species	Pharmacokinetic effect(s)	Reference
Cremophor EL			
Cyclosporin	baboon	4.2-fold increased AUC	113
Doxorubicin	mouse	2-fold increased AUC	114
	mouse	increased concentrations in plasma, liver	115
	mouse	increased concentrations in heart, liver	116
	human	1.2-fold increase in AUC	117
Epirubicin	mouse	increased levels in spleen	118
Etoposide	rat	4.6-fold increased AUC	111
SN-38	mouse	2-fold increased AUC	119
C8KC	mouse	increased C_{max} and $T_{1/2\beta}$	120
Oxaliplatin	rat	1.6-fold increased AUC	121
Paclitaxel	mouse	7-fold increased AUC	122
	rat	9-fold increased AUC	109
	human	2-fold increased AUC	123
Tween® 80			
Doxorubicin	mouse	increased levels in plasma, spleen	116,124,125
	human	2-fold reduced AUC	126
Etoposide	rat	1.2-fold increased AUC	118
Methotrexate	mouse	increased uptake in brain	127
Vigabatrin	rat	increased GABA in brain	128

AUC = area under the plasma concentration-time curve; C_{max} = peak plasma concentration; GABA = γ-aminobutyric acid; $T_{1/2\beta}$ = half-life of the terminal disposition phase.

4. Modulation of drug disposition patterns

4.1 Intravenous administration

Various studies have shown that CrEL alters the pharmacokinetic behaviour of many drugs administered intravenously, including cyclosporin, anthracyclines, etoposide, the irinotecan metabolite SN38, the photosensitiser C8KC, and paclitaxel (Table 4). The most common effect is a substantial increase in the systemic exposure to the studied agent with a concomitantly reduced systemic clearance, as was first described for paclitaxel in a mouse model (Figure 5). Various proposed causes of the CrEL-drug interactions have been put forward in recent years, including altered

protein binding characteristics,[52] altered hepatobiliary secretion,[109] and inhibition of endogenous P-glycoprotein mediated biliary secretion, thereby reducing elimination of drugs.[110] In the isolated perfused rat liver, CrEL inhibited the hepatic elimination of paclitaxel, preventing the drug from reaching the sites of metabolism and excretion,[109] and the same effect was noted for Tween® 80.[111] However, recent studies indicate that drug-transporting P-glycoproteins are not essential for normal hepatobiliary secretion of paclitaxel,[112] suggesting that this protein does not play a major role.[8]

In view of the very small volume of distribution of CrEL, it is likely that the pharmacokinetic interaction observed with some drugs takes place within the central blood compartment. This was recently confirmed by *in vitro* experiments demonstrating that encapsulation of the model drug paclitaxel within the hydrophobic interior of CrEL micelles takes place in a concentration-dependent manner, causing changes in cellular partitioning and blood:plasma concentration ratios of paclitaxel (Table 5).[8,19] It was shown that the affinity of paclitaxel was (in decreasing order) CrEL > plasma > human serum albumin, with CrEL present above the critical micellar concentration (i.e., ~0.01%). Since the effect was also observed in the absence of plasma proteins, it could not have been caused by altered protein binding or by an increased affinity of paclitaxel for protein dissociation products that are produced by the action of CrEL on native lipoproteins.[51,52] These findings are consistent with the hypothesis that paclitaxel can be entrapped within micelles, and that these micelles act as the principal carrier of paclitaxel in the systemic circulation.

An intriguing feature of paclitaxel pharmacokinetics is a distinct dose-dependent pharmacokinetic behaviour, with clearance values decreasing substantially with an increase in drug dose. This effect is particularly evident with 3-hour infusion regimens, and CrEL has been linked to this phenomenon. It has been shown that the percentage of total paclitaxel trapped in micelles increases disproportionally with higher doses of CrEL administered,[8] thereby influencing the unbound drug concentration and making it less available for distribution to tissues, metabolism, and biliary and intestinal secretion. Indeed, the free fraction of paclitaxel is inversely related to CrEL concentrations *in vitro,*[129] and CrEL has also been shown to alter the blood : plasma concentration ratios *in vivo* by reducing drug uptake in red blood cells.[130] Interestingly, when paclitaxel dissolved in another vehicle was administered to mice, no pharmacokinetic nonlinearity in plasma concentration profiles was evident.[122] The concentrations in tissues

also increased linearly with increasing dose even when dissolved in CrEL, suggesting linear kinetics for the unbound drug.

Table 5. Effect of Cremophor® EL (CrEL) and derivatives on the blood:plasma concentration ratio of paclitaxel[a]

Compound added (μg/mL)	Blood : plasma ratio	Change (%)	p[b]
None	1.07 ± 0.004		
CrEL (0.1)	1.09 ± 0.009	+1.83	0.387
CrEL (0.5)	0.990 ± 0.015	-9.35	0.012
CrEL (1)	0.901 ± 0.017	-15.8	0.003
CrEL (5)	0.690 ± 0.005	-35.5	<0.0001
CrEL (10)	0.625 ± 0.008	-41.6	<0.0001
Castor oil (5)	1.23 ± 0.171	+13.0	0.061
CrEL fraction 1 (5)[c]	1.06 ± 0.008	-0.94	0.520
CrEL fraction 2 (5)	0.926 ± 0.018	-13.5	0.043
CrEL fraction 3 (5)	0.763 ± 0.055	-28.7	0.010
CrEL fraction 4 (5)	0.645 ± 0.051	-39.7	0.003
CrEL fraction 5 (5)	0.943 ± 0.039	-11.9	0.103

a Paclitaxel was used at an initial concentration of 1 μg/mL and incubated in whole blood for 15 min at 37°C before fractionation and analysis by high-performance liquid chromatography. Ratio data are presented as mean values ± SD of (at least) triplicate measurements and were obtained from Sparreboom et al.[8]

b Probability of significant difference versus control (unpaired two-sided Student's t test).

c Five CrEL fractions, each with progressively increased hydrophobicity, were isolated as chromatographic peaks, as described elsewhere.[8] The fractionation process was based on reversed-phase high-performance liquid chromatography of crude CrEL. The first fractions mainly contain polyoxyethyleneglycerol and oxyethylated glycerol, and the pharmacologically active fraction 4 contains the micelle-forming component, polyoxyethyleneglycerol triricinoleate along with fatty acid esters of polyethyleneglycerol.

Earlier, the nonlinearity in paclitaxel pharmacokinetics had been described by empirical models using both saturable elimination and saturable distribution, where the saturable distribution has been described as saturable transport,[131] or saturable binding.[132] A recent study demonstrated that a mechanistic model could be used to describe the nonlinear kinetics of the drug using simultaneous description of total and

unbound plasma concentrations, whole blood concentrations, and concomitant CrEL concentrations.[133] This pharmacokinetic model has a foundation in the known properties of paclitaxel as determined with micellar trapping of paclitaxel, distribution to red blood cells and binding to serum albumin, α_1-acid glycoprotein and platelets. The results of that study showed that the nonlinear pharmacokinetics are predominantly explained by nonlinear binding to CrEL and that the unbound drug displayed linear pharmacokinetics when administered over a 3-hour period.

The drug fraction not bound to serum proteins or CrEL is a rather small fraction of the total under normal physiological conditions, and at high concentrations, paclitaxel is mainly bound to CrEL. From simulated concentration components in patients treated with 24-hour infusions, it was demonstrated that because CrEL concentrations are rather low, the linear binding to serum proteins and binding to blood cells are of greater importance than the CrEL binding.[133] Because of the schedule-dependent clearance of CrEL, this has serious clinical ramifications in that the systemic exposure to unbound paclitaxel will be a function of infusion duration. This was recently confirmed in a randomised comparative clinical trial evaluating drug disposition characteristics following 1- versus 3-hour infusions.[102] The area under the plasma concentration-time curve (AUC) of unbound paclitaxel was 24% (P = 0.009) reduced as compared with the 3-hour infusion group (Figure 6), despite significantly higher peak concentrations (0.26 ± 0.007 μM *vs* 0.15 ± 0.07 μM; P = 0.0002). Most importantly, this effect translated into more severe haematological toxicity with the 3-hour schedule of drug administration,[102] suggesting that the various infusion schedules currently employed for paclitaxel dosing are not interchangeable nor pharmacologically equivalent.

The existence of CrEL in blood as large polar micelles with a highly hydrophobic interior also raises the possibility of interactions occurring with other (poorly water-soluble) drugs. For example, the combination of paclitaxel with anthracycline drugs may result in altered cellular distribution and a concomitantly increased plasma concentration, because of incorporation of the anthracycline drug into CrEL micelles.[134] In this respect, several studies have demonstrated significant pharmacokinetic interactions between paclitaxel and/or CrEL and doxorubicin.[110,114,117,135,136] Although not tested explicitly, it is likely that the presence of CrEL in the clinical formulation of certain drugs contributes, at least in part, to various pharmacokinetic interactions described with other agents (Table 6).

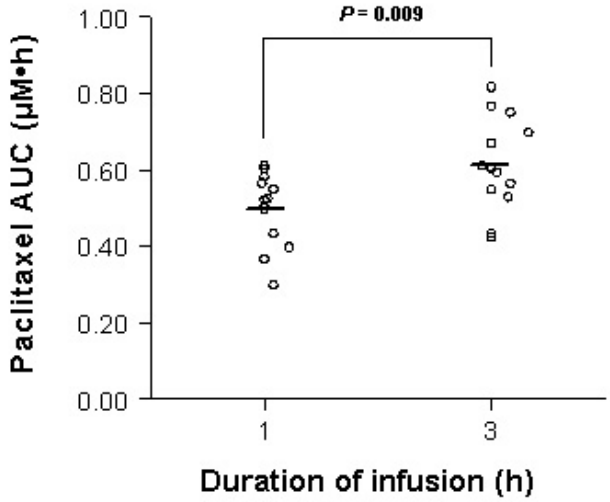

Figure 6. Effect of infusion duration of the systemic exposure (AUC) to unbound paclitaxel. Data were obtained from 29 cancer patients receiving a 1-hour [n = 15; mean AUC (± SD), 0.50 ± 0.10 µM•h] or a 3-hour infusion [n = 14; mean AUC (± SD), 0.62 ± 0.12 µM•h] and were obtained from Gelderblom et al.[102] Each symbol represents the AUC of an individual patient and the horizontal lines indicate mean values for each group. AUC=area under the concentration-time curve.

Table 6. Clinically relevant drug interactions attributable (partially) to Cremophor® EL.

Agents	Pharmacokinet effect(s)	Reference
Paclitaxel[a]		
Doxorubicin	1.4-fold increased AUC	137
Epirubicin	1.7-fold increased AUC	138
Gemcitabine/epirubicin	1.7-fold increased epirubicin AUC	139
Irinotecan	1.4-fold increased SN-38 AUC	140
Cyclosporin[a]		
Etoposide	1.8-fold increased AUC	141
Etoposide/mitoxantrone	1.5-fold increased etoposide AUC	142
Doxorubicin	1.5-fold increased AUC	143
Vinblastine	increased myelosuppression	144
Valspodar[a]		
Etoposide	1.9-fold increased AUC	145
Doxorubicin	2.0-fold increased AUC	146

[a] Formulated for clinical use in a Cremophor® EL-containing vehicle, and administered intravenously. AUC = area under the plasma concentration-time curve.

There are conflicting reports in the literature on the effects of Tween® 80 on the distribution and elimination of drugs administered intravenously (Table 4). In mice it was demonstrated that Tween® 80 caused an increase of doxorubicin plasma concentrations by decreasing the plasma volume as a result of the osmotic effect of Tween® 80 on total blood volume.[124,125] However, in patients receiving the same relative amount of Tween® 80 (administered concomitantly with etoposide at a dose of 100 mg/m^2), both the volume of distribution and the clearance of doxorubicin were increased, due to reduced plasma concentrations of doxorubicin in the early phase of the concentration-time profile.[126] In the isolated perfused rat liver, Tween® 80 decreased the clearance and the volume of distribution of etoposide,[111] but it increased the renal and biliary excretion of methotrexate.[127] The majority of clinical investigations have shown minimal alteration in the pharmacokinetic profiles of agents when used in combination with drugs formulated in Tween® 80.[135,147,148] This is most likely the result of the rapid degradation of Tween® 80 in plasma by esterases, such that it can not interfere to any significant extent with the pharmacokinetic behaviour of other agents.

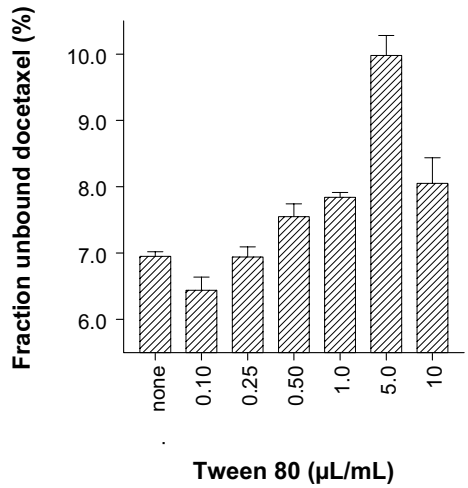

Figure 7. Extent of docetaxel binding to human plasma in vitro expressed as the unbound drug fraction as a function of Tween® 80 concentration. Data are expressed as mean values (bars) ± SD (error bars) of triplicate observations and were obtained from Loos et al.[149]

However, recent observations indicate that Tween® 80, at concentrations observed in patients treated with docetaxel, causes a profound and significant alteration of the fraction unbound docetaxel, which increased by 50% (Figure 7).[149] The mechanistic basis for the decreased binding of docetaxel in the presence of Tween® 80, contrary to that observed with CrEL and paclitaxel, is as yet unclear. It is possible, however, that with time Tween® 80 is able to form micellar complexes with proteins, including serum albumin and α_1-acid glyoprotein, so that the binding of docetaxel becomes saturable on single sites.[150] Similar observations have been reported for the binding of several other drugs that bind with high affinity but low capacity to α_1-acid glycoprotein in the presence of structurally-related mixed-micellar systems.[151] Alternatively, the phenomenon might be the result of Tween® 80 metabolism by serum esterases and subsequent oleic acid-mediated protein-binding displacement of docetaxel, causing increases in unbound drug.[152] Regardless of the mechanism underlying this effect, it is consistent with recent observations that, similar to paclitaxel, also in the case of docetaxel nonlinear distribution pathways exist that may be related to the presence of non-ionic surfactants in the clinical formulated product.[153]

4.2 Extravascular routes of administration

There have been many reports highlighting the ability of Tween® 80 to increase the absorption in *in vitro* systems, animals and humans of numerous agents involving various classes of drug. Typical examples of this phenomenon are provided in Table 7. The main overall conclusion from these studies is that Tween® 80 acts as an enhancer of the systemic exposure to orally administered agents by increasing biomembrane permeability,[154,155] as has also been described for intravesical instillation of thiotepa in the presence of Tween® 80 in cancer patients.[156] It has also been proposed that agents like Tween® 80 and CrEL not only support solubilisation, but also may inhibit the activity of P-glycoprotein with oral administration.[157,158] This protein is a membrane-bound drug efflux pump, which is abundantly present in the gastrointestinal tract,[159,160] and mediates direct secretion of substrate drugs into the intestinal lumen, thereby limiting its oral uptake.[112] However, following oral administration, polyoxyethylated surfactants are known to be extensively metabolised in the intestine by pancreatic lipases into the free fatty acid and the polyol moiety, with only less than 3% of the administered dose being excreted into the urine.[108] This makes it unlikely that the modulating effects are

predominantly caused by a direct influence on active drug transport by the intact vehicles.

In contrast to the enhancing effects of Tween® 80, addition of CrEL to the formulation of oral drug preparations, in general, seems to result in significantly diminished drug uptake and reduced circulating concentrations (Table 7). One of the best studied examples is the influence of CrEL on the oral absorption of paclitaxel. Oral administration of this drug is an attractive alternative for the currently used intravenous regimen, because it is convenient and practical for patients and it may circumvent systemic exposure to CrEL, which is known to be not absorbed intact after oral administration.(173,174)

Table 7. Influence of formulation vehicles on oral drug absorption characteristics

Agents	Test system	Effect(s)	Ref
Cremophor® EL			
Acf(N-Mef)NH_2	Caco-2 cells	2.6-fold reduced permeability	157
Digoxin	Human	Decreased lag time	161
Paclitaxel	Human	2.0-fold decreased AUC[a]	162
	Mouse	1.4-fold decreased AUC[b]	163
Saquinavir	Human	5.0-fold increased AUC	164
Phytomenadione	Human (infant)	Decreased PIVKA-II	165
Tween® 80			
Albendazole	Rat	1.9-fold increased AUC	166
Cyclosporin	Rat	33-fold increased bioavailability[c]	167
Danazol	Dog	16-fold increased bioavailability	168
Digoxin	Rat intestine	Increased uptake	158
Griseovulvin	Human	1.5-fold decreased AUC	169
Indomethacin	Rat	1.6-fold increased AUC	170
Itazigrel	Rat	1.5-fold increased absorption	171
Methotrexate	Mouse	2.0-fold increased AUC	127
Tetracycline	Rat intestine	2.7-fold increased absorption	172

[a] As compared to a Tween 80 formulation; [b] As compared to a formulation containing 7-fold less CrEL; [c] As compared to a nanosphere formulation.
AUC = area under the plasma concentration-time curve; PIVKA-II = des-gamma-carboxyprothrombin.

A study of paclitaxel formulated in Tween® 80 resulted in a significant increase in the peak concentration and AUC of paclitaxel in comparison with the CrEL formulations.[162,163] Fecal elimination data revealed a decrease in excretion of unchanged paclitaxel for the Tween® 80 formulation compared to the CrEL formulations, suggesting that entrapment of paclitaxel in CrEL micelles is an important factor limiting the absorption of orally administered paclitaxel from the intestinal lumen. Obviously, this has significant clinical ramifications in that oral paclitaxel delineates very distinct apparent saturable absorption kinetics with no further increase of the AUC with a given increase in dose (Figure 8).[175-178] Similar dose-dependence was not observed with oral administration of docetaxel formulated in Tween® 80,[179] suggesting that the effect is CrEL specific, and that other formulations should be developed in order to increase the usefulness of oral paclitaxel administration.

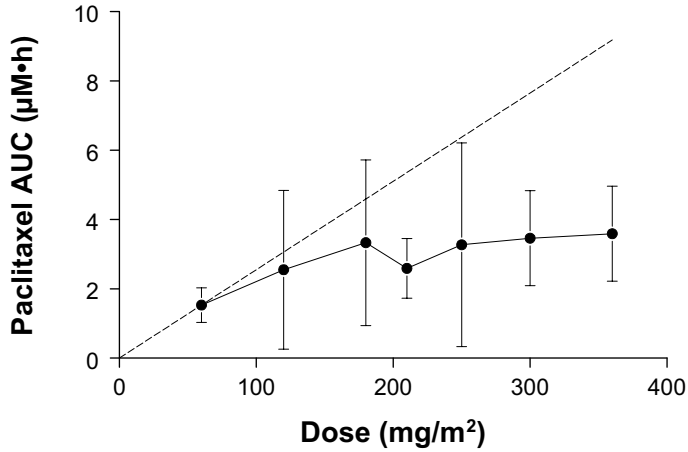

Figure 8. Effect of oral drug dose on the systemic exposure to paclitaxel in cancer patients. Data are expressed as mean values (symbols) ± SD (error bars) and were obtained from Malingre et al.[175] The broken line indicates the hypothetical dose-proportional increase in the area under the plasma concentration-time curve (AUC).

Entrapment of drug in CrEL micelles has also been demonstrated for several agents delivered intraperitoneally [e.g. O^6-benzylguanine in mice[180] and paclitaxel in cancer patients[123]] or intravesically [e.g. paclitaxel in dogs[181]]. The major goal of intraperitoneal therapeutic strategies is to expose

tumours within the peritoneal cavity to higher concentrations of antineoplastic agents for longer periods of time than can be achieved by systemic drug administration.[182,183] Treatment with paclitaxel given intraperitoneally is attractive in patients with ovarian carcinoma, since paclitaxel has proven single-agent activity in this disease.[184] With this route of drug administration, the presence of CrEL as an integral component of the clinical formulation may actually be advantageous as it prolongs exposure to the tumour cells and reduces transport across the peritoneal/blood barrier (Table 8).

Table 8. Influence of Cremophor® EL (CrEL) on the pharmacokinetics of intraperitoneal paclitaxel[a]

Parameter	With CrEL	Without CrEL	p[b]
C_{max} (µmol/L)	0.14 ± 0.08	0.26 ± 0.07	0.062
AUC (µmol•h/L)	5.04 ± 1.92	7.55 ± 3.38	0.044
F (%)	31.4 ± 5.18	98.8 ± 16.6	0.005

a Data were obtained from 4 cancer patients treated in a randomised cross-over setting with paclitaxel administered at a dose of 125 mg/m^2 in the presence and absence of CrEL and represent mean values ± SD; from Gelderblom et al.[123]

b Probability of significant difference versus control (two-sided test for matched pairs).

AUC = area under the plasma concentration-time curve; C_{max} = peak plasma concentration; F = bioavailability.

5. Conclusion

Numerous investigations have studied the role of pharmaceutical vehicles such as CrEL and Tween® 80 in the pharmacological behaviour of the formulated drugs. These investigations have yielded fundamental insight into modes of action, pharmacokinetic profiles and considerations of dosage and scheduling. Indeed, the administration of CrEL and Tween® 80 to patients presents a number of serious concerns, including unpredictable intrinsic adverse effects such as acute hypersensitivity reaction and peripheral neuropathy. Furthermore, these substances modulate the disposition profiles of various drugs using them as vehicles, and of other compounds administered concomitantly, by alteration of the blood

distribution resulting from entrapment of the compound in circulating micelles.

