Pharmacokinetic Optimization of Docetaxel Dosing

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‘So here it is at last, the distinguished thing!’
(Henry James, 1843 - 1916)

Voor papa

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Chapter 1

Introduction to the Thesis

Introduction to the Thesis

In 1986, French researchers succeeded in the development and upscaling of a semi-synthetic process using the readily available inactive precursor, 10-deacetylbaccatin III isolated from the needles of the European yew tree *Taxus Baccata*, to produce the second taxane (the first being paclitaxel), docetaxel (Taxotere®). Docetaxel was initially approved for the treatment of patients with locally advanced or metastatic breast cancer. Now, approximately 10 years later, approval also includes the treatment of patients with non-small-cell lung cancer, androgen-independent prostate cancer and advanced gastric cancer, making this anticancer drug one of the most widely active agents currently available.

An important limitation associated with docetaxel chemotherapy, the recommended treatment being a 1-hour intravenous infusion once every 3 weeks ('3-weekly'), is the substantial and, more importantly, highly unpredictable interindividual variability in toxicity and efficacy. Moreover, a large population pharmacokinetic-pharmacodynamic (PK-PD) analysis demonstrated that variability in toxicity is strongly associated with wide interindividual variability in docetaxel pharmacokinetics [1]. Against this background it was the aim of the work described in this thesis to assess whether purposeful modulation of docetaxel pharmacokinetics could help to improve docetaxel-based treatment and to lay the foundation for the development of future improved (individualized) dosing strategies.

Although the majority of research aimed to improve the risk-benefit ratio for docetaxel treatment has focused on the pharmacokinetics of docetaxel since the drug's early clinical development, several other pharmacological strategies, including optimizing route and schedule of administration, reversing drug resistance, and the development of structurally related second-generation taxanes and alternative, solvent-free formulations, have also been investigated in an attempt to overcome the limitations associated with 3-weekly docetaxel treatment. These strategies are reviewed in **Chapters 2, 3 and 4**.

Hepatic cytochrome P450 (CYP) isozyme 3A activity, responsible for the extensive metabolism of docetaxel to several pharmacologically inactive metabolites [2], has been identified as the strongest predictor of docetaxel clearance, accounting for the majority of the interpatient pharmacokinetic differences [3]. Indeed, wide variation has been observed in phenotypic CYP3A activity and this has been attributed to a combination of factors including variations in both basal content and catalytic activity of total CYP3A, disease-related differences [4], drugs inducing or repressing transcription, and possibly genetic and ethnic differences [5-8]. Moreover, CYP3A activity can be readily induced or inhibited by concomitantly administered drugs, food constituents and complementary and alternative medicine, thus rendering docetaxel subject to a host of enzyme-mediated pharmacokinetic

drug-interactions. The influence of medicinal cannabis (variety Bedrocan®) on the pharmacokinetics of docetaxel was investigated in **Chapter 9** as we anticipated an increased use of medicinal cannabis in combination with anticancer drugs following the introduction, September 2003 in The Netherlands, of this standardized, legal product, with reported beneficial effects for cancer patients [9-12].

Recently attempts have been made to individualize docetaxel dosing through CYP3A phenotyping and genotyping strategies [13-16]. Despite the availability of phenotypic probes, all current phenotyping techniques are associated with practical disadvantages, and attempts to individualize docetaxel dosing through CYP3A genotyping strategies have, to date, yielded controversial results, in part due to underpowered studies. Thus presently, interindividual variability in CYP3A activity is not taken into account when dosing docetaxel. An alternative approach to optimize docetaxel dosing strategy is to temporarily eliminate the source of interindividual variability in CYP3A metabolic activity, i.e. to purposefully inhibit CYP3A temporarily. Theoretically, inhibiting CYP3A using a potent CYP3A-inhibitor such as ketoconazole [17], could provide a tool to reduce the substantial interindividual variability in docetaxel pharmacokinetics, thereby leading to a uniform pharmacokinetic profile and thus ultimately to a more predictable toxicity profile, while maintaining cytotoxic efficacy. Furthermore, this approach also has the potential to achieve a considerable reduction in drug costs (anticancer drug expenditure accounts for up to approximately 30 % of overall hospital drug costs) since a markedly reduced docetaxel dose is necessarily administered, due to ketoconazole-induced prolonged systemic docetaxel exposure. To investigate this strategy, intended modulation of docetaxel pharmacokinetics through oral ketoconazole administration was first evaluated using the standard antifungal ketoconazole dose [18] as described in **Chapter 5**. Evaluation of this strategy was continued in **Chapter 6** with a higher ketoconazole dose as interindividual variability in docetaxel pharmacokinetics was not reduced upon standard-dose ketoconazole, possibly due to wide interindividual variability in ketoconazole systemic exposure. In addition, high-dose ketoconazole in combination with docetaxel is currently under investigation for the treatment of patients with androgen-independent prostate cancer [19], allowing us to evaluate the undesirable clinical consequences of this drug-interaction.

Following extensive CYP3A-mediated metabolism, the administered docetaxel dose is primarily excreted into the faeces via the biliary route [20]. Indeed, only less than 10 % of the administered dose is excreted unchanged into the faeces and total urinary excretion urinary accounts for less than 5 % [21,22]. Temporarily inhibiting CYP3A activity therefore not only affects the plasma pharmacokinetic profile of docetaxel, but also influences the faecal and urinary excretion of the drug. Moreover, ketoconazole, besides being a potent CYP3A inhibitor, is also a modest inhibitor of the ATP-binding cassette membrane-localized

transporter, ABCB1 [23,24], for which docetaxel is also a substrate [25,26], and which plays a prominent role in the faecal elimination of docetaxel [27]. Collection of both urinary and faecal samples of the patients treated with standard- and high-dose ketoconazole in combination with docetaxel (**Chapters 5 and 6**) allowed us to assess purposeful ketoconazole-induced modulation of docetaxel faecal and urinary disposition and subsequently total excretion (i.e. mass balance) using [^{3}H]-docetaxel. Such an evaluation, described in **Chapter 8**, can contribute to a better overall understanding of the mechanistic aspects involved in docetaxel metabolism and elimination.

Ideally assessing a mass balance is undertaken by administering a non-toxic tracer amount of radiolabelled drug to a patient, allowing one to quantify the drug in biological samples (e.g. faeces, urine) at concentrations below the lower limit of quantification for routinely used analytical techniques. However, most radiolabelled biological samples, including faeces, require extensive sample preparation to reduce quenching interference before quantification of radioactivity is possible. Clearly, a more rapid and simple method has important advantages. **Chapter 7** describes the development and validation of a rapid and simple combustion method to quantify [^{3}H]-docetaxel excreted in human faeces and urine. The validated method was successfully applied in cancer patients administered docetaxel and a tracer amount of [^{3}H]-docetaxel as described in **Chapter 8**.

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Chapter 2

Docetaxel Administration Schedule: From Fever to Tears? A Review of Randomized Studies

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Abstract

The anticancer agent docetaxel is approved for the treatment of patients with locally advanced or metastatic breast cancer, non-small-cell lung cancer (NSCLC) and for the treatment of androgen-independent prostate cancer patients. At the recommended dose of 60 mg/m^2 to 100 mg/m^2 given once every 3 weeks, severe neutropenia is the dose-limiting toxicity and a major concern, especially when treating patients at high-risk for myelotoxic complications. A less toxic schedule, involving weekly docetaxel administration was developed for patients with a poor performance status, multiple comorbidities, poor haematological reserves, or those who were heavily pretreated and/or elderly or for patients for whom palliation is the focus of treatment. Recent randomized trials allow a comparison of efficacy and toxicity between weekly and 3-weekly treatments. Efficacy appears to be similar for the two schedules regardless of the disease, while weekly docetaxel is significantly less myelotoxic. However, this benefit comes at the cost of cumulative increases in hyperlacrimation, skin- and nail-toxicity and negatively affects quality of life. Currently, 3-weekly docetaxel remains the standard schedule for treatment, whereas the weekly schedule offers a possibility for treatment individualization for those patients where the risk of myelosuppression is considered unacceptable.