The drawbacks presented by the presence of CrEL or Tween® 80 in drug formulations have instigated extensive research to develop alternative delivery forms, and currently, several strategies are in progress to develop formulations of the anticancer agents docetaxel and paclitaxel that are free from Tween® 80 and CrEL, respectively.(185)

Table 9. Examples of alternative approaches to development of taxane drugs

Strategy	Example(s)	Stage	Reference
Pharmaceutical			
Co-solvents	HSA-paclitaxel	Preclinical (in vivo)	188
Emulsions	S8184	Clinical (phase I)	189
	LDE-paclitaxel	Preclinical (in vivo)	190
Liposomes	LEP	Clinical (phase I)	191
Cyclodextrins	PTX-CYD	Preclinical (in vivo)	192
Nanoparticles	ABI-007	Clinical (phase II)	187,193
Microspheres	Paclimer	Preclinical (in vivo)	194
Chemical			
Analogues	BMS-184476	Clinical (phase II)	195
	BMS-275183 (oral)	Clinical (phase I)	196
	IDN5109/BAY59-8862 (oral)	Clinical (phase I)	197
	RPR 109881A	Clinical (phase II)	198
Prodrugs	DHA-paclitaxel[c]	Clinical (phase II)	199,200
	PNU-166945[d]	Discontinued	201
	CT-2103[e]	Clinical (phase I)	202
Biological			
Oral administration	paclitaxel + cyclosporin	Clinical (phase II)	203

a Poly(ethylene glycol)-human serum albumin-paclitaxel conjugate.
b Cholesterol-rich emulsion that binds to low-density lipoprotein receptors.
c Docosohexaenoic acid-paclitaxel.
d Water-soluble polymeric conjugate of paclitaxel.
e Polyglutamated paclitaxel.

A recent dose-finding study with a new submicronic Tween® 80-free dispersion formulation of docetaxel suggested a lower incidence and severity of haematological and non-haematological toxicity (fluid retention) at equimolar doses compared to the current formulation of docetaxel with

Tween® 80.[186] Likewise, the absence of CrEL in a novel formulation of paclitaxel (ABI-007) permitted drug administration without premedication routinely used for the prevention of hypersensitivity reactions, as well as increases in the maximum tolerable dose as compared with paclitaxel formulated in CrEL.[187] A summary of various approaches currently pursued to eliminate non-ionic surfactants from taxane formulations is provided in Table 9. 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

Disposition of polyoxyethylated excipients in humans: Implications for drug safety and formulation approaches

A.J. ten Tije[a], S.D. Baker[b], K. Dinh[b], W.J. Loos[a], W.D. Figg[c], J. Verweij[a], A. Sparreboom[c]

[a] Department of Medical Oncology, Erasmus MC – Daniel den Hoed Cancer Center, Rotterdam, the Netherlands; [b] Division of Experimental Therapeutics, the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA; [c] Clinical Pharmacology Research Core, National Cancer Institute, Bethesda, MD, USA.

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Introduction

Pharmaceutical excipients have a vital role in drug formulations, a role that has tended to be neglected as evidenced by the lack of procedures to assess excipient safety outside a new drug application process.[1] In contrast to earlier views, excipients are not inert vehicles, but can exert a range of intrinsic adverse effects and have the potential to cause clinically significant drug interactions.[2-4] Polyoxyethylene-20-sorbitan monooleate (polysorbate 80, Tween 80; ICI Group, London, United Kingdom; Figure 1), and polyoxyethylated castor oil (Cremophor EL -CrEL-; BASF, Ludwigshafen, Germany, Figure 1) are widely used as drug formulation vehicles, a.o for the taxanes paclitaxel and docetaxel.[5] Recent experimental data have indicated that that both solubilisers are biologically and pharmacologically active compounds with clinically important side effects including hypersensitivity reactions (HSR)[6-9] and peripheral neuropathy.[10-16]

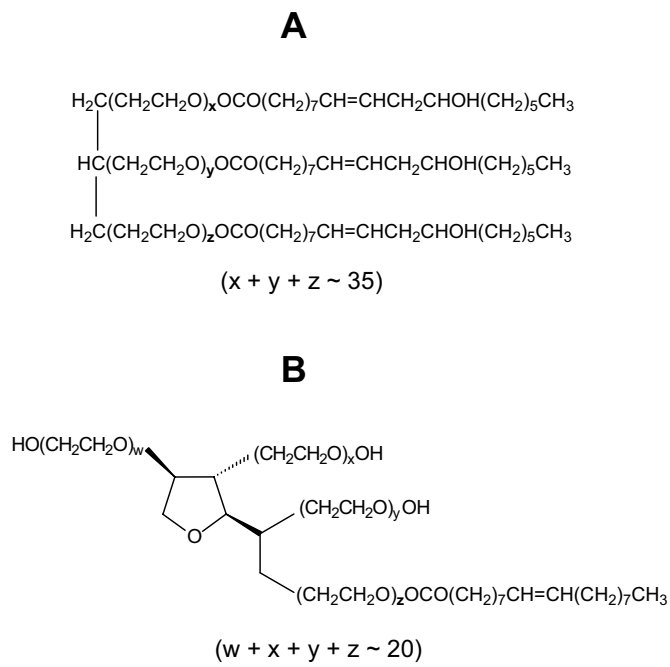

Figure 1. Chemical structures of Cremophor EL (A) and Tween 80 (B)

Both vehicles have been demonstrated to influence the disposition of the solubilised drugs.[17-19] This effect is possibly caused by entrapment of the drug in large spherical micellar structures consisting of the polyoxyethylated vehicles.[2,20] In general this mechanism results in a highly increased systemic drug exposure and a simultaneously decreased clearance.

It was hypothesised that the possibility for excipients to affect drug disposition and toxicity patterns is inversely linked to their rate of elimination.

Here, we performed a comparative pharmacokinetic analysis of these two commonly used and structurally-related excipients with validated and sensitive analytical methods for the determination of both vehicles in human plasma.

Sample Collection and Pharmacokinetic analysis

For Tween 80, samples were obtained from 32 cancer patients treated with a 30-minute or 1-hour infusion of docetaxel (Taxotere; Aventis Pharma, Vitry-sur-Seine Cedex, France; 26.0 mg of Tween 80 per mg of drug) at dose levels ranging from 25 to 75 mg/m^2 (Table 1). For Cremophor EL, samples were obtained from 31 cancer patients treated with a 1-hour infusion of paclitaxel (Taxol; Bristol-Myers Squibb Company, Princeton, NJ; 87.8 mg of CrEL per mg of drug) at dose levels ranging from 70 to 100 mg/m^2 (Table 1). Blood sampling for Tween 80 and CrEL analysis was performed on day 1 of the first chemotherapy course from a vein in the arm opposite to that used for docetaxel or paclitaxel infusion. Blood samples were collected in vials containing lithium heparin up to 48 h after infusion. Immediately after sampling the plasma was separated by centrifugation for 5 min at 3000*g*, transferred to a clean polypropylene tube, and then stored frozen at T<-70°C until the time of analysis.

Tween 80 and CrEL concentrations in plasma were determined by validated assays based on liquid chromatography-tandem mass spectrometry[21] and Coomassie brilliant blue staining,[22] respectively.

In brief, for CrEL aliquots of 25 µL plasma were extracted by addition of subsequently 500 µL acetonitrile and 2 mL *n*-butylchloride, followed vigorous vortex-mixing. After centrifugation, the organic layer was evaporated to dryness and the residue was redissolved in 50 µL water, from which an aliquot of 25 µL was transferred into a 96-well plate. After addition of 250 µL of 5-fold water diluted-diluted Coomassie Briliant Blue G-250, detection of

Table 1. Summary of pharmacokinetic data

Excipient	Excipient dose* (mg/m^2)	C_{max} (mg/ml)	AUC (mg.h/ml)	CL (L/h)	V_{ss} (L)	$T_{1/2,z}$ (h)	N
Tween 80	650 (25)	0.139 ± 0.0174	0.200 ± 0.0799	7.63 ± 2.72	8.30 ± 3.72	0.640 ± 0.071	3
	780 (30)	0.160	0.213	8.34	4.56	0.421	1
	910 (35)	0.181 ± 0.0645	0.180 ± 0.0417	8.58 ± 1.63	7.63 ± 2.44	0.568 ± 0.114	4
	1300 (50)	0.302 ± 0.0516	0.385 ± 0.133	6.53 ± 2.03	6.25 ± 1.93	0.739 ± 0.328	7
	1560 (60)	0.300 ± 0.118	0.286 ± 0.0650	10.2 ± 3.14	5.64 ± 2.45	0.386 ± 0.143	5
	1950 (75)	0.457 ± 0.233	0.576 ± 0.252	7.27 ± 3.22	2.87 ± 1.47	0.629 ± 0.246	19
Cremophor EL	6150 (70)	2.48	40.8	0.219	5.05	16.0	1
	7025 (80)	2.51 ± 0.332	70.4 ± 28.6	0.196 ± 0.073	8.08 ± 1.09	32.2 ± 14.7	7
	8780 (100)	2.98 ± 0.647	85.8 ± 38.5	0.216 ± 0.075	9.48 ± 2.59	35.7 ± 18.9	23

Mean values ± standard deviation

C_{max} peak plasma concentration; AUC, area under the plasma concentration-time curve extrapolated to infinity;

CL, 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.

* Data were obtained from cancer patients treated with docetaxel formulated in Tween 80 or paclitaxel formulated in Cremophor EL; between brackets the dose of docetaxel and paclitaxel.

the complex formation between CrEL and Coomassie Briliant Blue G-250 was performed by UV measuring the ratio of absorbances at 595 nm over 450 nm. The lower limit of quantitation was established at 0.05% (v/v; ~525 µg/mL).

Since a lower limit of quantitation for Tween 80 in the same range was insufficient for pharmacokinetic analysis, a new analytical method was developed based on liquid chromatography coupled to mass spectrometry. Aliquots of 1 mL plasma were extracted, after the addition of the internal standard paclitaxel, with 7 ml of a mixture of acetonitril-*n*-butylchloride (1:4, v/v). After vigorous vortex-mixing and centrifugation, the organic layer was evaporated to dryness. The residue was subsequently redissolved in 100 µL of a mixture of methanol-water (1:1, v/v), from which an aliquot of 10 µL were injected into the HPLC system. The analytes were separated on a Waters X-Terra MS column packed with ODS material, and eluted with methanol-water (9:1, v/v) containing 0.1% formic acid.
The column effluent was monitored using a triple-quadruple mass spectrometric detector, equipped with an electrospray probe, resulting in a lower limit of quantitation of 1 µg/mL.

Data were evaluated by standard non-compartmental analysis using *WinNonLin 4.0 using 1/y weighing* (Mountain View, CA, USA).

Statistical Considerations

The correlation between peak plasma concentrations of Tween 80 and CrEL and the administered dose level or the corresponding AUC values were analysed by means of Pearson's correlation coefficient, and linear regression analysis. Interpatient differences in PK parameters were assessed by the coefficient of variation, expressed as the ratio of the SD and the observed mean. Variability in PK parameters between the various dose levels was evaluated by the Wilcoxon signed-rank test and the Kruskal-Wallis statistic followed by Dunn's multiple comparison test, respectively.

Results

Pharmacokinetic studies were completed in 32 patients treated with docetaxel, and 31 patients treated with paclitaxel in several pharmacokinetic trials. Both taxanes were administered as single agent. All patients had a histologically confirmed solid malignancy suitable for single agent treatment with docetaxel or paclitaxel, or for whom no other treatment options existed.

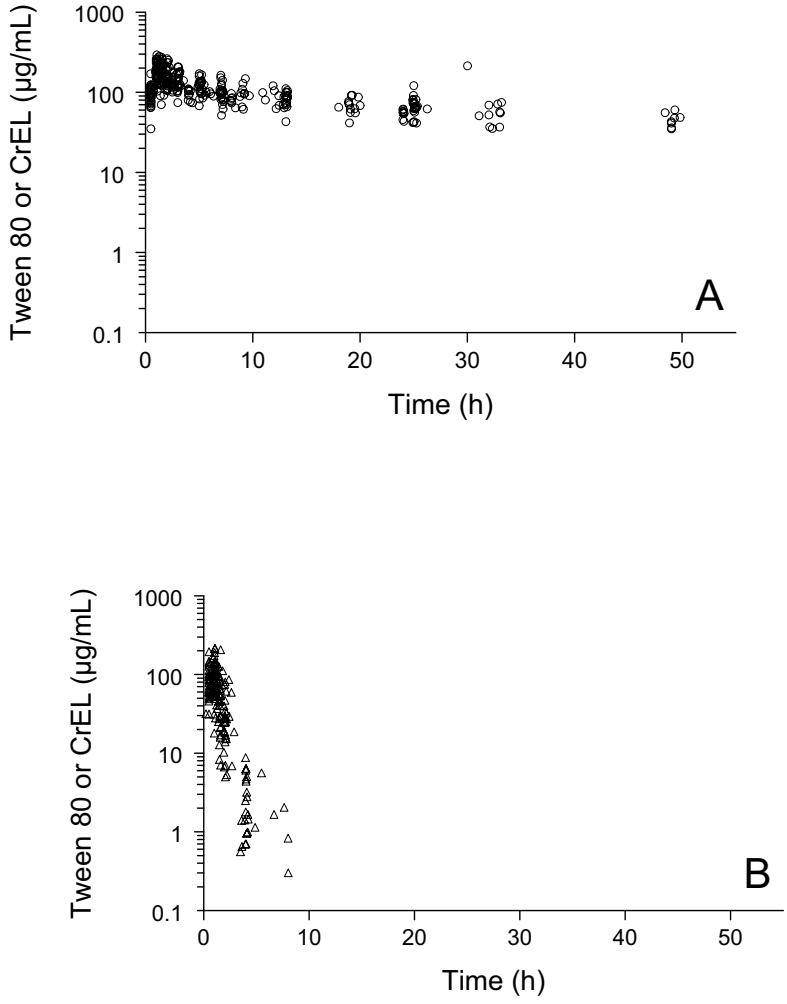

Figure 2. Pharmacokinetic profiles of CrEL (A) and Tween 80 (B) after dose adjustments to 1000 mg absolute dosing.

Measures of exposure to Tween 80 and Cremophor EL increased in near proportion with an increase in dose (Table 1), and clearance was independent of infusion duration (Tween 80, P = .298) and administered dose in the range studied (Tween 80, P = .355; Cremophor EL, P = .797). The plasma concentration versus time profiles of both Tween 80 and CrEL after

taxane infusion were similar for all patients in the separate groups. The disappearance of Tween 80 from the central compartment was characterised by a short terminal half-life with a mean (± standard deviation) value of 0.607 ± 0.245 hours and a total plasma clearance of 7.70 ± 2.90 L/h (Figure 2). In contrast, elimination of Cremophor EL was significantly slower, with values for half-life and clearance of 34.2 ± 17.9 hours and 0.211 ± 0.072 L/h, respectively (P < .00001) (Figure 2). The volume of distribution at steady-state was similar for Tween 80 and Cremophor EL (4.78 ± 2.76 L versus 9.02 ± 2.46 L, respectively), indicating limited distribution of both excipients outside the central compartment, as was suggested previously[23].

Discussion

Despite the widespread and long-term use of Tween 80 as a formulation vehicle for several IV drugs, such as etoposide and docetaxel, pharmacokinetic data on this vehicle are sparse.[16,24,25] Recently a new simple method for the quantitative determination of Tween 80 in human plasma by using liquid chromatography-tandem mass spectrometry was developed.[21] This novel technique permitted us to compare the pharmacokinetics of Tween 80 to CrEL to gain insights in the toxicity patterns of both vehicles.

Overall, the results from this study suggest that the relative systemic exposure to Tween 80 in humans is much lower as compared to Cremophor EL, as a result of different rates of elimination. In vitro experiments have shown that the rapid elimination of Tween 80 is caused by a rapid carboxylesterase-mediated hydrolysis in the systemic circulation, cleaving the oleic acid side chain from the molecule.[24] Little is know about elimination routes of CrEL. Possibly, the elimination pathways for CrEL are also determined by serum carboxylesterase-induced degradation. This route apparently is involved at a low rate, most likely caused by a lower affinity of CrEL for carboxylesterases compared to Tween 80 and the rapid forming of micellar complexes, which explains the typical time-dependent non-linear pharmacokinetics of CrEL.

This difference in elimination rate is consistent with studies reporting that the use of Cremophor EL as a formulation vehicle is more likely to result in excipient-related toxic side effects than Tween 80,[26] including hypersensitivity reactions[9] and neuropathy.[16] The recognition of the slow clearance of CrEL compared to Tween 80 also has implications for the

clinical use of CrEL containing drugs with respect to combination chemotherapeutic regimens. Several studies demonstrated the influence of CrEL on the pharmacokinetic and –dynamic profile of co-administered drugs, such as doxorubicin[19,27,28] and cisplatin.[29-31] It is proposed that proper pharmacokinetic evaluation is needed as an integral component of the preclinical screening package for new excipients, and to enable a more rational approach in the development of formulations for poorly water-soluble agents.

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Chapter 4

Effect of valspodar on the pharmacokinetics of unbound paclitaxel

A.J. ten Tije[1], T.W. Synold[2], D. Spicer[3], J. Verweij[1], J.H. Doroshow[2], A. Sparreboom[1]

[1]Department of Medical Oncology, Erasmus MC - Daniel den Hoed Cancer Center, Rotterdam, the Netherlands; [2]City of Hope Cancer Center, Duarte, CA; [3]USC/Norris Comprehensive Cancer Center, Los Angeles, CA

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Summary

The aim of this multicenter study was to determine whether valspodar (Amdray™; code designation, SDZ PSC 833), a potent P-glycoprotein (P-gp) inhibitor, affects the pharmacokinetics of unbound paclitaxel (C_u). Data were obtained from 31 patients with advanced breast cancer. Thirteen patients were treated with paclitaxel alone (3-h infusion at 175 mg/m^2) and another 18 received paclitaxel (3-h infusion at 70 mg/m^2) in combination with a 21-day cycle of oral valspodar (5 mg/kg given four times a day) starting 1 day before administration of paclitaxel. Serial blood samples were taken in the first course and C_u in plasma determined using equilibrium dialysis with a [G-^{3}H]paclitaxel tracer. The apparent clearance of C_u was not significantly different between the two groups, with mean ± standard deviation (± SD) values of 230 ± 56.0 and 202 ± 49.9 L/h/m^2 in the absence and presence of valspodar, respectively (P = 0.17). The volume of C_u distribution was slightly larger in the presence of valspodar (1160 ± 474 vs 1620 ± 552 L/m^2; P = 0.025), which contributed to a minor difference in the terminal disposition half-life (6.12 ± 3.42 vs 8.50 ± 2.06 h; P = 0.028). These data indicate that (i) valspodar lacks the significant interaction with paclitaxel observed previously with other P-gp modulators, (ii) the majority of the increased toxicity of the combination does not appear to be attributable to increased levels of C_u, and (iii) provide further evidence of the conjecture that the plasma concentration of paclitaxel may not be an appropriate measure to monitor the impact of P-gp inhibition.

Introduction

Resistance of malignant cells to various classes of anticancer drugs has been linked to the mechanism of multidrug resistance (MDR). This MDR phenotype is associated with the overexpression of a drug-transporting P-glycoprotein (ABCB1; P-gp), a member of the family of adenosine triphosphate (ATP) binding cassette transporters, which also includes multidrug resistance associated protein [MRP1 (ABCC1)], its homologues [MRP2 (ABCC2) to MRP9 (ABCC12)], and the breast-cancer resistance protein [BCRP (ABCG2)]. These proteins act as a cellular drug-efflux pump transporting many naturally-occurring cytotoxic agents, including paclitaxel.[1] Studies performed over the last several years have shown that intrinsic and acquired expression of P-gp might play an important role in

clinical drug resistance in specific solid tumors and hematological malignancies.[2] Consequently, numerous clinical studies have been performed to develop inhibitors of P-gp with the aim to overcome resistance to the anticancer agents. Unfortunately, the majority of the studied P-gp inhibitors have shown substantial pharmacokinetic interactions with the coadministered anticancer agent, characterized by a considerable decrease in systemic clearance, which resulted in a need to reduce the dose of the anticancer agent because of exacerbated toxicity.[3] This dose reduction not only complicated the interpretation of toxicity and response data, but also presented a serious obstacle in the development and rational use of P-gp inhibitors. It is now evident that the pharmacokinetic interference between anticancer drugs and P-gp inhibitors is due primarily to competition for drug metabolizing enzymes. Indeed, a wealth of recent experimental data shows that many of the previously tested P-gp inhibitors, including verapamil,[4] cyclosporin A,[5] and valspodar (Amdray™; code designation, SDZ PSC 833),[6] are prototypical substrates and/or potent inhibitors of CYP3A4. Previous work has shown that valspodar, a nonimmunosuppressive and nonnephrotoxic cyclosporin D analogue, is approximately 10-fold more potent as a P-gp inhibitor than cyclosporin A.[7] It has also been documented that valspodar significantly alters the clinical pharmacokinetics of various agents including etoposide[8] and doxorubicin.[9] Here, we studied the comparative pharmacokinetics of unbound paclitaxel, a known partial substrate of cytochrome P450 isozyme 3A4 (CYP3A4),[10] given as a 3-h intravenous (i.v.) infusion to two separate conhorts of patients receiving paclitaxel alone or in the presence of oral valspodar.

Patients and methods

Eligibility

Patients with a histological confirmed diagnosis of breast cancer with proven metastasis for whom paclitaxel monotherapy was a viable therapeutic option or for whom other treatment options were not available could enter this study. Additional eligibility criteria included (*i*) age >18 years; (*ii*) World Health Organization performance status <3; (*iii*) life expectancy of at least 3 months; (*iv*) off previous anticancer therapy for at least 4 weeks; (*v*) no previous treatment with taxanes or intensified chemotherapy with stem cell support; (*vi*) adequate bone marrow function (absolute neutrophil count >1.5 $\times$ 10^9/L, and platelet count >100 $\times$ 10^9/L), renal function (serum creatinine <2 $\times$ upper limit of normal), and liver function (normal bilirubin; aspartate

and alanine aminotransferases, and alkaline phosphatase <2.5 × upper limit of normal); (*vii*) symptomatic peripheral neuropathy graded ≤2 (according to the National Cancer Institute common toxicity criteria); and (*viii*) off any medication known to interfere with paclitaxel pharmacokinetics. Written informed consent was obtained from all patients, and the study was approved by the Ethics Board of the participating institutions. Clinical and toxicological profiles have been reported elsewhere.[11]

Drug administration

Paclitaxel (Bristol Myers Squibb, Wallingford, CT) was supplied as a concentrated sterile solution in a mixture of Cremophor EL (CrEL)-ethanol (1:1, v/v) at 6 mg/ml (Taxol). The drug was administered intravenously over 3 h at a dose of 175 mg/m^2 to one cohort of patients or at 70 mg/m^2 when administered in combination with oral valspodar in another cohort of patients. The latter patients were concomitantly treated with valspodar tablets (Novartis Pharmaceutical Corporation, East Hanover, NJ) at a daily dose of 5 mg/kg given four times a day for 21 days, starting 1 day prior to the administration of paclitaxel. All patients received pre-medication with dexamethasone (8 mg twice daily), starting 12 h prior to infusion of paclitaxel and continuing 3 days thereafter. Diphenhydramine (50 mg) and ranitidine (50 mg) were routinely administered intravenously 1 h before paclitaxel infusion. Patients were eligible to continue treatment until there was evidence of progressive disease.

Sample collection and processing

Blood specimens were obtained from all patients only during the first treatment cycle. Sample volumes of 6 ml were drawn directly from a peripheral venous access device into tubes containing lyophilized sodium heparin as anticoagulant. The samples were collected directly before infusion, and at 0.5, 1, 2, 3, 3.25, 3.5, 4, 5, 6, 9, 15, and 27 h after start of infusion. All samples were centrifuged immediately for 10 min at 1000g to separate plasma, which was stored at –20°C in polypropylene vials until analysis.

Drug analysis

The unbound fraction of paclitaxel (fu) was measured by equilibrium dialysis as described earlier.[12] The concentrations of total paclitaxel in plasma (C_p) were determined by isocratic reverse-phase high-performance liquid chromatography with ultraviolet detection (230 nm), as described.[13]

Unbound paclitaxel (C_u) was estimated from the product of C_p and fu in each individual pharmacokinetic sample, including the blank sample. The analytical procedure for CrEL in plasma samples was based on a colorimetric dye-binding micro assay using Coomassie Brilliant Blue G-250,[14] with modifications as described.[15]

Pharmacokinetic analysis

Paclitaxel concentration-time profiles of unbound and total drug in plasma were analyzed using the Siphar V4.0 package (Innaphase, Philadelphia, PA) by determination of slopes and intercepts of the plotted curves with multi exponential functions. The program determined initial parameter estimates, and these were improved using an iterative numerical algorithm based on Powell's method. Model discrimination was assessed by a variety of considerations including visual inspection of the predicted curves, dispersion of residuals, minimization of the sum of weighted squares residuals, and the Akaike information criterion. Final values of the iterated parameters of the best-fit equation were used to calculate pharmacokinetic parameters, including the disposition half-lives, area under the plasma concentration-time curve (AUC) from time zero and extrapolated to infinity using the terminal rate constant, and clearance (defined as dose divided by AUC). The peak concentration was put on par with the observed drug level at the end of infusion. Threshold concentrations for paclitaxel total and unbound drug, that is, the time that plasma concentrations remain higher than 0.05 and 0.0167 μM, respectively, were determined as described previously.[16] Noncompartmental analysis of CrEL plasma concentration data was performed as described previously.[17]

Statistical considerations

Pharmacokinetic parameters of paclitaxel and CrEL are reported as mean values ± standard deviation (±SD), unless stated otherwise. An unpaired (two-sided) Student's *t*-test was used to evaluate statistical significance between the two treatment groups using the NCSS V5.X package (J.L. Hinze, East Kaysville, UT; 1992). Probability values of <0.05 were regarded as statistically significant.

Results

Patient characteristics

A total of 31 patients with measurable or evaluable metastatic breast cancer was studied, and all patients were pharmacokinetically evaluable. Paclitaxel was administered as single agent (175 mg/m^2) to 13 patients, and another 18 patients received the combination treatment of paclitaxel (70 mg/m^2) with oral valspodar. Patient characteristics and baseline clinical chemistry values were similar between the two patient groups (Table 1).

Table 1. Patient demographics

Characteristics	PAC		PAC/valspodar	
No. of patients	13		18	
Age (years)	42	(29-63)	51	(36-68)
Weight (kg)	65	(40-84)	68	(48-117)
BSA (m^2)	1.69	(1.28-1.86)	1.71	(1.42-2.17)
Paclitaxel dose (mg)	295	(224-326)	118	(99-152)
Performance score	0	(0-2)	0	(0-1)
Serum creatinine (mg/dl)	0.7	(0.6-0.9)	0.7	(0.2-1)
Total bilirubin (mg/dl)	0.5	(0.3-1.2)	0.5	(0.3-0.8)
ASAT (units/L)	23.5	(17-47)	22.5	(13-75)
ALAT (units/L)	26.5	(11-58)	30.0	(10-59)
Total protein (g/dl)	7.5	(6.5-8.9)	7.3	(6.9-9.0)
Serum albumin (g/dl)	4.1	(3.2-4.6)	4.0	(3.3-4.6)

Abbreviations: PAC: paclitaxel; BSA: body-surface area; ASAT: aspartate amino-transferase; ALAT: alanine aminotransferase.

Paclitaxel disposition

A summary of model-independent parameter estimates for paclitaxel pharmacokinetics is shown in Table 2. This analysis revealed that the times that paclitaxel concentrations remained greater than the toxicity thresholds of 0.016 μM (unbound drug) and 0.05 μM (total drug) were similar in the presence and absence of valspodar in spite of the reduced dose of paclitaxel in the former group (Table 2).

Table 2. Noncompartmental pharmacokinetics of paclitaxel (mean ± SD with range)

Parameter	PAC	PAC/valspodar
No. of patients	13	16
Dose (mg/m^2)	175	70
Unbound paclitaxel		
C_{max} (µM)	0.230 ± 0.116 (0.057-0.543)	0.105 ± 0.072 (0.048-0.357)
$AUC_{0-\infty}$ (µM h)	0.836 ± 0.321 (0.257-1.58)	0.427 ± 0.113 (0.257-0.672)
$T_{>0.0164}$ (h)	7.89 ± 2.56 (5.56-14.61)	5.11 ± 1.32 (3.43-8.08)
Total paclitaxel		
$AUC_{0-\infty}$ (µM h)	15.9 ± 7.01 (2.95-31.4)	4.79 ± 0.84 (3.35-6.72)
$T_{>0.05}$ (h)	25.4 ± 6.37 (7.30-35.5)	21.7 ± 5.49 (11.8-28.6)

Abbreviations: PAC: paclitaxel; C_{max}: peak plasma concentration; AUC: area under the plasma concentration versus time curve; $T_{>0.0167}$: time above the unbound paclitaxel concentration thresholf of 0.0164 µM; $T_{>0.05}$: time above the total paclitaxel concentration threshold of 0.05 µM.

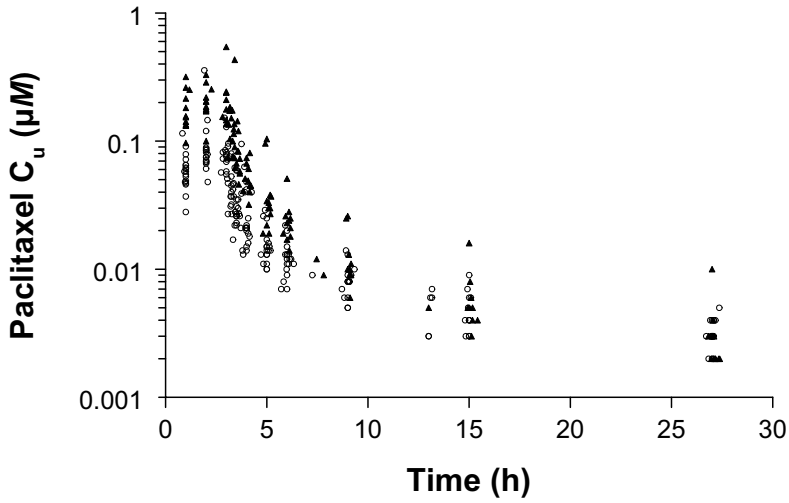

Figure 1. Observed plasma concentrations of unbound paclitaxel in patients receiving paclitaxel alone (175 mg/m^2; triangles) or paclitaxel (70 mg/m^2; circles) in combination with valspodar.

The plasma concentration-time profiles of unbound paclitaxel given alone or in combination with valspodar could be best described with a linear three-compartment model, in agreement with previous findings.[18,19] The observed unbound drug concentrations in both groups are shown in Figure 1. The apparent clearance of unbound paclitaxel was not significantly different between the two groups, with values of 230 ± 56.0 vs 202 ± 49.9 L/h/m^2 in the absence and presence of valspodar, respectively (P = 0.17) (Table 3). The volume of distribution of unbound paclitaxel was larger in the presence of valspodar (1160 ± 474 vs 1620 ± 552 L/m^2; P = 0.001), which contributed to a significant difference in the terminal disposition half-life (6.12 ± 3.42 vs 8.50 ± 2.06 h; P = 0.028).

Table 3. Compartmental parameter estimates of unbound paclitaxel (mean ± SD with range)

Parameter	PAC	PAC/valspodar	P*	Diff (± SE)**	95% CL
CL (L/h/m^2)	230 ± 56.0 (122-301)	202 ± 49.9 (126-305)	0.17	-28.0 ± 19.7	-68.4 | 12.4
MRT (h)	5.03 ± 1.34 (3.56-8.74)	8.15 ± 2.07 (2.50-12.3)	0.001	3.12 ± 0.67	1.75 | 4.49
Vd (L/m^2)	1160 ± 474 (531-2510)	1620 ± 552 (266-2470)	0.025	461 ± 194	63.3 | 858
$T_{1/2,z}$ (h)	6.12 ± 3.42 (2.84-17.0)	8.50 ± 2.06 (2.94-11.6)	0.028	2.38 ± 1.03	0.28 | 4.49

Abbreviations: PAC: paclitaxel; CL: apparent plasma clearance; MRT: mean residence time; Vd: steady-state volume of distribution; $T_{1/2,z}$: terminal disposition half-life.

* Unpaired (two-tailed) Student's t-test; ** mean difference ± standard error with the 95% confidence limits for the mean difference.

CrEL concentrations

Substantial interindividual variability in apparent clearance of CrEL was observed, with overall mean values of 267 ± 107 *vs* 294 ± 165 ml/h/m^2 (Table 4 and Figure 2), consistent with previous findings.[20]

Table 4. Plasma levels of CrEL (mean ± SD with range)

Parameter	PAC	PAC/valspodar
No. of patients	13	18
Dose (ml/m^2)	14.6	5.83
C_{max} (µM)	4.15 ± 1.42 (1.51-7.31)	2.22 ± 1.85 (1.08-7.58)
AUC_{0-t} (µL.h/mL)	59.9 ± 21.3 (11.2-85.9)	17.4 ± 7.11 (8.17-31.6)
CL (ml/h/m^2)	267 ± 107 (170-520)	294 ± 165 (185-714)

Abbreviations: PAC: paclitaxel; C_{max}: peak plasma concentration; AUC: area under the plasma concentration versus time curve; CL: apparent plasma clearance.

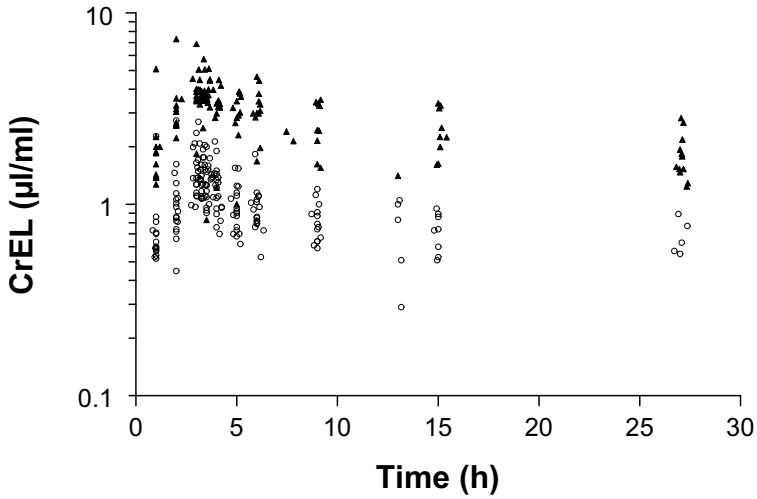

Figure 2. Observed plasma concentrations of CrEL in patients receiving paclitaxel alone (175 mg/m^2; triangles) or in combination with valspodar (70 mg/m^2; circles).

Discussion

In the current study, we obtained clinical pharmacokinetic data that increase our insight into the role of P-gp in paclitaxel disposition as well as the effects of a potent P-gp inhibitor and CYP3A4 substrate on the pharmacokinetics of paclitaxel. Overall, our data indicate that the clearance of unbound paclitaxel, following administration as a 3-h i.v. infusion, is not significantly altered by oral valspodar. Previously, a phase I trial combining

oral valspodar with paclitaxel demonstrated a maximum-tolerated dose of the anticancer agent of 70 mg/m^2 when given as a 3-h i.v. infusion.[21] Interestingly, this ~60% reduced dose for equivalent degrees of myelosuppression was not accompanied by any significant alteration in total paclitaxel plasma clearance.[22] Unfortunately, a clear-cut interpretation of the magnitude of the pharmacological interaction between paclitaxel and valspodar has been hampered by a number of important issues, including (*i*) the concomitant administration of other cytotoxic agents, including carboplatin[23] and doxorubicin,[24] (*ii*) the lack of a control group of patients treated without valspodar,[23] (*iii*) and/or variability and unpredictability of kinetic profiles associated with 96-h continuous paclitaxel-infusion regimens.[25] In addition, the data generated in these trials have without exception been based on comparison of two different dose groups in patients treated either with or without valspodar administration. This latter issue is of particular importance for paclitaxel in view of the profound nonlinear drug disposition in plasma,[26,27] which suggests that the pharmacological consequences of the combination treatment can not be predicted based on total plasma levels alone when different dose groups are compared. Since the AUC of unbound paclitaxel (i.e. not bound to serum proteins, its formulation vehicle CrEL or other macromolecules in the systemic circulation) is a linear function of the paclitaxel dose administered,[12,16] we focused here on comparing the fraction-unbound paclitaxel between the two treatment groups. This was made possible by the recent development of a novel robust and validated technique to measure unbound drug levels,[12] allowing for a better understanding of the clinical pharmacology of paclitaxel.[28]

Indeed, the nonlinear pharmacokinetic behavior of total plasma concentrations of paclitaxel in cancer patients has been demonstrated in a number of studies. Although the exact mechanism underlying this nonlinear disposition has not yet been fully elucidated, the presence of CrEL, used as formulation vehicle of the clinical paclitaxel preparation, is thought to play a principal role in this process.[29] This phenomenon is most likely associated with micellar entrapment of paclitaxel in the systemic circulation, thereby reducing the cellular accumulation of paclitaxel in blood cells, and thereby potentially altering drug distribution, metabolism and excretion pathways.[30] Similar to data obtained in clinical trials, it has also been shown previously that CrEL has a remarkable influence on the disposition of paclitaxel in mice.[31] An intriguing observation from these murine studies has been that the effect of *mdr1a* P-gp on the plasma pharmacokinetics of paclitaxel was not observed with a drug formulation vehicle containing CrEL, whereas its

impact on concurrent tissue concentrations, including heart and brain was clearly identifiable.[32] Thus, although plasma is usually the only biological matrix available from patients, these findings suggest that the total plasma concentration of paclitaxel may not be an appropriate measure to monitor the impact of P-gp inhibition by the use of modulating agents. This is also in keeping with previous knowledge from data generated in clinical trials of combined treatment with the related agent docetaxel and the P-gp inhibitor R101933 indicating unaltered drug clearance of the taxane with either oral[33] or i.v. dosing of the modulator,[34] whereas fecal elimination pathways were significantly altered. Despite these observations, recent work on the combination of paclitaxel with valspodar still applied the total plasma concentration of paclitaxel to study the potential for pharmacokinetic interactions.

Consistent with previous observations,[22] this study demonstrated that the systemic exposure (AUC) to unbound paclitaxel was unaffected when combined with valspodar. The difference in AUC between the two treatment groups in Table 2 are explained by the different dosage of paclitaxel. In contrast, valspodar had a pronounced effect on the duration of time that the total plasma concentrations of paclitaxel remained greater than the toxicity threshold level of 0.05 μM, which was not significantly different in spite of the 60% dose reduction in the presence of valspodar. As paclitaxel elimination is almost entirely caused by metabolic breakdown through CYP3A4 and CYP2C8 isoforms,[35] this may relate to interference by valspodar of CYP3A4-mediated paclitaxel metabolism causing changes in the terminal disposition phase. Indeed, Kang et al.[36] have shown that inhibition of CYP3A4 by valspodar increases the plasma concentrations of 6α-hydroxypaclitaxel, the formation of which is dependent on CYP2C8 activity.[37] Although the mechanistic explanation for this effect has not been conclusively elucidated, it is plausible that inhibition of one of two principal metabolic routes results in shunting of parent drug to alternative metabolites. However, other possible mechanisms, including altered enterohepatic recirculation through inhibition of intestinal P-gp or by promoting valspodar-induced cholestasis, cannot be excluded.[36]

It is likely that the observed differences in the terminal phase in unbound concentrations between treatment with or without valspodar are likewise related to changes in drug metabolism. As an alternative explanation for the altered terminal disposition phase, we considered a possible influence of valspodar on CrEL pharmacokinetics. However, the apparent clearance of CrEL was not significantly altered by valspodar, ruling

out this possibility as a potential contributing source. In this context, it is worth mentioning that since the apparent clearance of CrEL increases significantly with prolonged duration of the paclitaxel infusion, the systemic exposure to unbound paclitaxel is also schedule dependent.[18] For example, the AUC of unbound paclitaxel was substantially reduced following doses of 100 mg/m^2 administered as a 1-h i.v. infusion as compared to a 3-h i.v. infusion (0.50 ± 0.10 vs 0.62 ± 0.12 μM.h; P = 0.009). This suggests that the effect of valspodar on paclitaxel elimination may depend on the duration of drug infusion. In addition, it provides, at least in part, an explanation for the extensive effects of valspodar on circulating concentrations of 6α-paclitaxel with 96-h i.v. infusions,[36] when concomitant levels of CrEL are expected to be very low, as compared to those observed after 1-h i.v. infusions.[24]

As outlined, the exposure-toxicity relationships of paclitaxel in cancer patients have been most commonly described with a threshold model, although other models have been proposed, including those using a general model for the time-dissociated effects.[38,39] According to the threshold models, the severity of neutropenia is related to the duration of exposure above a certain threshold concentration, such that a duration of 17.4 h above 0.05 μM (total paclitaxel)[26] or 11.3 h above 0.0164 μM (unbound paclitaxel)[16] were predicted to yield a 50% decrease in absolute neutrophil count. Based on this kind of modeling exercise, it has been proposed by various investigators that the reduction in paclitaxel dose required when administered in combination with valspodar is directly attributable to the altered values for the threshold duration.[22-25] More recent work, however, has shed light on some important mechanistic aspects of paclitaxel-induced myelosuppression, and has clearly indicated the importance of unbound paclitaxel AUC as a pharmacokinetic parameter to delineate exposure-toxicity relationships, both with 1-h[28] and 3-h infusion regimens.[16] Since our data indicate unchanged clearance of unbound paclitaxel by valspodar, alternative mechanisms, in addition to changes in pharmacokinetics, contributing to the exacerbated toxicity cannot be excluded. More specifically, a direct pharmacodynamic effect of valspodar, by interacting with P-gp, might increase the cellular uptake and retention of paclitaxel in subpopulations of normal peripheral blood and bone marrow cells.[40] For example, recent work from Tidefelt et al.[41] has provided very compelling evidence that only patients with P-gp positive leukemia showed a significantly increased ratio of daunorubicin AUC in the hematopoietic cells to the exposure in plasma after the start of valspodar administration. This strongly supports the hypothesis that valspodar can cause an increased

intracellular accumulation of P-gp substrate drugs, not only by its effect on plasma pharmacokinetics, but also by interacting directly with P-gp expressing cells. Furthermore, pharmacodynamic modeling studies with data obtained from patients treated with high dose cyclosporin A and etoposide suggest that a certain degree of the enhanced myelotoxicity observed in this regimen is attributable to inhibition of P-gp in bone marrow precursor cells.[42] Clearly, this would be consistent with the *in vitro* observations where valspodar was shown to enhance the toxicity to and modulates cellular accumulation of paclitaxel in normal (human) cellular systems expressing P-gp.[43] Hence, it is hypothesized that due to P-gp inhibition by valspodar, the intracellular accumulation and retention of paclitaxel in bone marrow precursor cells is prolonged, thus leading to extensive myelosuppression when the two drugs are given concomitantly. This increase in toxicity requires dose reductions of the concomitantly administered paclitaxel and other chemotherapeutic agents, possibly leading to a less effective cytotoxic treatment.

Collectively, our study demonstrates that a linear three-compartment model best described the unbound paclitaxel concentration-time profiles, and that the apparent clearance of unbound paclitaxel is not significantly different in the absence and presence of valspodar. This indicates that valspodar lacks the profound interaction with paclitaxel observed previously with other modulators, including cyclosporin A and verapamil.[3] The increased toxicity of the combination regimen appears to be, at least partially, attributable to inhibition of P-gp function in bone marrow precursor cells and not solely to pharmacokinetic interactions resulting in increased levels of (unbound) paclitaxel, as suggested previously. In view of our current findings, it is concluded that the total plasma concentration of paclitaxel is not an appropriate measure to monitor the impact of P-gp inhibition.