Introduction

The anticancer drug docetaxel (Taxotere®) is a semi-synthetic taxane, with antitumour activity against a broad range of human malignancies. It is approved for the treatment of patients with locally advanced or metastatic breast cancer or non-small-cell lung cancer (NSCLC) and more recently for the treatment of androgen-independent metastatic prostate cancer. The docetaxel dose recommended for treating cancer patients ranges from 60 mg/m^2 to 100 mg/m^2 given as a 1-hour infusion once every 3 weeks (hereafter referred to as '3-weekly'). Severe myelosuppression is common and a concern. Neutropenia occurs in virtually all patients regardless of dose and, treated with 100 mg/m^2 docetaxel, a substantial number of patients require a dose reduction to control grade 4 neutropenia lasting 1 week or longer. Management of neutropenic infection requires patient hospitalization and treatment with intravenous antibiotics. The other major side-effect is neuropathy that is related to cumulative dose and which can potentially limit the number of cycles that can be given. In general, non-haematological toxicities are rarely severe and mostly manageable.

In view of the myelosuppression, there are specific patient groups who are expected not to tolerate the full-dose 3-weekly regimen. In general, this applies to patients with a poor performance status, patients with multiple comorbidities, patients with poor haematological reserves, whether or not due to heavy pretreatment, and elderly patients. Indeed, treatment recommendations for the heterogeneous group of elderly patients are inconsistent or lacking

as the elderly are largely underrepresented in clinical trials coupled to a general reluctance to administer chemotherapy to this group [1,2]. In addition, prolonging survival without excessive treatment-related toxicity is a major challenge in the management of patients where treatment focus is not curative. For these reasons, soon after the introduction of docetaxel in 1996, clinical trials were initiated investigating alternative docetaxel schedules with infusions given once every week (hereafter referred to as 'weekly'). This was in spite of the fact that during the initial development of docetaxel, studies with a day-1 and day-8 schedule every 3 weeks were not pursued due to excessive toxicity [3]. The primary goal of the new set of studies was to reduce severe haematological toxicity while preserving dose intensity. Although, some investigators even hoped to increase dose intensity and achieve a higher cumulative dose. In the end, the recommended phase II dose for weekly docetaxel was established at 36 mg/m^2/week based on a schedule of 6 consecutive weekly administrations followed by a 2-week rest interval [4]. This dose is equivalent to a (planned) dose intensity of 27 mg/m^2/week and comparable to the (planned) dose intensity for the recommended 3-weekly doses, 75 mg/m^2 and 100 mg/m^2 (25 mg/m^2/week and 33 mg/m^2/week, respectively) [5]. The landmark phase I trial demonstrated that the toxicity profile of weekly docetaxel was significantly altered and that dose-limiting toxicities were fatigue/asthenia, while overall myelosuppression was mild and severe haematological toxicity uncommon. This new side-effect profile suggested a potential for better tolerance. Since then, numerous clinical trials have further investigated the activity of weekly administrations of docetaxel, both as single agent and, given its favourable toxicity profile, in combination with other (myelosuppressive) cytotoxic drugs, new biological agents and radiotherapy.

This review aims to discuss the efficacy and toxicity of weekly docetaxel and to compare these features to the 3-weekly regimen when used to treat patients with metastatic breast cancer, NSCLC and hormone refractory prostate cancer.

Efficacy of docetaxel treatment

3-weekly docetaxel – metastatic breast cancer, NSCLC, hormone refractory prostate cancer

Treatment of patients with locally advanced or metastatic breast cancer after failure of prior chemotherapy was the first indication for which docetaxel was granted approval [6,7], followed by approval for the treatment of locally advanced or metastatic NSCLC patients after failure of prior platinum-based chemotherapy [8,9], and treatment of chemotherapy-naïve NSCLC patients in combination with cisplatin [10,11]. In addition, based upon a recent randomized phase III trial it was concluded that first-line treatment with single agent docetaxel could be a reasonable option for patients who do not tolerate cisplatin [12]. After early phase II trials demonstrated activity of single agent docetaxel in the treatment of patients with hormone refractory prostate cancer [13,14], approval was recently also granted for this indication based on a large phase III trial [15]. An overview of the efficacy of 3-weekly

docetaxel in the treatment of patients with metastatic breast cancer, NSCLC and hormone refractory prostate cancer is provided in Table 1.