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Chapter 5

Vascular binding limits cerebrospinal fluid penetration of docetaxel

A.J. ten Tije[1], W.J. Loos[1], M. Zhao[2], S.D. Baker[2], R.H. Enting[3], H. van der Meulen, J. Verweij[1], A. Sparreboom[4]

[1]Department of Medical Oncology, Erasmus MC - Daniel den Hoed Cancer Center, Rotterdam, the Netherlands; [2] Division of Experimental Therapeutics, The Sydney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore MD 21231, USA; [3]Department of Neurology, Erasmus MC - Daniel den Hoed Cancer Center, Rotterdam, the Netherlands; [4] Clinical Pharmacology Research Core, Medical Oncology Clinical Research Unit, National Cancer Institute, Bethesda MD 20892, USA

Anticancer Drugs; in press

Abstract

Purpose: To investigate the cerebrospinal fluid (CSF) penetration of docetaxel in cancer patients.

Methods: Docetaxel was administered as a 1-h infusion at a dose of 75 mg/m^2 to 2 patients with metastatic breast cancer and leptomeningeal carcinomatosis. CSF samples were obtained using a lumbar puncture up to a 72-h time period. Total and unbound docetaxel concentrations in plasma and CSF were determined by liquid chromatography (lower limit of quantitation, 0.5 nM for plasma and 0.050 nM for CSF) and equilibrium dialysis, respectively. *Results:* The pharmacokinetics of docetaxel in plasma are in line with data of previous studies. The concentrations of docetaxel in CSF did not follow the general pattern in plasma, with relatively stable concentrations over the 72-h time period. The fraction unbound docetaxel in plasma ranged from 6 to 13%, while those in CSF ranged from 67 to 103%. For total and unbound docetaxel, the CSF to plasma concentration ratio progressively increased in 72 h from 0.01% to 0.6%, and from 0.1% to 9%, respectively.

Conclusions: These data suggest that measurement of unbound docetaxel is required to accurately assess the extent of drug penetration into CSF, and that the drug can produce distribution to CSF at levels associated with significant antitumor activity in experimental models.

Introduction

Knowledge on the penetration of anticancer drugs into the central nervous system is essential for tumor targets localized within the brain. The pharmacodynamics of the drug in the central nervous system depends largely on the concentration-time profile at the site of action. This concentration-time profile is determined by several factors, including transport across the blood-brain barrier and the blood-cerebrospinal fluid barrier.[5] For obvious reasons, direct measurement of drug concentrations in the brain tissue of cancer patients is highly restricted. Hence, in the clinical setting, drug concentrations in cerebrospinal fluid (CSF) are commonly used as a surrogate for drug concentrations in the brain.[5] However, the brain consists of multiple compartments and many factors, such as the presence of drug-transporting proteins and disruption of the blood-brain barrier by tumor cells, are involved in the process of altering the transport of drugs to these compartments. In the current study, CSF concentrations of docetaxel

were used to evaluate the extent of drug delivery to the brain and in particular the meninges in 2 patients with metastatic breast cancer, because docetaxel is a commonly used drug in this malignancy,[8] which is frequently associated with leptomeningeal carcinomatosis.[4]

Patients and methods

Patients and treatment

Pharmacokinetic studies were performed on 2 patients treated for metastatic breast cancer with single-agent docetaxel. The patients received docetaxel (Taxotere; Aventis, Hoevelaken, the Netherlands) as a 1-h infusion at a dose of 75 mg/m^2 (absolute dose, 120 and 125 mg, respectively). Both patients had a World Health Organization performance score < 2; normal kidney function (serum creatinine < 130 μmol/l); adequate hepatic function [total serum bilirubin < 1.5 x upper limit of institutional normal (ULN); transaminases < 2 times ULN; and alkaline phosphatase < 2 times ULN]; and adequate bone marrow function (absolute neutrophil count > 1.5×10^9/liter, and platelet count > 100×10^9/liter). Both patients gave written informed consent, and the Ethics Board of the Erasmus MC (Rotterdam, the Netherlands) approved the study.

Sample collection

Blood and CSF samples in the 2 patients were collected up to 25 and 72 h, respectively. Blood samples were drawn from a venous access site into heparinized tubes, separate from the site of the docetaxel infusion, while CSF samples were obtained by lumbar puncture. Blood samples were centrifuged immediately for 5 minutes at 2,500 × *g* to separate plasma, and CSF samples and plasma were stored at a temperature lower than -70°C in propylene vials, until analysis. Prior to analysis, it was confirmed that the CSF samples were not contaminated with blood (i.e., less than 1×10^6 erythrocytes), except for one sample, which was not taken into consideration in the final analysis.

Docetaxel analysis

Analytical measurement of total docetaxel concentrations in plasma was performed using a validated assay based on liquid chromatography with tandem mass-spectrometric detection [lower limit of quantitation, 0.5 nM (~ 0.4 ng/mL)], as described previously.[1] For determination of total docetaxel

in CSF, the method was slightly modified. In brief, aliquots of 1 mL were extracted using a mixture of acetonitrile and *n*-butyl chloride (1:4, v/v) following the addition of the internal standard, paclitaxel. Chromatographic separations were achieved on a Waters X-Terra MS column (20 × 2.1 mm internal diameter) packed with a 3.5-µm octadecyl stationary phase (Waters), and a mobile phase composed of acetonitrile and 0.1% aqueous formic acid (80:20, v/v) that was delivered at a flow rate of 0.15 ml/min. Sample extracts were analyzed using a Micromass Quattro LC triple-quadrupole mass-spectrometry detector (Beverly, MA, USA) with an electrospray probe in the positive ionization mode. The spectrometer was programmed to detect the protonated molecular ion / product ion pairs of docetaxel (*m/z* 808.5, *m/z* 527.2) and paclitaxel (*m/z* 854.5, *m/z* 509.4). Calibration curves were constructed in Elliott's B solution over the range 0.050 to 1.0 nM, and computed using the peak area ratio of paclitaxel and docetaxel by weighted (1/*x*) linear-regression analysis. The lower limit of quantitation of the assay for docetaxel in CSF is 0.050 nM (~ 40 pg/mL).

For the determination of the fraction unbound docetaxel in plasma and CSF a validated equilibrium dialysis method was used.[9] In brief, aliquots of 260-µL plasma or CSF were dialyzed against an equal volume of phosphate-buffered saline containing a [G-^{3}H]docetaxel tracer over a membrane with a 12,000-14,000 Da molecular weight cut-off (Spectrum Medical, Houston, TX, USA). Dialysis experiments were performed using 2-mL polypropylene Safe-Lock vials (Eppendorf, Hamburg, Germany) as dialysis chamber in a humidified atmosphere at 37°C. After the end of the 48-h dialysis period, the radioactivity was measured by liquid-scintillation counting for 20 minutes using a Wallac Oy 1409 counter (Turku, Finland). The fraction unbound docetaxel was expressed as a percentage, while the unbound docetaxel concentration was calculated as the product of the fraction unbound docetaxel and the concentration of total docetaxel.

Pharmacokinetic analysis

Pharmacokinetic parameters estimates of docetaxel were derived from weighted (1/*y*) non-compartmental analysis using WinNonlin version 4.0 (Pharsight Corp., Mountain View, CA, USA). The CSF to plasma concentration ratios for docetaxel were calculated using the concurrent plasma concentrations at the time point of CSF sampling.

Results

A summary of the pharmacokinetics of total and unbound docetaxel in plasma and CSF is presented in Table 1. The values for total docetaxel parameters showed wide variability with 2.5-fold variation in clearance between the 2 patients, but are in line with data from several previous studies on docetaxel pharmacokinetics.[3] Using the applied analytical method, docetaxel concentrations in CSF could not be quantified in the patient in which the drug was cleared fast (i.e., levels were below 40 pg/mL).

Table 1. Summary of docetaxel pharmacokinetics

Parameter	Patient 1[1]		Patient 2[2]	
Plasma	*total*	*unbound*	*total*	*unbound*
C_{max} (µg/mL)	1.18	0.119	3.21	0.169
AUC (µg·h/mL)	1.34	0.112	3.47	0.201
CL (L/h)	89.6	1067	36.0	620
$T_{1/2,z}$ (h)	12.4	13.3	33.9	23.6
CSF	*total*	*unbound*	*total*	*unbound*
$C_{1.35\ h}$ (pg/mL)	NQ			
$C_{2.62\ h}$ (pg/mL)	NQ			
$C_{1.53\ h}$ (pg/mL)			71.6	47.7
$C_{25.1\ h}$ (pg/mL)			56.5	42.9
$C_{72.0\ h}$ (pg/mL)			63.1	46.5

[1] Plasma samples taken up to 26 h; [2] Plasma samples taken up to 72 h.
Abbreviations: C_{max}, peak concentration; AUC, area under the plasma concentration-time curve extrapolated to infinity; CL, total clearance; $T_{1/2,z}$ (h), half-life of the terminal disposition phase; CSF, cerebrospinal fluid; $C_{i\ h}$, concentration of docetaxel at *i* h after the start of infusion; NQ, not quantifiable (i.e., total CSF concentration below 40 pg/mL).

The fraction unbound docetaxel in plasma ranged from 6 to 13% in samples from the two patients, while those in CSF ranged from 67 to 103%, presumably because of lower concentration of binding proteins in CSF compared to plasma. The concentration time curves of total and unbound docetaxel in plasma and CSF are presented in Figure 1.

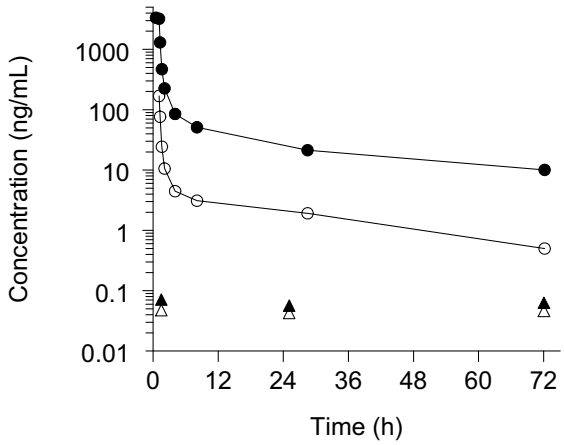

Figure 1. Plasma concentration time curves of total (closed circles) and unbound (open circles) docetaxel in patient 2. The triangles indicate the observed total (closed triangles) and unbound (open triangles) docetaxel concentrations in cerebrospinal fluid samples.

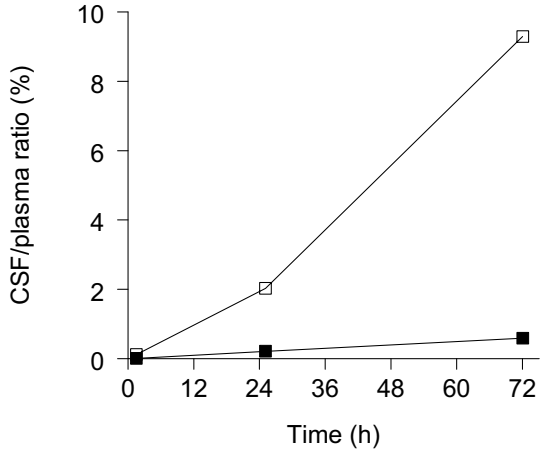

Figure 2. Cerebrospinal fluid (CSF) to plasma concentration ratios of total docetaxel (closed symbols) and unbound docetaxel (open symbols) in patient 2.

The concentrations of docetaxel in CSF did not follow the general pattern of docetaxel in the plasma compartment, with relatively stable levels being observed over the entire sampling-time period. As shown in Figure 2, the CSF to plasma concentration ratio of docetaxel varied in time with values for the total drug ratio increasing from 0.01% to 0.6%, and the unbound drug ratio increasing from 0.1% to 9%.

Discussion

Despite numerous studies describing the clinical pharmacokinetics and pharmacodynamics of docetaxel (reviewed in [3]), the CSF pharmacokinetics and penetration for this agent have only been described previously in a single case report.[6] This current investigation adds to that knowledge because it is the first to take into account the vascular binding of docetaxel by measuring unbound concentrations. In addition, it reports on the application of a recently developed, highly sensitive assay based on liquid chromatography coupled with tandem mass-spectrometric detection.[1]

In both patients with metastatic breast cancer and leptomeningeal carcinomatosis studied here, only very low concentrations of docetaxel were measured in CSF, despite plasma levels of total docetaxel being within the therapeutic range associated with this regimen.[2] Interestingly, the docetaxel concentrations in CSF remained relatively constant over time, suggesting a very slow clearance from the CSF compartment relative to that in the systemic circulation. As a result, apparent equilibrium for docetaxel could not be determined within the time-frame in which CSF samples were drawn. The limited surface area for docetaxel diffusion and the hydrophobic nature of the drug, combined with extensive vascular binding to serum proteins like alpha 1-acid glycoprotein[10] likely contributed to the slow equilibrium kinetics. For this reason, CSF represented only a relatively small additional compartment for docetaxel distribution, particularly in view of the large volume of distribution of docetaxel. It is of particular note that, because the CSF to plasma unbound concentration ratios are time-dependent, single-point data are clearly inappropriate to directly assess the extent of CSF penetration by docetaxel. Furthermore, analysis based on total drug levels in plasma as done previously,[6] potentially results in an estimated 10 to 20-fold underestimation of the extent of drug penetration in CSF.

Although the concentrations of docetaxel measured in CSF are relatively low, results of *in vitro* tests with several cell lines continuously exposed to docetaxel for 96 h previously suggested more than 50% inhibition of cell

growth (IC_{50}) at a mean concentration of 0.4 ng/mL (5,1 x 10^{-10} M).[7] Assuming a protein-bound fraction of approximately 90% for docetaxel in cell culture due to the presence of binding proteins in fetal calf serum, the IC_{50} for unbound docetaxel is around 40 pg/mL, which is comparable to values observed in the patient's CSF. Although the current data are limited to only two patients, the results suggest that docetaxel administered intravenously at doses commonly used in 3-weekly treatment regimens (i.e., ≥ 75 mg/m^2) produces unbound drug levels in CSF for prolonged time periods that are associated with significant antitumor activity in experimental models.

Acknowledgment

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Chapter 6

Effect of CYP3A4 inhibition on the pharmacokinetics of docetaxel

F.K. Engels,[1,2] A.J. ten Tije,[1] S.D. Baker,[3] C.K.K. Lee,[3] W.J. Loos,[1] A.G. Vulto,[2] J. Verweij,[1] A. Sparreboom,[4]

[1]Department of Medical Oncology, Erasmus MC - Daniel den Hoed Cancer Center, Rotterdam, the Netherlands; [2]Department of Clinical Pharmacy, Erasmus MC, Rotterdam, the Netherlands; [3]Division of Experimental Therapeutics, the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA; [4]Clinical Pharmacology Research Core, Medical Oncology Clinical Research Unit, National Cancer Institute, Bethesda, MD, USA.

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Abstract

Objective: In vitro studies indicate that the anticancer drug docetaxel is primarily eliminated by CYP3A4-mediated metabolism. Co-administration of drugs that modulate the activity of CYP3A4 is therefore likely to have undesirable clinical consequences. Here, we investigated the effects of the potent CYP3A4 inhibitor, ketoconazole, on the pharmacokinetics of docetaxel in cancer patients.

Methods: Seven patients were treated in a randomized crossover design with docetaxel (100 mg/m^2) followed 3 weeks later by docetaxel (10 mg/m^2) given in combination with orally administered ketoconazole (200 mg once daily for 3 days), or the reverse sequence. Plasma concentration-time data were analyzed using non-compartmental analysis.

Results: Ketoconazole co-administration resulted in a 49% decrease in clearance of docetaxel (P = .018). The mean (± SD) clearance values were 35.0 ± 11.8 L/h (95% confidence interval, 24.1 – 45.9 L/h) for docetaxel alone and 18.2 L/h (95% confidence interval, 9.22 – 27.1 L/h) in the presence of ketoconazole, respectively. The docetaxel clearance ratio in the presence and absence of ketoconazole was weakly related to the area under the curve of ketoconazole (R-squared, 0.529; P = .064).

Conclusion: Inhibition of CYP3A4 by ketoconazole in vivo results in docetaxel clearance values that were previously shown to be associated with a several-fold increase in the odds for febrile neutropenia at standard doses. Caution should be taken and substantial dose reductions are required if docetaxel has to be administered together with potent inhibitors of CYP3A4.

Introduction

Drug interactions are a major cause of morbidity and mortality in modern clinical practice.[1] Many anticancer drugs have a narrow therapeutic index and are administered to cancer patients who are also taking numerous concomitant medications.[2] An understanding of the implications of interactions is therefore particularly important in anticancer therapy. The human cytochrome P450 3A (CYP3A) subfamily, which is involved in the metabolism of more than 50% of currently prescribed drugs, plays a dominant role in many clinically-relevant drug interactions.[3] In adults, CYP3A activity represents the combined activities of the isoforms CYP3A4, CYP3A5, and CYP3A7.[4] In the majority of humans, however, CYP3A activity in the intestine and liver is predominately reflected by CYP3A4 activity.

Significant interindividual variability in the pharmacokinetics of CYP3A substrates has been observed both in vitro and in vivo. These differences are thought to be related to variations in both basal content and catalytic activity of total CYP3A.[4] Disease-related differences, drugs inducing or repressing transcription, and possibly inherited and ethnic differences are also factors contributing to CYP3A phenotype.[5]

The anticancer drug docetaxel is extensively metabolized by CYP3A.[6,7] The major metabolites and less than 10% of the parent drug are excreted into the feces, whereas total urinary excretion is also less than 10%.[8] The metabolites demonstrate substantially reduced cytotoxic activity as compared to the parent drug, making biotransformation by CYP3A a major route of inactivation.[9] Furthermore, total CYP3A activity has been identified as a strong predictor of docetaxel clearance and most likely accounts to a large extent for the observed interindividual variability in drug clearance and area under the plasma concentration-time curve (AUC).[10-12] Although the fact that docetaxel is predominately metabolized by CYP3A makes the agent subject to a host of enzyme-mediated drug interactions, data on potential interactions are lacking in humans. The aim of the current trial was to assess the effect of CYP3A inhibition on the pharmacokinetics of docetaxel in cancer patients, using the model inhibitor, ketoconazole.[13]

Methods

Patients selection

Eligible patients had a histologically or cytologically confirmed diagnosis of cancer for which docetaxel has proven efficacy, or for which no other treatment option was available. Additional eligibility criteria included: (*i*) a life expectancy of ≥12 weeks; (*ii*) a World Health Organization performance status ≤1; (*iii*) no chemotherapy, hormonal therapy, radiotherapy, or major surgery within four weeks prior to treatment; (*iv*) age above 18 years; (*v*) adequate contraception for women of child-bearing potential; and (*vi*) adequate bone marrow function (absolute neutrophil count, $>1.5 \times 10^9$/L; platelets, platelet count, $>100 \times 10^9$/L), renal function [serum creatinine, ≤1.5 × the upper limit of normal (ULN)], and hepatic function (serum bilirubin, ≤1 × ULN; alanine aminotransferase and aspartate aminotransferase, <2.5 × ULN; and alkaline phosphatase, ≤5 × ULN in the presence of only bone metastases and in the absence of any liver disorders). Simultaneous use of any medication, dietary supplements, or other

compounds known to inhibit or induce CYP3A was not allowed. The study protocol was approved by the Erasmus Medical Center ethical review board, and all patients provided written informed consent before study entry.

Study design

Treatment consisted of two courses of docetaxel (Taxotere; Aventis Pharma BV, Hoevelaken, the Netherlands), administered three weeks apart. Docetaxel was diluted in 250 mL of 0.9% (wt/vol) sodium chloride solution, and delivered as a 1-hour intravenous infusion. One course was given at a docetaxel dose of 100 mg/m^2, and the other at a dose of 10 mg/m^2 in combination with three 200-mg doses of orally administered ketoconazole (Nizoral; Janssen Pharmaceutical, Beerse, Belgium). Previously, it was shown that the AUC of docetaxel is dose-proportional over a large dose range (5 to 145 mg/m^2) in the tested 3-week regimen with the drug administered as a 1-hour intravenous infusion, indicating a linear pharmacokinetic behavior (reviewed by Clarke and Rivory).[8] Therefore, values for clearance of docetaxel between the treatment courses with and without ketoconazole co-administration were compared directly without any correction.

The first ketoconazole dose was administered 1 hour before start of the docetaxel infusion, and the second and third doses were given 24 and 48 hours later. The ketoconazole dose and schedule were based on previously published data.[14] We hypothesized that CYP3A inhibition would prolong the exposure to docetaxel, and that a significant dose reduction was required to prevent unacceptable toxicity in the combination cycle. The decision to administer docetaxel at a dose of 10 mg/m^2 (in combination with ketoconazole) was based on the mild toxicity profile seen at this dose level in a previous Phase I study with single agent docetaxel,[15] and the hypothesis that transient inhibition of CYP3A-mediated metabolism of docetaxel would result in associated exposure levels not exceeding those observed at the recommended single-agent dose for docetaxel in this regimen, while maintaining above the therapeutic threshold level. The allocation sequence of the courses for each patient was determined at study entry using a restricted-block randomization procedure. Premedication consisted of dexamethasone (dose, 8 mg orally) given twice daily for three consecutive days, starting on the evening before docetaxel infusion. Side effects were scored according to the National Cancer Institute common toxicity criteria (version 2.0; available at http://ctep.info.nih.gov). Patients benefiting from docetaxel treatment were offered continuation of treatment beyond cycle 2 at standard doses outside of the study protocol.

Sample size calculation

The mean clearance for docetaxel used in the sample size calculation was 23.99 L/h/m^2, estimated from a group of 56 cancer patients that had sampling for pharmacokinetics on at least 2 occasions (unpublished data). In this group of patients, the SD of the expected differences of the two measurements was estimated to be 4.89 L/h/m^2. It was assumed that the interval between treatments was an adequate washout period, with no carryover or period effect. The trial was designed to detect an effect size of 6.00/4.89, where 6.00 is 25% of the mean docetaxel clearance. Based on a pair-wise (two-sided) analysis, this results in a sample size of (at least) 6 for the prospective evaluation, with a significance level of 0.05 (5%) and power of 0.7 (70%). The statistical analysis was performed in the SISA-Binomial program (D. G. Uitenbroek, Hilversum, the Netherlands, 1997; available at http://home.clara.net/sisa/samsize.htm).