Weekly docetaxel – metastatic breast cancer

Several phase II clinical trials have assessed the activity of weekly docetaxel in patients with metastatic breast cancer, the majority of which had been pretreated with chemotherapy (Table 2) [16-22]. Unfortunately, only one trial was designed to directly compare safety and activity of weekly and 3-weekly treatment in a randomized phase II setting [21]. Two non-randomized trials specifically targeted the elderly and/or frail population [17,22]. The pivotal trials for the 3-weekly schedule in metastatic breast cancer patients had reported an overall response rate (ORR) of 47.8 % and 30 %, for patients previously treated with alkylating agent-containing or anthracycline-containing chemotherapy, respectively (Table 1) [6,7]. As summarized in Table 2, the activity of weekly schedules seems to be in the same range. Although not powered to detect a difference, the randomized phase II study also suggests similar activity [21]. The limitations of phase II trial designs are expressed in the fact that ORRs and time to progression (TTP) in the indicated studies range from 25 % to 41 %, and from 4 months to 9 months, respectively. Apart from differences in prognostic factors, part of the observed variation could be explained by selection bias. In that sense, the randomized phase II study [21] provides the most reliable information.

Weekly docetaxel – NSCLC

A number of phase II clinical trials have assessed weekly docetaxel in the treatment of patients with advanced NSCLC, both as first-line treatment, in combination with cisplatin, and as single agent second-line treatment (Table 3) [23-28]. Importantly, several randomized (phase II/III) trials are ongoing and definitive results are awaited [29-32].

Two recent clinical trials evaluated weekly docetaxel in combination with cisplatin [25,26]. The trial performed by Ohe and colleagues [25] specifically targeted elderly patients (≥ 75 years), who however, all had a good performance status (0-1). These two studies yielded activity in the same range as for 3-weekly docetaxel followed by cisplatin 75 mg/m^2 (Table 1) [10]. The results of randomized studies are again eagerly awaited. The importance of such phase III evaluations is further stressed by the fact that seemingly achieved activity is less than expected in chemotherapy-naïve elderly patients or (young) patients who were poor candidates for first-line combination therapy (Table 3) [23].

Lilenbaum and colleagues [24] evaluated second-line treatment with single agent docetaxel in a small (N = 30) group of patients, where 1 in 3 had a poor performance status (≥ 2). Importantly, it should be noted that all patients with a poor performance status progressed, underlining the 2003 treatment guidelines, which recommend that second-line treatment with docetaxel be confined to patients with adequate performance status [11], as well as the possible influence of selection bias on outcome parameters.

Table 1. Efficacy of 3-weekly docetaxel in metastatic breast cancer, non-small-cell lung cancer and hormone refractory prostate cancer patients