Pharmacokinetic analysis

Blood samples were collected in glass tubes containing lithium heparin as anticoagulant and immediately centrifuged (4000 g at 4°C for 10 minutes) to separate plasma, which was stored at –80°C until analysis. Samples were taken at the following time points: immediately prior to infusion, at 30 minutes after the start of infusion, immediately before the end of infusion, and at 10, 20, 30 minutes, and 1, 1.5, 2, 4, 8.5, 24, 32, 56, 64, and 72 hours after the end of infusion. Determination of docetaxel and ketoconazole concentrations in plasma was performed by high-performance liquid chromatography with tandem mass-spectrometric and UV detection, respectively, according to published procedures.[16,17] These assay for docetaxel has a lower limit of quantitation of 0.0004 µg/mL (0.5 nM), which is sufficiently sensitive to allow quantitation of docetaxel in samples (within the tested collection time period) obtained from patients treated with low drug doses. Determination of the fraction of unbound docetaxel was performed using equilibrium-dialysis with a tritiated-docetaxel tracer (Moravek Biochemicals, Brea, CA).[18]

Pharmacokinetic parameters for docetaxel and ketoconazole were calculated using non-compartmental analysis as implemented in the software package WinNonlin version 4.0 (Pharsight Corp., Mountain View, CA). For docetaxel, the parameters of interest included the peak plasma concentration (C_{max}), the area under the plasma concentration-time curve extrapolated to infinity (AUC), clearance (CL, defined as dose divided by AUC), and volume of distribution at steady-state (Vss), and the half-life of

the terminal phase ($t_{1/2}$). The latter parameter was calculated as $\ln(2)/k$, where k is the rate constant of the terminal phase estimated from log-linear regression analysis of the final 3 to 5 sampling time points. For ketoconazole, the parameters of interest included C_{max}, the time to C_{max}, and AUC over the first dosing interval.

Statistical considerations

Pharmacokinetic data are presented as mean values ± SD with 95% confidence intervals (CI), unless stated otherwise. The effect of ketoconazole co-administration on the pharmacokinetic parameters of docetaxel was evaluated statistically using a non-parametric, two-sided, Wilcoxon signed rank test for paired observations. The relationship between the exposure to ketoconazole and reduction of docetaxel clearance was evaluated using a least-squares linear regression analysis. The cut-off for statistical significance was set at $P < .05$. All statistical calculations were performed using NCSS 2001 (Number Cruncher Statistical System, Kaysville, UT).

Results

Patients and toxicity profiles

To determine the influence of ketoconazole co-administration on the pharmacokinetics of docetaxel, a total of seven patients entered the study (Table 1). All patients completed the study within the scheduled time. In three patients, uncomplicated grade 4 neutropenia was observed, and grade 3 leukocytopenia in another two patients during the single-agent cycle with docetaxel. During the combination course with ketoconazole administration, only minimal toxicity was noted.

Ketoconazole analysis

The median peak concentration and AUC for ketoconazole over the first dosing interval were 1.90 µg/ml (range, 0.886 – 7.37 µg/ml) and 7.80 µg·h/ml (range, 2.73 – 44.8 µg·h/ml), respectively, similar to previous findings.[13] The mean time to peak concentration on day one was observed at 2.24 hours (range, 1.50 – 3.47 hours), suggesting that high concentrations of ketoconazole were present during and immediately after the administration of docetaxel. Although ketoconazole is generally well absorbed, large inter- and intraindividual pharmacokinetic variation after the same oral dose has been reported.[13] This is partly due to differences in gastric acidity, as an increased pH in the stomach decreases the extent of

ketoconazole absorption. A large interindividual variation in peak concentration and AUC was also observed in the current population; for one patient this could be explained by administered co-medication (see below).

Table 1. Baseline patient characteristics*

Demographic variable	No.of patients	Median value	Range
Age, years		40	36 – 59
Sex			
Male	4		
Female	3		
BSA, m^2		1.8	1.6 – 2.1
WHO performance		1	0 – 1
0	3		
1	4		
Tumor type			
Head and neck	2		
Cervix	1		
Sarcoma	1		
Melanoma	1		
ACUP	1		
Rectum	1		
AST, U/L		24	17 – 79
ALT, U/L		18	6 – 30
Alk Phos, U/L		73	62 – 241
Total bilirubin, μM		6	4 – 11
WBC, $\times 10^{-9}$/L		8.4	6.7 – 12.9
ANC, $\times 10^{-9}$/L		7.1	2.6 – 15.1

Abbreviations used: BSA, body surface area; WHO, World Health Organization; ACUP, adenocarcinoma of unknown primary; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Alk Phos, alkaline phosphatase; WBC, white blood cell counts; ANC, absolute neutrophil count.

* The upper limits of institutional normal for the pretherapy clinical chemistry parameters are: AST, < 93 U/L for males and < 78 U/L for females; ALT, < 103 U/L for males and < 78 U/L for females; Alk Phos, ≤ 600 U/L; total bilirubin < 17μmol/L.

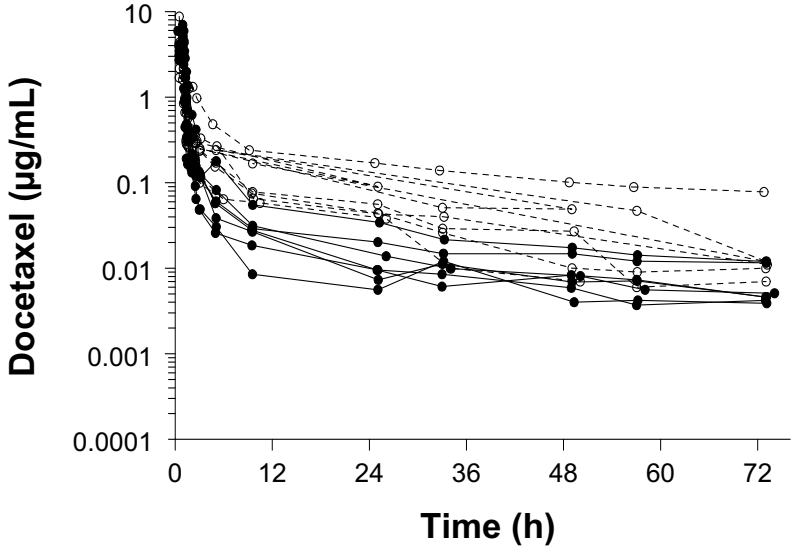

Figure 1. Observed plasma concentrations of docetaxel in the absence (closed circles, solid lines; dose, 100 mg/m²) and presence of ketoconazole co-administration (open circles, dashed lines; dose, 10 mg/m²; data normalized to 100 mg/m²).

Docetaxel analysis

The observed plasma concentrations of docetaxel for both treatments are shown in Figure 1. When ketoconazole was co-administered, the fractional change for clearance was 0.51 (95% CI, 0.36 – 0.65; range, 0.26 – 0.68), indicating that, overall, clearance was reduced by 49% (P = .018) (Table 2). However, large interindividual variability was seen in the reduction in clearance, which reached a maximum value of 74%. The fractional change in docetaxel clearance was weakly correlated to the corresponding AUC of ketoconazole (Figure 2), as determined by a linear regression analysis (R-squared, 0.529; P = .064). A similar relationship was not observed with the time to peak concentration of ketoconazole (R-squared, 0.047; P = .639), suggesting that the rate of absorption was unrelated to variability in effect. For one patient, the fractional change was only 0.68, which was attributable to a very low exposure to ketoconazole due to concomitant administration of

ranitidine, which is known to alter the gastrointestinal absorption of ketoconazole.[19]

It may seem paradoxical that, although docetaxel clearance is inhibited by ketoconazole, the terminal half-life for docetaxel was found to be slightly shorter in the presence of ketoconazole (Table 2). However, the elimination half-life also depends on intercompartmental rate constants. When these processes take place at a higher rate, the elimination half-life, which characterizes the decline in plasma concentration from the site of measurement, will decrease.

Docetaxel in plasma was approximately 94% bound in all patients (mean, 94.2 ± 1.45%; range, 88.8 – 96.7%), consistent with previous estimates.[18] The fraction unbound docetaxel was not significantly different in courses with and without ketoconazole (5.74 ± 1.96% versus 5.81 ± 1.45%; $P = .74$), indicating that protein binding of docetaxel is not significantly affected by ketoconazole.

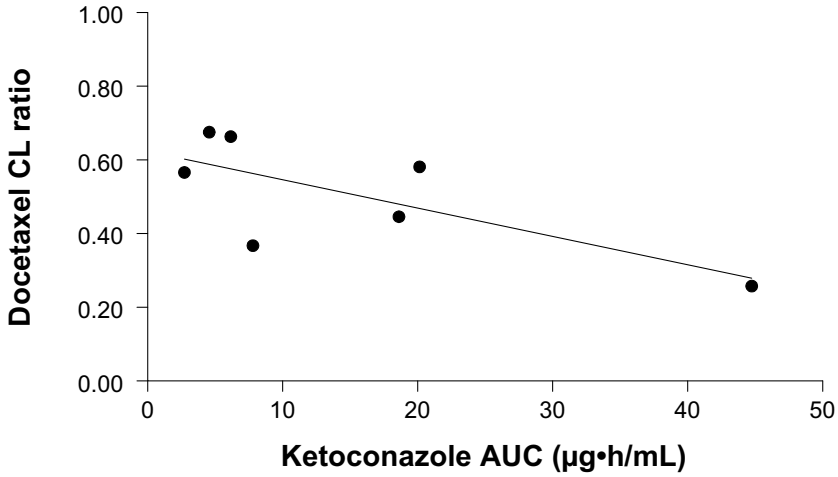

Figure 2. Relationship between ketoconazole area under the curve (AUC, µg·h/ml) and the fractional change in docetaxel clearance (CL), defined as the ratio of CL in the presence and absence of ketoconazola co-administration. The change is described by the following equation: (0.623 ± 0.0660) – (0.0077 ± 0.0033) × (ketoconazole AUC in µg·h/mL); R-squared = 0.529; P = .064).

Table 2. Pharmacokinetic parameters for docetaxel in the absence and presence of ketoconazole

Parameter	Docetaxel	Docetaxel/ketoconazole	Ratio	*P*
	Mean ± SD (95% CI)	*Mean ± SD (95% CI)*	*95% CI)*	
Dose (mg)*	190 (160 – 210)	19 (16 – 21)	N/a	N/a
Dose (mg/m^2)	100	10	N/a	N/a
T_{inf} (h)*	1.05 (0.95 – 1.07)	1.03 (0.59 – 1.15)	N/a	N/a
C_{max} (µg/mL)	4.42 ± 1.32 (3.19 – 5.64)	0.543 ± 0.221 (0.339 – 0.748)	N/a	N/a
AUC (µg·h/mL)	5.90 ± 2.56 (3.53 – 8.27)	1.28 ± 0.628 (0.699 – 1.86)	N/a	N/a
C_{max}/dose (ng/mL/mg)	23.8 ± 5.30 (18.9 – 28.7)	29.9 ± 13.7 (17.3 – 42.6)	1.27 (0.72 – 1.81)	.31
AUC/dose (ng·h/mL/mg)	31.7 ± 11.5 (21.1 – 42.4)	70.3 ± 37.8 (35.3 – 105)	2.19 (1.39 – 2.99)	.018
CL (L/h)	35.0 ± 11.8 (24.1 – 45.9)	18.2 ± 9.68 (9.22 – 27.1)	0.51 (0.36 – 0.65)	.018
Vss (L)	477 ± 265 (232 – 722)	388 ± 283 (126 – 649)	0.87 (0.52 – 1.22)	.50
$t_{1/2}$ (h)	41.0 ± 10.9 (30.9 – 51.0)	28.3 ± 7.89 (21.0 – 24.4)	0.73 (0.45 – 1.02)	.043

CI, confidence interval; N/a, not applicable; T_{inf}, infusion duration; C_{max}, peak plasma concentration; AUC, area under the plasma concentration time curve extrapolated to infinity; CL, systemic clearance; Vss, volume of distribution at steady-state; $t_{1/2}$, half life of the terminal phase.

* Median value with range in parenthesis.

Discussion

This study shows that the clearance of docetaxel is significantly reduced by 49% upon co-administration with ketoconazole, albeit at large interindividual variability. This degree of variability was shown to be related to interindividual differences in the systemic exposure to ketoconazole, with low AUC values leading to only minimal inhibitory effects.

The main toxic side effect associated with docetaxel treatment is a short-lasting neutropenia that reaches grade 3-4 in approximately 90% of patients (see: http://www.taxotere.com). Bruno et al have reported previously that the AUC of docetaxel is a significant predictor of severe neutropenia; a 50% decrease in clearance corresponds to a 4.3-fold increase in the odds for grade 4 neutropenia and in a 3.0-fold increase in the odds for febrile neutropenia.[20] In the present study, the maximum decrease in clearance observed was 74%, which translates into a 6.5-fold increase in the odds for grade 4 neutropenia, and in a 4.5-fold increase in the odds for febrile neutropenia. This could have had clinical consequences had docetaxel been administered in combination with ketoconazole at the full recommended dose. Calculation of the predicted AUC in combination with ketoconazole (ie, the AUC normalized to a 100 mg/m^2 dose) for this same patient resulted in a relative increase in exposure of approximately 290%, further supporting the potential for a substantially increased risk of severe toxicity. The current findings are inconsistent with previously published data that suggest that ketoconazole does not consistently affect docetaxel pharmacokinetics,[21] even though much higher doses of ketoconazole were administered. In that study, however, plasma concentrations of ketoconazole were not reported, making a direct comparison impossible.

As mentioned previously, human adult CYP3A activity reflects the heterogeneous expression of CYP3A4, CYP3A5, and CYP3A7, although the level of hepatic CYP3A4 seems to be the major determinant in the metabolism of docetaxel.[22] However, the polymorphic distribution of CYP3A5 indicates that metabolically active CYP3A5 is expressed in approximately 30% of Caucasians and in 50-73% of African Americans.[23,24] In these individuals, CYP3A5 expression accounts for at least 50% of the total CYP3A content, and likely contributes substantially to the metabolic clearance of many CYP3A substrates. However, CYP3A5 is less susceptible to inhibition by ketoconazole as demonstrated by increased K_i values.[25] Furthermore, the percentage of inhibition by ketoconazole is inversely related to the fractional percentage of CYP3A5 in total CYP3A.[25] The

presence of variable expression ratios of CYP3A4/CYP3A5 in other ethnic populations may therefore result in a different drug interaction between docetaxel and ketoconazole.

In conclusion, co-administration of the potent CYP3A4 inhibitor ketoconazole leads to a 49% decrease in docetaxel clearance and, as such, to an increased risk for severe neutropenia. The extent to which docetaxel clearance was reduced depends on the exposure to ketoconazole, as expressed by AUC. Further research is required to ascertain whether this measurement of ketoconazole exposure can be used *a priori* to identify patients potentially at risk for a clinically relevant interaction when being treated with ketoconazole and docetaxel, a strategy that is currently being pursued in the treatment of androgen-independent prostate cancer.[26,27] Most importantly, with concomitant use of docetaxel and ketoconazole, or other potent CYP3A4 substrates or inhibitors, potentially dangerous interactions are likely. Hence, caution should be taken and substantial dose reductions are necessary if these drugs need to be administered together.

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Chapter 7

Altered clearance of unbound paclitaxel in elderly patients with metastatic breast cancer

C.H. Smorenburg[1], A.J. ten Tije[1], J. Verweij[1], M. Bontenbal[1], K. Mross[2], D.M. van Zomeren[1], C. Seynaeve[1], A. Sparreboom[1]

[1]Department of Medical Oncology, Erasmus MC - Daniel den Hoed Cancer Center, Rotterdam, the Netherlands; [2]Department of Medical Oncology and Clinical Pharmacology, Albert-Ludwigs-University Freiburg, 79106 Freiburg im Breisgau, Germany.

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Abstract

The pharmacokinetic behaviour of anticancer drugs may be altered with aging due to (for example) differences in body composition and decreased hepatic and renal function. To address this issue for paclitaxel, we studied the pharmacokinetics of the drug in eight elderly women (≥70 years) with metastatic breast cancer [median age (range), 77 years (70 – 84)] and a control group of 15 patients aged <70 years [median age (range), 54 years (22 – 69)]. Paclitaxel was administered as a 1-h intravenous (i.v.) infusion at a dose of 80 (elderly) or 100 mg/m^2 (<70 years), and serial blood samples were obtained at baseline, and up to 24 h after the end of infusion. Paclitaxel concentration-time profiles were fitted to a linear three-compartment model without any demonstration of saturable behaviour. The clearance of unbound paclitaxel was 124 ± 35.0 (elderly) versus 247 ± 55.4 l/h/m^2 (<70 years) (P = 0.002), and was inversely related to patient's age (R^2 = 0.857; P <0.00001). Total plasma clearance of the formulation vehicle Cremophor EL (CrEL) was 150 ± 60.7 (elderly) versus 115 ± 39.2 ml/h/m^2 (< 70 years) (P = 0.04). These data indicate an approximately 50% change in total body clearance of unbound paclitaxel and a concomitant significant increase in systemic exposure with age, most likely as a result of altered CrEL disposition. The clinical relevance of these observations with respect to toxicity profiles and antitumour efficacy requires further evaluation.

Introduction

As the incidence of breast cancer rises with advancing age, and populations in Western countries are aging, the total number of women with breast cancer will increase substantially.[1] Unfortunately, elderly patients are still underrepresented in trials on cancer therapies, especially on breast cancer treatment.[2] This holds true even after the exclusion of trials restricted to patients younger than 65 years.[2] Moreover, as elderly patients frequently suffer from impaired organ functions and/or comorbidity, extrapolating standard recommendations for chemotherapy in metastatic breast cancer patients to the elderly might result in excessive toxicity.[3] Notwithstanding the large number of elderly patients, and the known impact of impaired renal and hepatic functions on the absorption, distribution, metabolism and excretion of various anticancer agents, including taxanes, there have been only a few pharmacological studies conducted in this subgroup of patients.[4]

The cytotoxic agent paclitaxel (Taxol) is registered for the treatment of advanced breast cancer, for which it is usually administered in second-line therapy as a single agent every 3 weeks at a dose of 175–225 mg/m^2. Frequently encountered side-effects are neutropenia, neuropathy, asthenia and alopecia. Weekly administration of paclitaxel has demonstrated sustained efficacy together with a more favourable toxicity profile lacking severe myelotoxicity.[5] While the related agent docetaxel, despite a dose reduction of 75% of the standard dose of 100 mg/m^2 every 3 weeks, appeared to be too toxic in non-pretreated patients aged >70 years with metastatic breast cancer, a weekly schedule at a dose of 36 mg/m^2 in heavily pretreated elderly patients indeed appeared effective and well tolerated.[6-8] Recently published data suggest similar efficacy for weekly paclitaxel.[9,10] This way of administering paclitaxel therefore seems an attractive chemotherapeutic alternative for elderly women with metastatic breast cancer, although no pharmacological data are yet available. Here, we studied the pharmacokinetics of paclitaxel and its formulation vehicle Cremophor EL (CrEL) in patients with breast cancer aged ≥70 years treated in a weekly schedule, and compared the results with a control group of patients aged <70 years treated in a similar way.

Patients and methods

Eligibility criteria

Two groups of patients were studied based on age; patients aged ≥70 years were eligible if they had histologically or cytologically confirmed breast cancer, unresponsive to hormonal therapy, while patients aged between 18 and 70 years were eligible if they had any histologically or cytologically confirmed metastatic solid tumour for which treatment with paclitaxel was a viable option. Prior to recruiting male patients in the control group, it was confirmed that there are no sex-related differences in unbound paclitaxel clearance. This was investigated in unpublished data from a historical patient population treated at the Erasmus MC – Daniel den Hoed Cancer Center (Rotterdam, the Netherlands) with single agent paclitaxel given as a 1-h intravenous (i.v.) infusion at dose levels ranging between 70 and 200 mg/m^2. The group consisted of 10 males (median age, 58 years; range, 46–70 years) and 30 females (median age, 57 years; range, 29 – 71 years). The mean (± SD) values for clearance of unbound paclitaxel in male and female patients were 200 ± 35.6 and 195 ± 48.3 l/h/m^2, respectively, which is a not

statistically significant difference [P = 0.75; mean difference (± SE), 5.26 ± 16.3 l/h/m^2; 95% confidence limits for the mean difference, -27.8 and 38.3; unpaired two-tailed Student's *t*-test].

Other criteria for patient enrollment were (*i*) acceptable performance status according to the World Health Organization criteria (WHO) (0-2), (*ii*) an adequate bone marrow function (defined by pretherapy values of haemoglobin ≥ 6.0 mM, absolute neutrophil count (ANC) >1.5 × 10^9/l, and platelet count >160 × 10^9/l), (*iii*) adequate renal function (creatinine levels <175 μM) and (*iv*) adequate hepatic function (bilirubin levels < 25 μM). Patients with other malignancies during the past 5 years, neuropathy graded ≥2, symptomatic cardiac disease, and/or signs of central nervous system involvement were excluded. All patients gave written informed consent, and the study protocol was reviewed and approved by the Erasmus MC – Daniel den Hoed Cancer Center review board (Rotterdam, the Netherlands).

Treatment schedule and patient evaluation

Paclitaxel was administered as a 1-h i.v. infusion at a dose of 80 mg/m^2 (elderly patients) or 100 mg/m^2 (< 70 years) on days 1, 8 and 15 with treatment cycles repeated every 4 weeks until progressive disease or the occurrence of serious treatment-related side-effects. All premedication, consisting of dexamethasone (10 mg), clemastine (2 mg) and ranitidine (50 mg), was administered by the i.v. route at 30 minutes prior to paclitaxel infusion. Pretreatment evaluation consisted of a complete history and physical examination, complete blood cell counts, serum chemistry analysis, electrocardiogram, chest X-ray. Complete blood cell counts were measured on a weekly basis, while other tests were repeated before the next full cycle. Toxicity in each patient following paclitaxel administration was evaluated using the National Cancer Institute common toxicity criteria (NCI CTC) version 2.0.

Pharmacokinetic and pharmacodynamic analysis

Blood samples for pharmacokinetic analysis were collected from all patients only on day 1 of the first administration from a vein in the arm opposite to the one used for drug infusion. Blood samples of 5 ml were obtained at the following time points: before infusion, at 0.5 h after the start of infusion, 5 min before the end of infusion, and at 5, 15, 30 min and 1, 2, 4, 8, 12 and 24 h after the end of infusion. Samples were collected in tubes containing lithium heparin as anticoagulant and were subsequently

centrifuged at 3000g for 10 min at 4°C to separate plasma and cells. Plasma samples were stored frozen at –80°C until analysis.

In view of the profound non-linear disposition of paclitaxel in patients,[11] the pharmacological consequences of the treatment in patients with increasing age can not be predicted based on total plasma levels alone when different dose groups are compared. Since the area under the plasma concentration-time curve (AUC) of unbound paclitaxel is a linear function of the dose administered,[12,13] we focused here on comparing the fraction unbound paclitaxel between the two groups. Concentrations of total paclitaxel in plasma samples were determined by a validated reversed-phase high-performance liquid chromatography with ultraviolet detection as described earlier.[14] The free drug fraction of paclitaxel was measured by using a reproducible equilibrium dialysis method using a tritiated-paclitaxel tracer.[12] Coinciding levels of CrEL were measured by a colorimetric dye-binding microassay, as published.[15] The kinetics of paclitaxel and CrEL were evaluated for each patient separately by a linear three-compartment model and by model-independent methods, respectively, using the Siphar version 4.0 software package (InnaPhase, Philadelphia, PA, USA). This program determines the slopes and intercepts of the logarithmically plotted curves of multiexponential functions using non-linear least-squares, iterative steps. Initial parameter estimates were determined by an automated curve-stripping procedure. The mathematical equations describing the drug concentration $C_{(t)}$ at any time *t* during (eq. 1) and after i.v. administration (eq. 2) are given by:

$$C_{(t)} = \Sigma \{C_i / (\lambda_i \times T_{inf}) \times (1 - e^{(-\lambda_i \times t)})\} \quad \text{(eq. 1)}$$

$$C_{(t)} = \Sigma \{C_i / (\lambda_i \times T_{inf}) \times (e^{(-\lambda_i \times [t - Tinf])} - e^{(-\lambda_i \times t)})\} \quad \text{(eq. 2)}$$

In these equations, λ_i is the component of the *I*-th exponential term, C_i is the initial concentration of the *i*-th component of the curve, and T_{inf} is the infusion duration. In all cases, paclitaxel-concentration-time curves were best described with a tri-exponential model, which gave the lowest Akaike information criterion, without any demonstration of saturable behaviour (R^2 = 0.996 ± 0.002, root mean square error = 13.5 ± 3.53%). The curve fitting procedure with this model yields the parameters C_1, C_2, C_3, λ_1, λ_2, and λ_3. The AUC values were determined on the basis of the parameters of equations 1 and 2 with extrapolation to infinity using the terminal disposition rate constant. The clearance was defined as dose (expressed in μmol/m^2) divided

by AUC. The volume of distribution at steady-state was calculated as the product of clearance and the mean residence time, also estimated from equations 1 and 2. Peak plasma concentrations were put on par with observed (experimental) drug levels immediately following the end of infusion. The fraction unbound paclitaxel was defined as the ratio of unbound paclitaxel AUC and total paclitaxel AUC. Pharmacodynamics was assessed by calculation of the relative haematological toxicity of white blood cell count (WBC) and absolute neutrophil count (ANC), defined as:

$$\%\text{decrease} = [(\text{pretherapy value} - \text{nadir value}) / (\text{pre-therapy value})] \times 100\% \qquad \text{(eq. 3)}$$

Statistical evaluation

All pharmacological parameters are expressed as mean values ± SD. Differences in any of the studied pharmacokinetic and pharmacodynamic parameters between the two age groups or within the control group between male and female patients were evaluated statistically using an unpaired two-tailed Student's *t*-test after testing for normality. The relationship between clearance of unbound paclitaxel and age was evaluated using least-squares linear regression analysis and adjusted R^2 values to compensate for the expected chance prediction when the null hypothesis is true. The level of significance was set at $P<0.05$. All statistical calculations were performed using Number Cruncher Statistical System v5.X (Jerry Hintze, Kaysville, UT, USA).

Results

Patient characteristics

A total of 8 elderly patients and 15 patients aged < 70 years was studied (Table 1), and all were evaluable for paclitaxel pharmacokinetics and toxicity. The median age in the groups was 77 years (range 70 – 84) and 54 years (range 22 – 69), respectively. Other patient characteristics and baseline clinical chemistry values were similar between the two groups (Table 1). In the elderly group, 7 of 8 patients had received prior hormonal therapy for metastatic disease, and a median number of four cycles (range, 1 to 6 cycles) was administered per patient.

Table 1. Patient characteristics and baseline clinical chemistry values (median with range)

Characteristic	patients ≥70 years	patients <70 years
No. studied	8	15
Age (years)	77 (70-84)	54 (22-69)
BSA (m^2)	1.75 (1.45-1.91)	1.76 (1.31-2.37)
Weight (kg)	71.6 (54.0-84.3)	68.1 (36.6-116)
Height (cm)	160 (150-167)	165 (157-185)
Sex (M/F)	0/8	7/8
Serum albumin (g/L)	42 (38-47)	38 (24-47)
Total serum protein(g/L)	74 (69-80)	69 (49-79)
Hematocrit (l/l)	0.35 (0.27-0.40)	0.35 (0.29-0.44)

BSA, body-surface area; M, male; F, female.

Pharmacokinetics

Unbound paclitaxel concentration-time curves for both groups are shown in Figure 1. Overall, the interpatient variability in unbound paclitaxel clearance was moderate (coefficient of variation, 30.8%). A summary of pharmacokinetic data of unbound paclitaxel, total paclitaxel and CrEL is shown in Table 2. In the control group, there were no significant sex-related differences in unbound paclitaxel clearance (males vs females, 251 ± 74.3 vs 237 ± 43.0 l/h/m^2; P = 0.67), total paclitaxel clearance (18.4 ± 5.63 vs 16.6 ± 2.69 l/h/m^2; P = 0.43), the fraction unbound paclitaxel (0.084 ± 0.007 vs 0.085 ± 0.005; P = 0.76), and the clearance of CrEL (115 ± 41.7 vs 114 ± 39.2 ml/h/m^2; P = 0.94). Therefore, pharmacokinetic data were directly compared between the groups despite the distribution of males and females being unequally represented in the elderly and younger patient groups.

The clearances of unbound paclitaxel and total paclitaxel were significantly different between the two age groups, with mean values (elderly vs younger) of 124 ± 35.0 vs 247 ± 55.4 l/h/m^2 (P = 0.002) and 13.9 ± 2.31 vs 17.4 ± 4.52 l/h/m^2 (P = 0.04), respectively (Table 2). The difference in unbound paclitaxel clearance remained significant when the eight females in the elderly group were compared with the eight females in the control group (124 ± 35.0 vs 237 ± 43.0 l/h/m^2; P = 0.002). In the entire patient population, a significant negative correlation was observed between age and unbound paclitaxel clearance [Figure 2; clearance (in l/h/m^2) = (-4.127 ×

age) + 457.5; adjusted R^2 = 0.847; P < 0.00001]. The unbound paclitaxel volume of distribution at steady state was also significantly smaller in the elderly patients (1105 ± 300 vs 2546 ± 754 l/m^2; P < 0.001), whereas the terminal disposition half-life was similar (18.0 ± 7.40 vs 21.7 ± 4.33 h; P = 0.14). The clearance of CrEL was significantly faster in the elderly patients compared with the control group (150 ± 60.7 vs 115 ± 39.2 ml/h/m^2; P = 0.04).

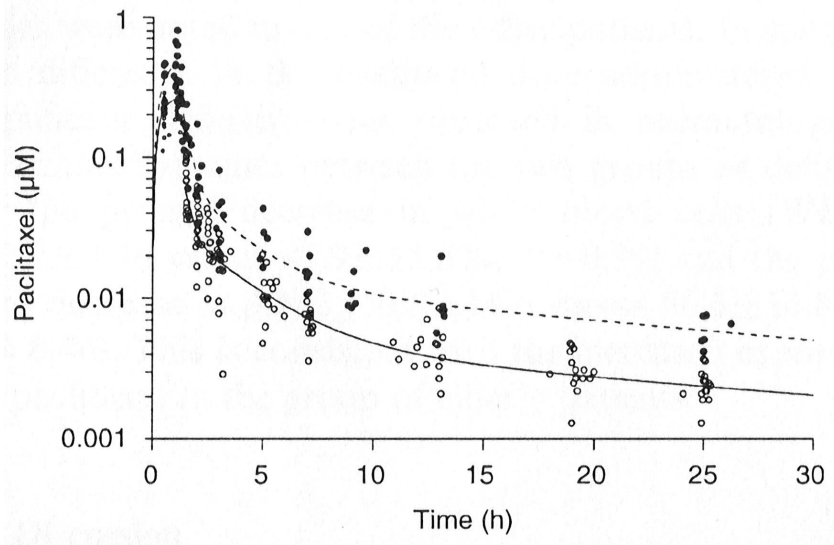

Figure 1. Plasma concentration-time profiles of unbound paclitaxel in elderly (≥ 70 years) patients (n = 8; closed symbols and dotted line) and patients < 70 years (n = 15; open symbols and solid line) receiving a 1-h i.v. infusion of paclitaxel at dose levels of 80 and 100 mg/m2, respectively. Data from the elderly group were normalized to a paclitaxel dose of 100 mg/m2, by multiplying unbound paclitaxel concentrations (Cu) by the dose difference [Cu × (100/80)]. The mathematical equations describing the drug concentration ($C_{(t)}$) at any time (t) during (eq. 1) and after i.v. administration (eq. 2) are given by: $C(t) = \Sigma \{Ci / (\lambda I \times Tinf) \times (1 - e(-\lambda I \times t))\}$ (eq. 1) and $(t) = \Sigma \{Ci / (\lambda I \times Tinf) \times (e(-\lambda I \times [t - Tinf]) - e(-\lambda I \times t))\}$ (eq. 2). The model parameters were C1 = 1.19 μM, C2 = 0.076 μM, C3 = 0.013 μM, λ1 = 2.96 h-1, λ2 = 0.444 h-1, and λ3 = 0.033 h-1 for elderly patients, and C1 = 0.976 μM, C2 = 0.033 μM, C3 = 0.005 μM, λ1 = 4.26 h-1, λ2 = 0.350 h-1, and λ3 = 0.029 h-1 for younger patients.

Table 2. Summary of paclitaxel and CrEL pharmacokinetics (mean ± SD)

Parameter	patients ≥70 years	patients <70 years
No. of patients	8	15
Paclitaxel dose		
(mg/m^2)	80	100
(mg)[a]	140 (105 - 170)	170 (130 - 226)
Infusion duration (h)[a]	1.00 (0.90 - 1.21)	1.00 (0.98 - 1.19)
Unbound paclitaxel		
C_{max} (µM)	0.366 ± 0.155	0.262 ± 0.079
AUC (µM•h) 5	0.749 ± 0.231	0.503 ± 0.095
CL (l/h/m^2)	124 ± 35.0	247 ± 55.4[b]
V_{ss} (l/m^2)	1105 ± 300	2546 ± 754[c]
$T_{1/2}$ (h)	18.0 ± 7.40	21.7 ± 4.33
fu	0.095 ± 0.014	0.085 ± 0.006
Total paclitaxel		
C_{max} (µM)	3.22 ± 1.30	3.37 ± 0.730
AUC (µM•h)	*6.92 ± 1.25*	*5.99 ± 1.12*
CL (1/h/m^2)	*13.9 ± 2.31*	*17.4 ± 4.52[d]*
CrEL		
C_{max} (µl/ml)	2.51 ± 0.34	2.82 ± 0.76
AUC (µl•h/ml)	51.8 ± 22.0	80.2 ± 27.3
CL (ml/h/m^2)	150 ± 60.7	115 ± 39.2[e]

C_{max}, peak plasma concentration; AUC, area under the plasma concentration-time curve; CL, plasma clearance; $T_{1/2}$, half-life of the terminal disposition phase; fu, unbound drug fraction (AUC unbound drug / AUC total drug).

[a] Median with range; [b] P = 0.002; [c] P < 0.001; [d] P = 0.04; [e] P = 0.04.

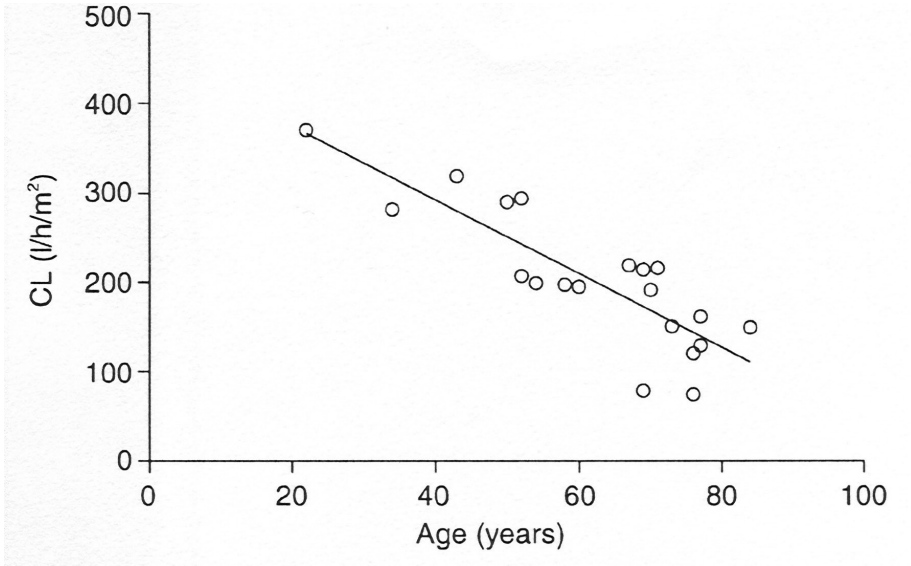

Figure 2. Relationship between patient age and unbound paclitaxel clearance (CL). The solid line indicates the fit of a least-squares linear regression analysis [CL = (-4.127 × age) + 457.5; adjusted R2 = 0.847; P < 0.00001].

Toxicity profiles

Only four administrations (5%) were delayed, of which one was due to erysipelas and three were due to non-therapy-related morbidity. Dose reductions were not required in any patient from both groups, and no cumulative toxicity of any kind was seen. In the elderly group grade 2 fatigue was common, in line with previous findings,[16] and resulted in the discontinuation of treatment in 2 patients. One patient experienced a grade 3 toxicity (neutropenia and skin toxicity with generalized erythroderma), while no grade 3 or 4 toxicities were noted in any of the other patients. In spite of the difference in the paclitaxel dose administered, no significant difference was observed in haematological pharmacodynamics between the two groups as defined by the percent decrease in white blood cells (WBC) (40.7 ± 7.96 vs 45.9 ± 15.5%; P = 0.39) and the percent decrease in ANC (50.8 ± 14.6 vs 56.3 ± 14.8%; P = 0.40). This is consistent with the increased exposure to paclitaxel in the group of elderly patients.

Discussion

In the present study, we have described for the first time the pharmacokinetics of unbound paclitaxel in cancer patients as a function of age. Overall, our data indicate that the clearance of unbound paclitaxel, following weekly administration as a 1-h i.v. infusion, is approximately 50% reduced in elderly patients (≥ 70 years) compared with younger patients, and that age is a significant predictor of paclitaxel disposition in the population studied. These data complement previous knowledge on the clinical pharmacology of paclitaxel, and may have important practical implications for its optimal use. Indeed, while some studies examined the efficacy and feasibility of chemotherapy in elderly patients with metastatic breast cancer, little is known about the pharmacokinetic behaviour of the anticancer agents involved, with the notable exception of some anthracyclines and *Vinca* alkaloids.[4] For doxorubicin, a trend for delayed clearance in elderly cancer patients has been documented, while the AUC of daunorubicinol, an active metabolite of daunorubicin, was significantly increased in 13 elderly patients with acute leukaemia.[17,18] In patients aged ≥ 70 years, the clearance of vinorelbine was reduced by 30-40%, compared with adult patients.[19] To adjust for decreasing renal function with age, a study investigating combination chemotherapy with cyclophosphamide, methotrexate and 5-fluorouracil in women aged ≥ 65 years used creatinine clearance for calculation of appropriate doses of cyclophosphamide and methotrexate.[20] While indeed less toxicity resulted, unfortunately no pharmacokinetic analysis was performed.

For paclitaxel, only scarce data are available on the effect of aging on the agent's pharmacokinetic behaviour. Nakamura and colleagues performed a retrospective analysis investigating total paclitaxel pharmacokinetics in 120 lung cancer patients, of whom 28 were elderly, treated at a dose of 210 mg/m^2 given over 3 h in a 3-weekly regimen.[21] These authors could not detect any differences in AUC, peak concentration, terminal disposition half-life, and time above the threshold of 0.1 μM between patients aged <70 years and those >70 years.[21] Likewise, Fidias and colleagues recently reported that the clearance of total paclitaxel in a group of 8 patients with non-small cell lung cancer (age ≥ 70 years) treated with a dose of 90 mg/m^2 as a 1-h i.v. infusion was comparable to values that have been reported for studies involving younger patients.[22] However, these apparent inconsistencies with our current findings need to be interpreted with great caution as, in the study performed by Fidias and colleagues, no control group involving

younger patients was studied, and a host of confounding factors might influence their overall conclusions, including differences in the paclitaxel dose administered between the comparative trials, variability in analytical methods employed, and parameter calculation procedure used. In contrast to conclusions drawn in the above studies,[21,22] Lichtman and colleagues recently reported in abstract form a significant difference in AUC and clearance of total paclitaxel with advancing age in 113 patients treated with paclitaxel at a dose of 175 mg/m^2 administered as a 3-h infusion.[23] The total paclitaxel clearances in patients aged 55-64 years and in 28 patients >75 years were 10.9 and 8.21 l/h/m^2, respectively, which was significant at P = 0.012. Unfortunately, these investigators used a strategy for AUC calculation based on the use of only a few timed samples early (i.e. a limited-sampling strategy) up to 7 h after dosing, which may have caused a serious flaw in that any alteration in drug elimination as a result of aging (e.g. metabolic and excretory routes) may remain undetected by such methodology. Moreover, as it cannot be excluded that any alteration in paclitaxel disposition is (partially) associated with changes in CrEL pharmacokinetics as a function of age (see below), the use of total plasma concentrations and subsequent calculation of total plasma clearance, as was done in the mentioned studies,[21-23] may be essentially less meaningful. The results of the various investigations performed to date further emphasise the need to simultaneously study paclitaxel pharmacokinetics in a control group of younger patients when evaluating the role of patient age in drug disposition.

Previous investigations have demonstrated the importance of unbound paclitaxel AUC as a pharmacokinetic parameter to delineate exposure-toxicity relationships, both with 1- and 3-h infusion schedules.[13,24] Although intuitively the unbound fraction of paclitaxel accounts for the (cyto)toxic actions of the treatment, its concentration has never been investigated in elderly patients. We have recently shown that CrEL, the vehicle used for i.v. paclitaxel administration, has a substantial impact on the fraction unbound paclitaxel.[25,26] Although the exact mechanism underlying this interaction has not yet been fully elucidated, the presence of CrEL in the circulation as large polar micelles is thought to entrap paclitaxel, thereby reducing cellular accumulation of paclitaxel in blood cells (e.g. erythrocytes) and altering the fraction of unbound paclitaxel in whole blood. Since CrEL clearance increases with prolonged duration of infusion from 1- to 3- and 24-h, the systemic exposure to unbound paclitaxel and CrEL significantly depends on the duration of drug infusion.[27] Our current

data on unbound paclitaxel levels in elderly patients should therefore not be compared with studies using other infusion schedules. In any event, the demonstration that CrEL clearance is significantly increased by 30% in elderly patients, combined with the notion that CrEL micelles act as the principal carrier of paclitaxel in the systemic circulation,[28] suggests that this phenomenon likely contributes substantially to the changes in unbound paclitaxel clearance.

The mechanisms underlying the age-dependent pharmacokinetics of CrEL are not clear. In fact, the faster clearance of CrEL in the group of elderly patients is rather unusual, because for most xenobiotics that exhibit age-dependent pharmacokinetics, clearance tends to decrease with advancing age.[29] It has been previously shown that elimination routes of polyoxyethylated surfactants like CrEL are associated with esterase-mediated metabolic breakdown within the systemic circulation.[26] One possibility to explain the age-dependent pharmacokinetics of CrEL would be that CrEL biotransformation takes place at an accelerated rate as a result of elevated enzyme levels in the systemic circulation in elderly patients. This would be consistent with the observation that the clearance of CrEL is significantly higher (approximately 3 to 4 fold) in adult patients with moderate to severe hepatic dysfunction compared with patients with normal hepatic function.[30] This and several other possibilities, including diminished liver volume and blood flow,[31] are currently under investigation.

As paclitaxel elimination is almost entirely caused by metabolic breakdown through cytochrome P450 (CYP) isoforms 3A4 and 2C8,[32] an alternative explanation for the altered paclitaxel clearance is an impaired hepatic function with advancing age. Although eligibility criteria excluded patients with an elevated bilirubin and all patients entered had normal values of aspartate and alanine aminotransferases, these laboratory values do not represent the actual capacity of hepatic metabolism.[33] A previous investigation in a group of 226 patients with equal histopathological conditions has shown a significant decline in total CYP content with age and a concomitant approximately 30% reduction of drug metabolism in patients after 70 years of age.[34] Thus, one possibility to investigate the role of altered liver function in relation to the current findings would be to determine pretreatment CYP3A4 and CYP2C8 activity in each patient using a functional surrogate such as the erythromycin breath test.[35] Additional clinical and pharmacological information is currently being collected by implementation of such assays in ongoing trials with paclitaxel as well as docetaxel to

further explore the role of enzyme capacity in taxane disposition in elderly patients.