Reference	Chan [6]	Nabholtz [7]	Shepherd [9]	Fossella [8]	Fossella [10]	Georgoulias [12]	Friedland [13]	Picus [14]
N	161	203	55	125	408	152	21	35
Median age (range)	52 (32-74)[1]	51 (30-73)[2]	61 (37-73)	59	61 (30-81)	63 (41-77)	69 (55-79)	70 (49-85)
ECOG PS								
0-1			41 (74)	103 (82)		137 (90)	20 (95)	
≥ 2			14 (26)	23 (18)		15 (10)	1 (5)	
Karnofsky PS (range)	90 (60-100)	90 (60-100)			≥ 80[3]			≥ 60[4]
Extent of disease								
Visceral metastasis	121 (75)	153 (75)					2 (10)	
Bone metastasis	89 (55)	116 (57)					20 (95)	
Measurable lesions	129 (80)[5]	151 (74)[5]						25 (71)
Stage IIIB	NA	NA	15 (27)	12 (10)	135 (33)	54 (36)		
Stage IV	NA	NA	40 (73)	113 (90)	273 (67)	98 (64)		
Median PSA ng/ml, range	NA	NA	NA	NA	NA	NA	67 (9-2489)	96 (24-2070)
Prior Treatment								
Any chemotherapy	161 (100)[6]	203 (100)	55 (100)	125 (100)	0 (0)	9 (6)[7]	11 (52)	0 (0)
Anthracycline	0 (0)	203 (100)	NA	NA	NA	NA	NA	NA
Platinum-based	NA	NA	55 (100)	125 (100)	0 (0)	0 (0)	NA	NA
Dose, mg/m^2	100	100	75	75	75[8]	100	75	75
ORR, % (95% CI)	49 (40-56)	30 (24-36)	5.5	6.7	32 (27-36)	21 (15-29)		28
Median OS, months	15	11.4				8.0		27
1-year survival rate, %						43	33	
PSA response[9], %	NA	NA	NA	NA	NA	NA	38	46

Abbreviations: N, number of patients; ECOG PS, Eastern cooperative group performance status; NA, not applicable; ORR, overall response rate; CI, confidence interval; OS, overall survival; PSA, prostate specific antigen. Values between brackets are percentage of total number of patients, unless otherwise specified; [1]13 % aged > 65 years; [2]11 % aged > 65 years; [3]96 % of patients PS ≥ 80; [4]PS ≥ 60 inclusion criterion, majority of N PS 80; [5]≥ 1 measurable lesion; [6]Alkylating agent-containing therapy; [7]Adjuvant therapy, otherwise chemotherapy-naïve; [8]Followed by cisplatin 75 mg/m^2; [9]PSA response defined as ≥ 50 % decline from baseline PSA confirmed by second value.

Table 2. Efficacy of weekly docetaxel in patients with metastatic breast cancer

Reference	**Burstein [16]**	**Hainsworth [17]**	**Stemmler [18]**	**Aihara [19]**	**Mey [20]**	**Tabernero [21]**		**D'Hondt [22]**
N	29	41	35	37	20	41	42	47
Median age (range)	57 (35-75)	74 (50-88)	53 (42-66)	53 (31-74)	57 (28-80)	56 (25-75)	55 (33-72)	63 (43-82)
Age ≥ 70 years		27 (66)			1 (5)			11 (23)
ECOG PS								
0-1	28 (97)	32 (78)	Median[1]	36 (97)	16 (80)	39 (95)	40 (95)	17 (36)
≥ 2	1 (3)	9 (22)		1 (3)	4 (20)	2 (5)	1 (2)	30 (64)
Visceral metastasis								
Liver	19 (66)		20 (57)	7 (19)	13 (65)	17 (42)	20 (48)	30 (64)
Lung	14 (48)		23 (66)	11 (30)	5 (25)	13 (32)	17 (41)	19 (40)
Overall		30 (73)			16 (80)			34 (72)
Prior Treatment								
Any chemotherapy	19 (66)	20 (49)	35 (100)	34 (92)	20 (100)	40 (98)	41 (98)	37 (79)
Anthracycline	9 (31)	7 (17)	32 (91)	17 (46)	18 (90)	30 (73)	28 (67)	35 (74)
No chemotherapy	10 (35)	21 (51)	0 (0)	3 (8)	0 (0)	1 (2)	1 (2)	10 (21)
Dose, mg/m^2; schedule[2]	40; 6 / 2	36; 6 / 2	35; 6 / 2[3]	40; 3 / 1	40; 6 / 2	40; 6 / 2	100; 3-weekly	36; 6 / 1[4]
Dose intensity,mg/m^2/wk	30	27	26	30	30	30	33.3	31
Relative median dose intensity (range)	1.0			0.95 (0.73-1.00)		0.95 (0.54-1.07)	0.96 (0.68-1.00)	0.78
Median cumulative dose, mg/m^2 (range)	720 (80-1440)			560 (120-1080)	380	620 (80-2400)	614 (100-1200)	278
ORR, % (95% CI)	41 (24-61)	36	34 (18-51)	38 (22-53)	25	34 (21-51)	33 (20-50)	30
Median TTP, months (range or *95% CI*)		5 (3-21)	2.6 (1.5-≥5.5)	5	8.8	5.7 *(4-7.5)*	5.3 *(4.3-6.2)*	3.5 (0-25)
1-year survival rate, %		61			40			29
Median OS, months (95% CI)				12		29 (24-34)	20 (15-25)	7