Collectively, our study demonstrates that CrEL and unbound paclitaxel clearance are subject to considerable changes depending on age. In our patient population, haematological toxicity was relatively mild and not clinically relevant due to the low paclitaxel doses, precluding detection of statistically significant differences between both age groups. More insight will be provided by the ongoing Cancer Leukaemia Group B (CALGB) 9762 study, evaluating paclitaxel pharmacology in relation to patient age with drug administration over 3-h in a 3-weekly schedule at higher doses.[23] As the unbound fraction of paclitaxel is responsible for its cellular actions and its clearance is remarkably reduced in the elderly, this observation warrants further studies on the efficacy and feasibility of paclitaxel in aged patients using dose-dense regimens.

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Chapter 8

Prospective evaluation of the pharmacokinetics, CYP3A phenotype, and toxicity profile of docetaxel in the elderly

A.J. ten Tije, J. Verweij, M.A. Carducci, T. Rogers, T. Pronk, M.P. Verbruggen, F. Dawkins, S.D. Baker

From Erasmus University Medical Center, Rotterdam, the Netherlands; The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland; Howard University Cancer Center, Washington DC; and National Cancer Institute, Bethesda, Maryland.

Present address A.J. ten Tije: Gooi-Noord Hospital, Rijksstraatweg 1, 1261 AN Blaricum, the Netherlands

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 20 elderly cancer patients aged ≥ 65 years and 20 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/m2 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 February 12, 2004); in patients with non-small cell lung cancer receiving 75 mg/m2, 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 x ULN; 6) 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; 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 °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 ($t1/2,\lambda z$) and systemic clearance (CL). The AUC was calculated as dose divided by CL. Maximum plasma concentration (Cmax) 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 January 16, 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	(1.45-2.45)
Sex[a]				
Female	10		9	
Male	10		11	
AAG (mg/dL)[a]	159	(86-257)	126	(60-201)
Liver Function Tests				
AST (x ULN)	0.95	(0.30-3.9)	0.80	(0.40-4.7)
ALT (x ULN)	0.70	(0.20-6.6)	0.50	(0.10-1.5)
Alkphos (xULN)	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)	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		5	
1-2 regimens	14		14	
≥3 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
C_{max} (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).

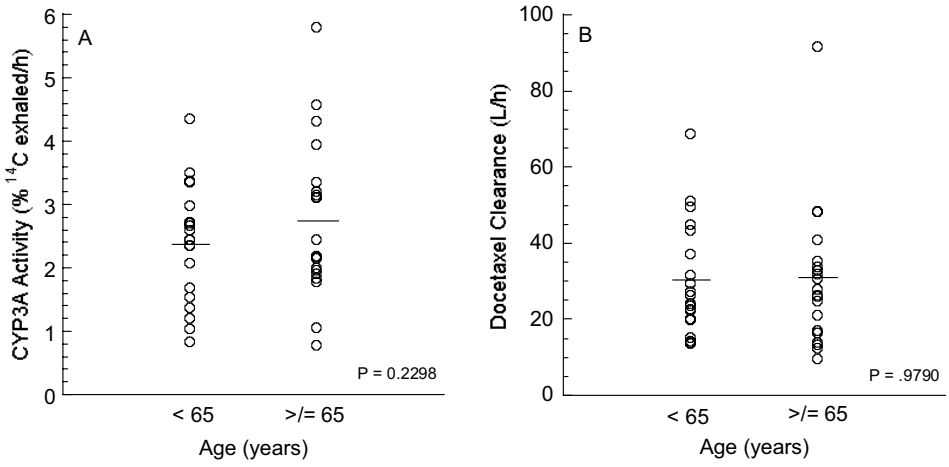

*Figure 1. [**A**] CYP3A activity (% ^{14}C exhaled/h) and [**B**] docetaxel clearance as a function of age group. Lines represent the mean values.*

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 ($x10^9/L$)[b]	%Decrease 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. Percentage decrements in ANC was 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.

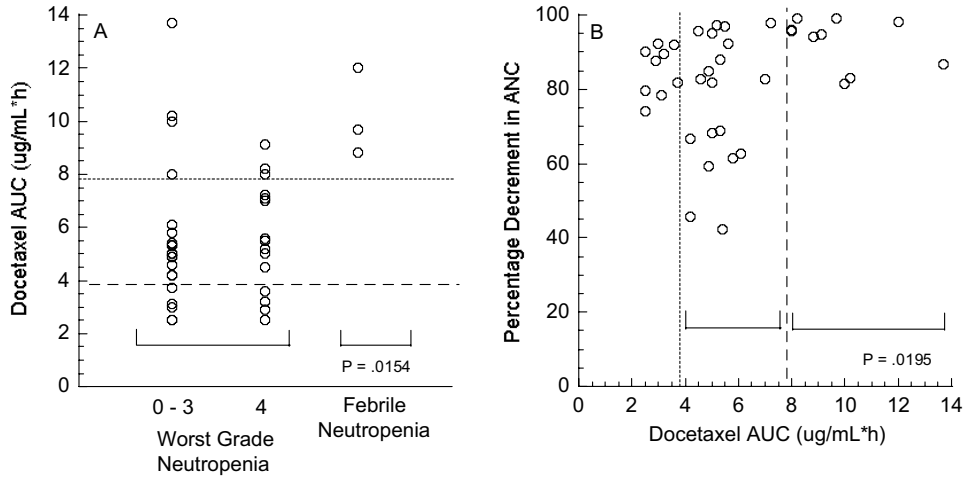

*Figure 2. [**A**] Worst grade of neutropenia (grade 0 – 3 versus grade 4 versus febrile neutropenia) and [**B**] percentage decrease in absolute neutrophil count as a function of docetaxel AUC. Dotted lines are the 25% quantile and dashed lines are the 75% quantile.*

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%)				

[a]Data is number of patients (% of patients)

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 (ie, 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 9

Weekly paclitaxel as first-line chemotherapy for elderly patients with metastatic breast cancer. A multicenter phase II trial

A.J. ten Tije,[1] C.H.Smorenburg,[1,2] C. Seynaeve,[1] A. Sparreboom,[1.3] K.L.C. Schothorst,[1] L.G.M. Kerkhofs,[4] A.G.P.M. van Reisen,[5] G. Stoter,[1] M. Bontenbal,[1] J. Verweij[1]

[1]Department of Medical Oncology, Erasmus MC - Daniel den Hoed Cancer Center, Rotterdam, the Netherlands; 2. Present address: Dept of Medical Oncology, Vrije Universiteit Medical Center Amsterdam, The Netherlands; 3. Present address: Clinical Pharmacology Research Core, Medical Oncology Clinical Research Unit, National Cancer Institute, Bethesda MD, USA; 4. Dept. of Internal Medicine, Ziekenhuis Walcheren, Vlissingen, The Netherlands; 5. Dept of Internal Medicine, Ziekenhuis Zeeuwsch-Vlaanderen, Terneuzen, The Netherlands.

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Abstract

Paclitaxel is a cytotoxic agent with proven antitumour activity in metastatic breast cancer. Weekly administration of paclitaxel has demonstrated sustained efficacy together with a more favourable toxicity profile (e.g. less myelotoxicity) than the 3-weekly administration. This study evaluates the activity and toxicity of weekly paclitaxel (Taxol®) as first-line chemotherapy in elderly patients (>70 years of age) with hormone-refractory metastatic breast cancer. Patients with metastatic breast cancer received 80 mg/m^2 paclitaxel administered weely on days 1, 8, and 15 of a 28-day cycle. Additional cycles were given until disease progression, or unacceptable toxicity. A dose increase to 90 mg/m^2 was allowed in the absence of toxicity. 26 Patients received a total of 101 cycles (median 4, range 1 – 11). 22 patients completed at least two cycles (six administrations). In 23 patients who were evaluable for response, there were 10 partial responses (38%), 9 patients with stable disease (35%), while 4 patients had disease progression (15%). The median duration of response was 194 days (>6 months). Overall treatment was relatively well tolerated, but 8 patients (32%) had to prematurely discontinue treatment because of fatigue. Neuropathy > grade 1 was noted only after five or more cycles in 4 patients). Weekly paclitaxel at this dose and schedule is an effective treatment regimen in the elderly patient with metastatic breast cancer, and is feasible but yields relevant fatigue in a subset of patients.

Introduction

The incidence of breast cancer increases with age. Because the population is ageing, the number of elderly women with breast cancer is expected to rise significantly in the near future. The treatment of cancer in elderly patients is increasingly recognised as an important challenge to the medical community.[1] Despite the fact that patients older than 70 years of age account for >25% of all breast cancer cases, only a small fraction of this group is generally entered into clinical studies.[2,3] Consequently, our knowledge of the use of chemotherapy in the elderly is based on very sparse data. Therefore, there is an urgent need to develop chemotherapy regimens that are well tolerated by elderly patients.

Taxanes have been used in a large number of trials investigating their activity in cancer patients. Studies with docetaxel in these patients were limited to patients younger than 75 years of age.[4] Only one trial on weekly

docetaxel in elderly breast cancer patients (>65 years) has demonstrated that docetaxel at a dose of 36 mg/m^2 is feasible in this group of patients, with 36% of patients achieving an objective response.[5]

Paclitaxel is an active drug in first-line therapy of metastatic breast cancer, as well as in patients with relapsed or refractory disease.[6-8] Response rates of 21–61% in previously untreated patients have been reported in phase II and III trials evaluating paclitaxel at doses of 135-250 mg/m^2 in a 3-weekly schedule.[6,8-16] *In vitro* experiments and clinical studies have suggested that prolonged exposure to paclitaxel, through either a continuous infusion schedule or a weekly administration, can lead to enhanced cytotoxicity, while maintaining a favourable toxicity profile.[17-19] The weekly schedule of administrating paclitaxel therefore seems an attractive chemotherapeutic regimen for elderly patients. Paclitaxel has been used in elderly patients, but specific trials for this population, exploring the weekly administration schedule as first-line treatment, were lacking. We performed such a study in patients >70 years of age with hormone-refractory metastatic breast cancer to assess the activity and toxicity.

Patients and methods

Eligibility

Patients who were previously chemotherapy-naïve with respect to their metastatic disease and refractory to hormonal treatment were eligible for this study. Other eligibility criteria included age of at least 70 years, histologically documented and measurable (or evaluable) metastatic breast cancer; a baseline World Health Organization (WHO) performance score (PS) of ≤ 2; a life expectance of at least 3 months; bilirubin <25 µmol/l; creatinine <175 µmol/l; white blood cells (WBC) count >1.5 x 10^9/l; platelet count >100 x 10^9/l; haemoglobin >6.0 mmol/l; no signs of central nervous system (CNS) involvement; or neuropathy > WHO grade 1. All patients gave their written informed consent. The institutional ethical boards of the participating hospitals approved the study.

Pre-treatment evaluations included a medical history, complete physical examination, a complete blood count with differential, and the following serum chemistry tests: electrolytes, creatinine, glucose, alkaline phosphatase, aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), lactate-dehydrogenase (LDH), and total and direct bilirubin. The cardiological function was evaluated by electrocardiogram (ECG), and by a multiple gated acquisition (MUGA) scan when indicated. All sites of disease

were documented by computerised tomography (CT), X-ray, or bone scan, depending on the site of disease activity.

Treatment

Paclitaxel (Taxol®, Bristol-Myers Squibb, Woerden, The Netherlands) was infused intravenously (I.V.) over 1 hour, at a dose of 80 mg/m^2, and given on day 1, 8 and 15 of a 28 day cycle. Standard intravenous premedication to prevent hypersensitivity reactions (HSR) consisted of dexamethasone 8 mg, clemastine 2 mg and ranitidine 50 mg administered approximately 30 minutes before the paclitaxel infusion.[20] During treatment blood cell count and toxicity assessment were performed weekly. Toxicity was evaluated using the National Cancer Institute common toxicity criteria version 2.0 (URL:https://webapps.ctep.nci.nih.gov/ ctcv2/plsql/ctc000w$.startup). The dose of paclitaxel was modified depending on the haematological and non-haematological effects observed. The treatment was postponed in case of a neutrophil count < 0.5 x 10^9/l, and/or platelet count < 50 x 10^9/l, febrile neutropenia (temperature > 38^{o}C and neutrophil count < 1.0 x 10^9/l), any grade > 1 non-haematological toxicity, except nausea and vomiting or alopecia. When treatment had to be postponed for a second week, the patient went off study. In case the administration had to be postponed for 1 week, the dosage of paclitaxel was reduced with 10 mg/m^2 in the next course. In case more than two dose reductions were necessary, the patients went off study. In patients who tolerated the weekly regimens for 3 consecutive administrations without delay, a dose escalation to 90 mg/m^2 per administration was allowed at the discretion of the treating physician.

Response evaluation and follow-up

Response evaluation criteria in solid tumours (RECIST) criteria were used to define measurable and evaluable disease and response.[21] Response was evaluated after every two cycles of treatment (i.e. every 8 weeks), and every 2 months thereafter for the first year and every 3 months for the following years, for all responding and stable patients until progression. Paclitaxel treatment was stopped in the case of progressive disease, stable disease (SD) after 16 weeks (four cycles), at patient's preference at any time, or at unacceptable toxicity.

Statistical analysis

The primary endpoint in this study was the overall response rate of weekly administered paclitaxel.

The sample size was calculated based on the assumption that a 40% objective response rate would be detected. The accrual consisted of two stages. If there were no complete or partial responses in the first 6 enrolled patients, the study would be terminated. In the case of one or more responses in these 6 patients, 19 additional patients would be enrolled (for a total of 25 patients), so that the standard error of response rate would be less than or equal to 0.10. This scheme ensured that if the drug is active in at least 40% of the patients, the chance of erroneously rejecting the drug after the first 6 patients is less than 5%. The advantage of such two-stage scheme is that it allows early rejection of an ineffective drug.

Time to disease progression (TTP) was estimated from the beginning of paclitaxel therapy, while duration of response (DR) was determined from the date the response [complete response (CR) or partial response (PR)] was initially reported. Patients who discontinued treatment for any reason or died from probable disease-related causes were considered, at that time, as having disease progression.

The Kaplan-Meier analysis method was used to calculate duration of response, and TTP curves.

Results

Patient characteristics

The demographics of the 26 enrolled patients is depicted in Table 1. All but 5 patients presented with a PS of 0-1. The time from first diagnosis of breast cancer to study entry was more than 12 months in 19 patients (73%), 6 – 12 months in 1 patient (4%), and less than 6 months in 6 patients (23%). All patients were chemotherapy naïve for their metastatic disease.

Treatment characteristics

A total of 101 treatment cycles was administered to 26 patients. Since two responses were noted in the first 6 patients, a total of 25 patients had to be included according to the protocol. All patients were evaluable for toxicity. One patient was replaced due to the development of a severe HSR immediately at the start of the first paclitaxel infusion. One other patient developed erythema after paclitaxel infusion, but she was evaluable for toxicity evaluation after this single course. One patient received only 2 cycles due to vomiting (grade 3) and refused further treatment. In 6 out of the 23 remaining patients (26%), the dose was escalated to 90 mg/m^2. In 2 patients, the dose was lowered to 70 mg/m^2. The median delivered dose

intensity was 240 mg/m^2/4-week-cycle (range 210 – 270). The median number of cycles delivered was 4 (range 1 – 11). 22 patients completet at least 2 cycles, 15 patients completed 4 or more cycles. Treatment delay was uncommon and was most often related to patients' requests, rather than toxicity. Nine patients (35%) continued treatment after 4 cycles.

Table1. Patient and tumour characteristics

	n	(%)
n	26	(100)
Age in years		
Median	77	
Range	71-84	
Performance status		
0	4	(15)
1	17	(65)
2	5	(20)
ER/PR		
Positive	10	(39)
Negative	10	(38)
Unknown	6	(23)
Previous adjuvant		
Chemotherapy	1	(4)
HT	6	(23)
Previous hormonal treatment for metastatic disease		
0	7	(27)
1 line	2	(8)
2 lines	9	(35)
≥ 3	8	(31)
No of metastatic sites		
1 organ	2	(8)
2 organs	11	(42)
≥ 3 organs	13	(50)
Site of metastasis		
Locoregional only	1	(4)
Distant		
Bone	19	(73)
Lung	6	(23)
Liver	10	(38)
Lymph Node	11	(42)
Distant only	18	(69)
Locoregional + distant	7	(27)

HT = hormonal therapy; ER = oestrogen receptor; PR = progesterone receptor

Table 2. Worst grade toxicity per patient observed (% of patients)[a]

NCI toxicity grade Toxicity	1	2	3	4
Neutropena	50	23	12	-
Anaemia	15	27	12	-
Thrombocytopenia	-	-	-	-
Infection	-	4	-	-
Febrile neutropenia	-	4	-	-
HSR	-	4	4	-
Fatigue	27	38	4	-
Alopecia	15	73	NA	NA
Neuropathy	23	12	4	-
Myalgia	8	-	-	-
Nausea	4	12	-	-
Vomiting	-	4	4	-
Nail disorders	8	4	-	-
Stomatitis	23	8	-	-

[a]26 patients were evaluable for toxicity, of whom 1 patient received ony a few mg's of paclitaxel
HSR=hypersensitivity reactions; NCI=National Cancer Institue; NA=not available.

Toxicity

Toxicity data were evaluated in the 25 patients receiving at least one full cycle. Toxicity data are outlined in Table 2. Overall, paclitaxel therapy was relatively well tolerated and manageable on an outpatient basis (Table 2). Myelosuppression was mild and relatively infrequent. Fatigue constituted an important problem and occurred in 67% of patients. In 8 patients (32%), fatigue was the reason for treatment discontinuation. Fatigue could not be related to anaemia. In many of these elderly patients, the distinction between cancer-related and treatment-related fatigue was difficult to determine. Neuropathy occurred in 39% of patients and resulted in discontinuation of treatment in three patients. Neuropathy grades 2 and 3 were only seen after 5 or more courses in 4 out of 9 patients. Nausea was observed in 11 patients, but not during all their paclitaxel administrations. Alopecia grade 1 developed in 4 patients; grade 2 in 15 patients. Other toxicities consisted of oedema and nail changes in less than 10% of patients, and were all easy to manage (CTC grades 1 and 2). Two patients were withdrawn from the study

due to HSR. One patient developed a grade 3 toxicity (neutropenia and generalised erythroderma) after the first administration; the other patient suffered a severe allergic reaction with hypotension after the infusion of a small amount of paclitaxel and was not evaluable for further toxicity.

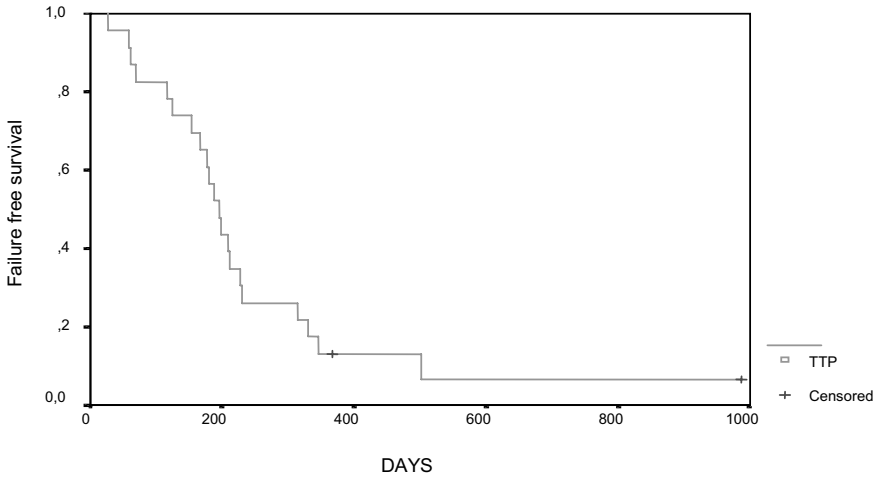

Figure 1. Kaplan-Meier analysis of time to progression (TTP) of patients treated with weekly paclitaxel. Median time to progression = 194 days. Data were censored at May 1, 2003; at this time, 2 patients were still free of progression.

Tumour response and survival

In 23 out of 26 patients (88%) enrolled, the response could be assessed. A total of 3 patients were not evaluable for response due to early treatment discontinuation because of severe HSR in 2 patients, and vomiting grade 3 in 1 patient. Two patients withdrew informed consent because of side-effects after 2 treatment courses. Ten patients achieved PR (38% - intent-to-treat-analysis), complete responses were not seen. In addition, SD was observed in 9 patients.

Time-to-progression (TTP) analysis (Figure 1) was performed on May 1, 2003, at which time 15 patients had died, including the 3 non-evaluable patients. Two patients were still in remission. The median time-to-progression was 6.5 months. Median follow-up time for surviving patients was 557 days (range 196-1141).

Discussion

Weekly paclitaxel is clearly active as first line treatment in patients with metastatic breast cancer, and has several suggested advantages over 3-week schedules in terms of both toxicity and probably efficacy.[16,22-24] In the current study, we assessed toxicity and efficacy of weekly paclitaxel as first line treatment in patients older than 70 years with metastatic breast cancer. This is a clearly underrepresented age group in trials for chemotherapeutic treatment of metastatic breast cancer, which is probably due to the high incidence of co-morbidity, and the reluctance of physicians to treat elderly patients with chemotherapy. Nevertheless, in this phase 2 study a sufficient number of patients was included, although the accrual was relatively slow.

Weekly paclitaxel at this dose and schedule yielded a response rate of 38%. This response rate seems relatively high compared to the response rate of 20% reported by Perez and colleagues,[25] but might be explained by the differences in pre-treatment. All of our patients except one were chemotherapy-naïve, while 82% of patients in the study of Perez received prior chemotherapy. Response rates of 21 to 49% have been reported from other multicentre trials of single agent paclitaxel administered at different doses and with different infusion schedules every 3 weeks to patients with metastatic breast cancer.[6,8-16] Thus, our response results are within the range observed in other trials with paclitaxel. In addition, weekly treatment with both docetaxel[5] and vinorelbine[26] in elderly patients revealed similar response levels.

The regimen appears relatively feasible, but the observation of fatigue in 67% of patients is of concern. In other studies with weekly administrations of paclitaxel in elderly patients, a similar incidence of asthenia was reported.[25,27] This side-effect following weekly docetaxel treatment in elderly appears to be even more severe when compared with paclitaxel treatment, since Hainsworth and colleagues reported grade 3 fatigue in 20% of patients, and grades 1 and 2 in 73% of patients.[5] Given the relatively short median treatment period of 16 weeks, the incidence of neuropathy in this weekly paclitaxel regimen is another reason for concern, although neuropathy > grade 1 was only noted after 5 or more cycles. By contrast, docetaxel causes hardly any neuropathy in the weekly regimen.[5]

In agreement with other studies with weekly paclitaxel, only a few patients (12%) developed serious haematological side-effects of neutropenia of more than grade 2. This is in line with the haematological side-effects

reported in the weekly docetaxel regimen.[5] In the weekly regimen with vinorelbine, haematological side-effects were the dose-limiting toxicity.[26]

The pharmacokinetic study reported by Smorenburg and colleagues performed in this group of patients revealed that the clearance of both unbound and total paclitaxel are significantly lower in elderly women with metastatic breast cancer, as compared with younger females (124 ± 35.0 versus 237 ± 43.0 l/h/m^2 (p=0.002), and 13.9 ± 2.31 versus 17.4 ± 4.52 l/h/m^2 (p=0.004) respectively.[28] In the entire population, a significant negative correlation was observed between age and unbound paclitaxel clearance. Therefore, we anticipated observing increased toxicity in this elderly population, compared with younger patients. Obviously, a formal comparison cannot be made. However, the number of treatment discontinuations based on fatigue, and to a lesser extent based on neuropathy, is of concern. It suggests a decreased tolerance in this elderly population. Whether this is related to the decrease in drug clearance remains to be elucidated. In addition, the same pharmacokinetic study revealed a significant and rather unusual increase in Cremophor EL (CrEL) clearance. Since neuropathy[29,30] and HSR[20,30] are partly related to this vehicle, a lower incidence of these side-effects could be expected. However, this was not the case.

In conclusion, the weekly administration of paclitaxel is an effective first line regimen for elderly patients with metastatic breast cancer, but yields relevant toxicity. Fatigue is the main toxicity, and, overall, is the main reason for treatment discontinuation. Weekly paclitaxel can be considered for elderly patients with metastatic breast cancer, although will not be tolerated in the longer run in an important sub-set of patients.

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Summary, conclusions

and future perspectives

From the early nineties both paclitaxel and docetaxel have obtained a prominent position in anticancer treatment. Many publications have since discussed their clinical pharmacological properties, although many aspects still remain to be elucidated, in particular those related to pharmacokinetic interference by other agents administered concomitantly.

Chapter 2 reviews the neglected role of the non-ionic formulation vehicles Cremophor EL and Tween 80, that are used to administer the poorly water-soluble drugs paclitaxel and docetaxel, respectively. In contrast to earlier views, these excipients are not inert, but can exert a variety of side effects and can cause clinically significant drug interactions.

In **chapter 3** the comparative pharmacokinetics of both vehicles is described in a group of patients with cancer. The study revealed that the relative systemic exposure to Tween 80 in humans is much lower as compared to Cremophor EL, as a result of different rates of elimination. The disappearance of Tween 80 from the central compartment was characterized by a short terminal half-life with a mean (± standard deviation (SD)) value of 0.607 ± 0.245 hrs and a total plasma clearance of 7.70 ± 2.90 L/h. In contrast, elimination of Cremophor EL was much slower, with values for half-life and clearance of 35.7 ± 18.9 hours and 0.216 ± 0.075 L/h, respectively. The slower clearance of the latter vehicle is consistent with Cremophor EL being more likely to be associated with drug interactions and excipient-related toxic effects. It is therefore recommended to evaluate the pharmacokinetic properties of excipients as an integral component of the development of poorly water-soluble agents.

In **chapter 4** the interaction of the P-glycoprotein inhibitor valspodar (PSC833; Amdray) with the pharmacokinetics of paclitaxel is described in order to explain the increased myelotoxicity of paclitaxel when it is given in combination with valspodar. In a clinical study, it was shown that valspodar lacks the significant interaction with paclitaxel as compared to studies with other P-glycoprotein modulators. The apparent clearance of unbound paclitaxel was not significantly different with mean (± SD) values of 230 ± 49.9 and 202 ± 49.9 L/h/m^2 in the absence and presence of valspodar, respectively. These findings further suggest that the plasma concentrations of paclitaxel may not be an appropriate measure to monitor the impact of P-glycoprotein inhibition.

In **chapter 5** an interaction study on docetaxel combined with ketoconazole is reported. This combination of drugs is currently being developed for the treatment of prostate cancer. Ketoconazole is a known potent inhibitor of the main enzyme involved in the elimination of docetaxel (i.e., CYP3A4), and hence an interaction was suspected. Indeed, the concomitant administration of ketoconazole with docetaxel resulted in a significant 32% decrease in clearance of the taxane. The mean clearance values of docetaxel were 33 L/h (range, 20 – 50 L/h) and 27 L/h (range, 7 – 46 L/h) in the absence and presence of ketoconazole, respectively. The interaction with ketoconazole resulted in an increase in docetaxel concentrations that were previously shown to be associated with an up to 4-fold increase in the odds to develop neutropenic fever at the recommended dose of docetaxel. This suggests that substantial dose reductions are required when docetaxel is combined with agents interfering with CYP3A4 activity.

The penetration of cytostatic agents into the central nervous system remains a controversial issue. In **chapter 6** the penetration of docetaxel in the cerebrospinal fluid (CSF) in 2 breast cancer patients with leptomeningeal metastasis is reported. Although the concentration of docetaxel in CSF remains well below those measured in plasma, the pharmacologically-relevant fraction unbound docetaxel in plasma samples ranged from 5.9 to 12.8%, while those in CSF ranged from 66.7 to 103%. Since the drug remained in CSF much longer than in plasma, the penetration and retention of docetaxel in CSF can potentially reach levels associated with significant antitumor activity.

Despite the increasing numbers of elderly patients presenting with cancer, only few pharmacological studies have been conducted in this subgroup of patients. Furthermore, elderly patients are underrepresented in trials on cancer therapy. The pharmacokinetics of anticancer drugs may be altered with aging due to several factors, including differences in end organ function and body composition. Besides that, elderly patients may be intrinsically more susceptible to toxic effects of certain cytostatic agents. In **chapter 7** the pharmacokinetics of both unbound and total paclitaxel in 8 elderly women (age ≥ 70 years) with breast cancer are compared to a control group of 15 patients aged < 70 years. In both groups paclitaxel was administered once weekly at a dose of 80 or 100 mg/m^2. The clearance (± SD) of unbound paclitaxel and total paclitaxel was 124 ± 35.0 and 13.9 ± 2.3 L/h/m^2 in the elderly group vs. 244 ± 58.8 and 17.4 ± 4.5 L/h/m^2 in the control group,

respectively. The total plasma clearance of Cremophor EL was 150 ± 60.7 vs. 115 ± 39.2 mL/h/m^2, respectively. These data indicate that the clearance of unbound paclitaxel is approximately 50% reduced in elderly patients as compared to younger patients, resulting in a significant increase in systemic exposure with age. The unexpected increase in clearance of Cremophor EL in the elderly may (in part) explain the altered clearance of unbound paclitaxel.

In contrast to paclitaxel, a prospective study on docetaxel administered at a dose of 75 mg/m^2 in a 3-weekly regimen in elderly patients (≥ 65 years) and patients < 65 years, described in **chapter 8**, 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 CYP3A4, 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.

In **chapter 9,** the results of a multicenter Phase II clinical trial is described on the chemotherapeutic treatment of elderly patients with breast cancer receiving single agent paclitaxel once weekly at a dose of 80 mg/m^2. The study revealed that with this schedule a response rate of 38% can be achieved, with 34% of patients showing stable disease. The median duration of response was > 6 months and overall treatment was well tolerated with only mild and infrequent myelosuppression. Fatigue constituted an important problem and occurred in 67% of patients.

Future perspectives

The work presented in this thesis aimed at identifying factors involved in pharmacokinetic alterations for taxane drugs, including formulation vehicles, concomitant medication and patient demographic characteristics like age. However, there is still a substantial degree of interpatient variation in the pharmacokinetics of both drugs, ultimately leading 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 plasma protein binding capacity and/or in hepatic metabolism by members of the cytochrome P-450 family.

Currently ongoing studies will focus on the role of inherited factors regulating the expression and function of these proteins, and will hopefully lead to a more predictable pharmacokinetic behavior of the taxanes. However, it is likely that in addition to genetic components, other environmental and physiological factors not studied here may influence the clinical pharmacology of the taxanes. Hence, it seems imperative to design additional prospective studies in the future employing both genotyping and phenotyping approaches of proteins crucial to drug elimination in order to eventually individualize and improve chemotherapeutic therapy with taxanes.

Finally, the drawbacks presented by the presence of Cremophor EL and Tween 80 as an integral component of the pharmaceutical formulation of paclitaxel and docetaxel have instigated extensive research to develop alternative delivery systems. These alternative formulations of the taxanes should 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, including those involving nanoparticles, 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, leading to a more favorable treatment outcome.

Samenvatting, conclusies

en toekomstige ontwikkelingen

Samenvatting, conclusies en toekomstige ontwikkelingen

Sinds de jaren 90 hebben zowel paclitaxel als docetaxel een belangrijke plaats verworven in de behandeling van kanker. In vele publicaties zijn de klinisch farmacologische eigenschappen van beide middelen besproken. Desondanks zijn er nog veel onopgehelderde aspecten, met name de interactie met andere, gelijktijdig toegediende geneesmiddelen.

In **hoofdstuk 2** wordt een overzicht gegeven over de onderschatte rol van de niet-ionogene oplosmiddelen Cremophor EL en Tween 80, die respectievelijk gebruikt worden om de slecht-wateroplosbare middelen paclitaxel en docetaxel toe te dienen. In tegenstelling tot vroegere opvattingen zijn beide oplosmiddelen niet inert, maar kunnen aanleiding geven tot vele bijwerkingen en leiden tot klinisch relevante interacties met (andere) geneesmiddelen.

In **hoofdstuk 3** worden de farmacokinetische eigenschappen van beide oplosmiddelen nader belicht aan de hand van een studie uitgevoerd bij kankerpatiënten. Deze studie leerde dat de relatieve blootstelling aan Tween 80 veel geringer is ten opzichte van Cremophor EL t.g.v. een verschil in afbraaksnelheid. Tween 80 heeft een korte halfwaardetijd met een gemiddelde waarde (± standaard deviatie (SD)) van 0,607 ± 0,245 uur en een plasmaklaring van 7.70 ± 2.90 L/uur. De eliminatie van Cremophor EL was daarentegen beduidend langzamer, met een halfwaardetijd van 35,7 ± 18,9 uur en een plasmaklaring van 0,216 ± 0,075 L/uur. De trage klaring van Cremophor EL is overeenkomstig observaties dat dit oplosmiddel vaker aanleiding geeft tot geneesmiddelinteracties en bijwerkingen dan Tween 80. Het is daarom aan te bevelen om bij de ontwikkeling van slecht-wateroplosbare geneesmiddelen ook de farmacologische eigenschappen van oplosmiddelen in ogenschouw te nemen.

In **hoofdstuk 4** wordt de interactie van de P-glycoproteineremmer valspodar (PSC833; Amdray®) beschreven, om inzicht te verwerven in de toegenomen paclitaxel-gerelateerde beenmergtoxiciteit bij gelijktijdige toediening van valspodar. In een klinische studie wordt aangetoond dat er geen duidelijke aanwijzingen zijn voor een farmacologische interactie tussen beide geneesmiddelen, i.t.t. studies met andere P-glycoproteineremmers. De gemeten klaring van ongebonden paclitaxel bedroeg 230 ± 49,9 L/uur/m^2 (gemiddelde (± SD)) in de afwezigheid en 202 ± 49,9 L/uur/m^2 in de

aanwezigheid van valspodar; hetgeen niet significant verschillend is. Deze resultaten suggereren dat het meten van paclitaxel concentraties in het plasma geen goede manier is om de gevolgen van P-glycoproteineremming te meten.

In **hoofdstuk 5** wordt een interactie-studie over de combinatie van docetaxel met ketoconazole beschreven. De combinatie van deze geneesmiddelen wordt momenteel ontwikkeld voor de behandeling van prostaatkanker. Ketoconazole is echter een krachtige remmer van het leverenzyme CYP3A4 dat betrokken is bij de afbraak van docetaxel, waardoor farmacologische interacties mogelijk zijn. Inderdaad bleek de gemiddelde docetaxelklaring in respectievelijk af- en aanwezigheid van ketoconazole 33 L/uur (uitersten, 20 – 50 L/uur) en 27 L/uur (uitersten, 7 – 45 L/uur). Deze significant vertraagde plasmaklaring van 33% resulteerde in een toename van docetaxelconcentraties die geassocieerd zijn met een 4-voudige toename in de kans op het ontwikkelen van neutropene koorts bij de aanbevolen standaarddosering van docetaxel. Het lijkt derhalve dat substantiële dosis reducties van docetaxel noodzakelijk zijn, indien het middel moet worden gecombineerd met remmers van CYP3A4.

De doordringbaarheid van het centraal zenuwstelsel voor cytostatica blijft een controversieel onderwerp. In **hoofdstuk 6** wordt de penetratie van docetaxel in het hersenvocht beschreven bij twee patiënten met borstkanker met leptomeningeale metastasering. Alhoewel de gemeten docetaxel-concentraties in het hersenvocht duidelijk lager zijn dan die in het plasma, bleek het farmacologisch relevante, ongebonden docetaxel in plasma te variëren van 5,9 tot 12,8%, terwijl die in de hersenvocht varieerde van 66.7 tot 103%. Omdat docetaxel veel langer in het hersenvocht verbleef dan in plasma, zou dit kunnen leiden tot antitumoractiviteit.

Ondanks de recent toegenomen prevalentie van ouderen met kanker, zijn er maar weinig farmacologische studies verricht in deze specifieke patiëntengroep. Verder zijn oudere patiënten duidelijk ondervertegen-woordigd in klinische onderzoeken gericht op de behandeling van kanker. De farmacokinetiek van cytostatica zou kunnen veranderen op hogere leeftijd t.g.v. een aantal factoren, zoals veranderingen van orgaanfuncties en lichaamssamenstelling. Daarnaast zouden ouderen meer gevoelig kunnen zijn voor de bijwerkingen van cytostatische behandeling. In **hoofdstuk 7** wordt de farmacokinetiek van paclitaxel in 8 oudere patiënten (≥ 70 jaar)

vergeleken met die van 15 patiënten < 70 jaar. In beide groepen werd paclitaxel toegediend in doseringen van respectievelijk 80 of 100 mg/m^2. De plasmaklaring van zowel vrij als totaal paclitaxel was 124 ± 35,0 and 13.,9 ± 2,3 L/uur/m^2 in de oudere groep vs. 244 ± 58,8 and 17,4 ± 4,5 L/uur/m^2 in de groep jongere patiënten. Daarentegen was de plasmaklaring van Cremophor EL in de oudere groep juist hoger: 150 ± 60,7 vs. 115 ± 39,2 mL/uur/m^2. Deze resultaten tonen dat de klaring van vrij paclitaxel in de oudere patiënten met ca. 50% is afgenomen t.o.v. de jongeren, hetgeen zal leiden tot een significante toename in blootstelling aan vrij paclitaxel. De onverwachte toename in Cremophor EL klaring zou een mogelijke verklaring kunnen zijn voor de afname in vrij paclitaxel klaring.

In tegenstelling tot paclitaxel, leerde een studie beschreven in **hoofdstuk 8**, dat de docetaxelklaring niet veranderd op hogere leeftijd. De gemiddelde (± SD) plasmaklaring van docetaxel in de ouderen was 30,1 ± 18,3 vs. 30,0 ± 14,8 L/uur in de jongere groep. Ter verdere ondersteuning voor de afwezigheid van een leeftijdseffect bleek ook de fenotypische activiteit van het lever enzym CYP3A4, gemeten m.b.v. de erythromycineademtest, niet veranderd op hogere leeftijd.

In **hoofdstuk 9** worden de resultaten beschreven van een klinisch fase II onderzoek verricht in samenwerking met diverse Nederlandse instituten naar de chemotherapeutische behandeling van oudere borstkanker patiënten met paclitaxel in een eenmaal-per-week toediening van 80 mg/m^2. Deze studie toont dat met dit schema een responspercentage van 38% kan worden bereikt, met daarnaast 34% van de patiënten met stabilisering van de ziekte. De mediane responsduur was > 6 maanden en over het algemeen werd de behandeling goed verdragen met relatief geringe beenmergtoxiciteit. Vermoeidheid vormde de belangrijkste bijwerking en trad op in 67% van de patiënten.

Toekomstige ontwikkelingen

Het werk gepresenteerd in dit proefschrift had tot doel het identificeren van factoren die de farmacokinetiek van taxanen kunnen beïnvloeden, zoals oplosmiddelen, gelijktijdig toegediende medicatie en patiëntkenmerken zoals leeftijd. Helaas is er nog steeds een substantiële interpatiëntvariatie in de farmacokinetiek van beide cytostatica, die zowel kunnen leiden tot onvoorspelbare toxiciteit en effectiviteit. Deze variatie wordt niet alleen

bepaald door de voornoemde factoren, maar waarschijnlijk ook door interpatiëntvariatie in metabolisme door het cytochroom P450 systeem en door variatie in de mate van eiwitbinding in plasma.

Momenteel zijn er studies gaande die de rol van erfelijkbepaalde factoren verder ontrafelen, zodat hopelijk in de toekomst het farmacokinetisch gedrag van beide taxanen beter wordt begrepen en dus ook beter kan worden voorspeld. Het is echter aannemelijk dat naast de erfelijkbepaalde factoren ook omgevingsinvloeden en fysiologische aspecten de farmacokinetiek van taxanen beïnvloeden. Het lijkt derhalve noodzakelijk om in de toekomst studies op te zetten die zowel genotypische als fenotypische factoren van eiwitten en enzymen betrokken bij het taxaanmetabolisme onderzoeken, om uiteindelijk taxanen op geïndividualiseerde basis te kunnen toedienen.

Tenslotte, de nadelen die de aanwezigheid van Cremophor EL en Tween 80 in de farmaceutische formulering van paclitaxel en docetaxel met zich meebrengen, hebben uitgebreide onderzoeken geïnitieerd om nieuwe toedieningsvormen te ontwikkelen. Deze alternatieve toedieningsvormen moeten uiteindelijk leiden tot minder oplosmiddelgerelateerde bijwerkingen en verminderde kans op het ontwikkelen van interacties met het actieve geneesmiddel. Dergelijke nieuwe formuleringen, bijvoorbeeld bestaande uit zogenaamde nanoparticles, moeten in de nabije toekomst toediening zonder pre-medicatie mogelijk maken en leiden tot langere blootstelling van de tumor aan het cytostaticum, met mogelijk verbeterde behandelingsuitkomst.

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Curriculum Vitae

Albert Jan ten Tije werd op 8 november 1964 geboren te Enschede. Na de brugklas in het Kottenpark College te Enschede werd het middelbaar onderwijs voortgezet aan het Jeanne d'Arc lyceum te Maastricht. In 1984 werd het eindexamen VWO-B behaald. Na inloting werd in 1985 gestart met de studie Geneeskunde aan de Rijksuniversiteit Limburg, te Maastricht. Tijdens de studie werd gedurende enkele maanden als Research Fellow op de afdeling nefrologie stage gelopen bij Sandoz (Novartis) in Bazel, Zwitserland. Aan het eind van de studie volgde een klinische stage oncologie bij Dr. J. Wils in Roermond, waarna de keuze voor de interne geneeskunde een feit werd.

Direct na het artsexamen werkte hij als AGNIO Interne Geneeskunde in het St. Clara Ziekenhuis te Rotterdam (opleider: Dr. A.F. Grootendorst) en het Reinier de Graaf Gasthuis te Delft (Dr. W. Hart). Januari 1994 werd begonnen met de opleiding tot internist in het Erasmus MC (Dijkzigt Ziekenhuis) (Prof.dr. M.A.D.H. Schalekamp).

In 1996 werd de opleiding gedurende 1 jaar onderbroken voor een research fellowship aan het James G. Brown Cancer institute in Louisville, KY in de Verenigde Staten. Onder leiding van V. Fingar† werden de systemische bijwerkingen van photodynamische therapie bij kanker patiënten bestudeerd. Verder werd een proefdiermodel ontwikkeld voor *in vivo* monitoring van longmetastastasering.

Vanaf januari 1997 tot januari 1999 werd de opleiding tot internist hervat in het St. Clara Ziekenhuis. Het laatste jaar van de opleiding vond wederom plaats in het Erasmus MC.

1 januari 2000 begon de vervolgopleiding in het aandachtsgebied Hematologie in hetzelfde centrum (Prof.dr. B. Löwenberg). Vanaf september 2000 tot november 2003 volgde de vervolgopleiding in het aandachtsgebied Medische Oncologie in het Erasmus MC – locatie Daniel den Hoed kliniek (Prof.dr. G Stoter). In deze periode kwam het proefschrift tot stand.

De auteur is thans werkzaam als internist-oncoloog in ziekenhuis Gooi-Noord te Blaricum.

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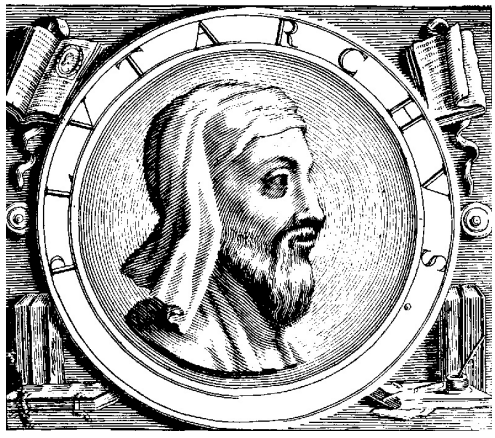
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