Abbreviations: TTP, time to progression; wk, week; If no value specified then not available. Values between brackets are percentage of total number of patients unless otherwise specified; [1]Range 0-2; [2]Number of consecutive weekly treatments / number of rest weeks; [3]First cycle only, following cycles 3 / 2; [4]First cycle only, following cycles 2-3 / 1.

Table 3. Efficacy of weekly docetaxel in patients with locally advanced or metastatic non-small-cell lung cancer

Reference	Hainsworth [23]	Lilenbaum [24]	Ohe [25]	Tsunoda [26]	Gridelli [27]		Gervais [28]	
N	39	30	33	38	110	110	62	63
Median age (range)	71 (55-82)	68 (47-84)	77 (75-86)	62 (34-73)	62 (26-74)	63 (28-75)	59 (37-72)	58 (26-74)
Age ≥ 65 years	20 (51)[1]							
Age ≥ 75 years			33 (100)[2]					
ECOG PS								
0-1	23 (59)	21 (70)	33 (100)	37 (97)	93 (85)	92 (84)	49 (79)	50 (79)
≥ 2	16 (41)	9 (30)	0 (0)	1 (3)	17 (15)	18 (16)	13 (21)	13 (21)
Stage								
IIIB	12 (31)		12 (36)	16 (42)	21 (19)	10 (9)	21 (34)	21 (33)
IV	27 (69)		17 (52)	22 (58)	89 (81)	100 (91)	41 (66)	42 (67)
Prior Treatment								
Radiotherapy	4 (10)		4 (12)	0 (0)				
Chemotherapy	0 (0)[3]	30 (100)	0 (0)	0 (0)	110 (100)	110 (100)	62 (100)	63 (100)
- platinum-based	--	13 (43)	--	--	94 (85)	92 (84)	62 (100)	63 (100)
- non-platinum-based	--	12 (40)	--	--	16 (15)	18 (16)		
None	35 (90)	0 (100)	24 (73)	38 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Dose, mg/m^2; schedule	36; 6 / 2	36; 6 / 2	20[3]; 3 / 1	25[4]; 3 / 1	75; 3-weekly	33.3; 6 / 2	75; 3-weekly	40; 6 / 2
Dose intensity, mg/m^2/wk	27	27	15	18.8	25	25	25	30
ORR, % (95% CI)	18	10 (1.6-29)	52 (31-67)	31.6 (17-46.4)	2.7	5.5[5]	4.8	3.2
Median OS, months (95% CI)	5	8.0 (5.6-13.9)	15.8	11.8 (9.4-15)	7.3 (5.3-9)	6.3 (4.5-8.5)	5.8	5.5
1-year survival rate, % (95% CI)	27	31 (17-58)	64	46.5 (30-63.8)	21	31	18	6

Values between brackets are percentage of total number of patients, unless otherwise specified; If no value specified then not available; [1]Aged > 70 years; [2]Inclusion criterion age ≥ 75 years; [3]Combined with cisplatin 25 mg/m^2 on days 1, 8 and 15; [4]Combined with cisplatin 80 mg/m^2 on day 1; [5]$P = 0.50$.

Most recently, two small randomized clinical trials comparing weekly and 3-weekly second-line treatment have been completed [27,28]. Gridelli and colleagues [27] focused on quality of life (QoL) issues and found no overall statistically significant differences in the assessed items. Disappointingly, both studies showed low response rates regardless of schedule and even opposite differences in 1-year survival rate (Table 3), again suggesting that larger trials will be needed to completely eradicate selection bias.

Weekly docetaxel – hormone refractory prostate cancer

Phase II clinical trials had demonstrated that weekly or 3-weekly docetaxel had single agent activity in hormone refractory prostate cancer patients (Tables 1 and 4) [13,14,33-36]. For this reason, a large phase III study was performed comparing weekly docetaxel-prednisone to 3-weekly docetaxel-prednisone and to mitoxantrone-prednisone [15]. In assessing the outcome one has to take into account that prostate cancer patients are frequently elderly and frail, and that treatment is often intended to be palliative. In the randomized phase III study there was no significant difference between weekly and 3-weekly efficacy and the percentage of patients who had an improvement in QoL was also similar in both docetaxel groups. Although based on phase II studies [34,36] age does not seem to affect treatment outcome (Table 4). In addition, a retrospective analysis of the pooled individual patient data from two trials [33,34] also showed no difference between older and younger patients with respect to efficacy endpoints [37]. Most importantly in this respect, survival benefit in the randomized phase III study was similar for patients younger than 65 years *vs* those 65 years or older [15].

Docetaxel Toxicity

The toxicity profile of docetaxel when given once every 3 weeks is well known. The dose is limited by dose-dependent, mostly short-lasting neutropenia, relatively frequently complicated by fever. Other side-effects include nausea, vomiting, stomatitis and diarrhoea. Docetaxel induces a peculiar type of skin- and nail-toxicity, peripheral oedema as well as frequent hypersensitivity reactions. All of these latter side-effects can be largely circumvented or diminished by adding a short prophylactic corticosteroid schedule [6-10,12-15]. Finally, the drug induces neuropathy related to cumulative dose rather than the cycle dose.

The toxicity profile of weekly docetaxel is significantly altered compared to 3-weekly treatment. Overall, acute toxicities are uncommon. However, chronic toxicities, which develop and increase with successive weekly dosing (i.e. related to cumulative dose) are (more) prominent. Although the randomized trials comparing weekly and 3-weekly docetaxel treatment were performed in three different patient categories, taken together they provide a relevant overview of the toxicities related to both schedules (Table 5).

Table 4. Efficacy of weekly docetaxel in patients with hormone refractory prostate cancer

Reference	**Beer** [33]	**Berry** [34]	**Beer** [37]		**Gravis** [35]	**Ferrero** [36]	**Tannock** [15]	
N	25	60	34	52	30	64	335	334
Median age (range)	72 (55-81)	72 (41-86)	< 70	≥ 70	67 (52-83)	73 (49-88)	68 (42-92)	69 (36-92)
Age ≥ 65 years		48 (80)				48 (75)		
Age ≥ 75 years							67 (20)	70 (21)
ECOG PS								
0-1	13 (52)	50 (83)	25 (74)	38 (73)		57 (89)		
≥ 2	12 (48)	10 (17)	9 (26)	14 (27)		7 (11)		
Karnofsky PS (range)					80 (70-90)		≥ 60[1]	≥ 60[1]
Median PSA ng/ml (range)	201 (1-1432)	92 (0-2737)	83 (1-1502)	159 (0-2737)	73 (1-895)	82 (2-857)	114	108
Extent of disease								
Bone metastasis	24 (96)	57 (95)	31 (91)	50 (96)	19 (63)	58 (91)	302 (90)	304 (91)
Visceral metastasis	1 (4)	9 (15)	4 (12)	3 (6)	5 (17)	12 (19)	74 (22)	80 (24)
Measurable lesions	5 (20)	6 (10)	16[2]		9 (30)	6 (9)	134 (40)	130 (39)
Prior Treatment								
Radiotherapy	14 (56)	42 (70)	30 (88)	31 (60)	20 (67)	8 (13)	174 (52)	147 (44)
Chemotherapy	0 (0)	16 (27)	8 (24)	9 (17)	15 (50)	16 (25)	64 (19)[3]	60 (18)[3]
Dose, mg/m^2; schedule	36; 6 / 2	36; 6 / 2	36; 6 / 2	36; 6 / 2	35; 6 / 2	40; 6 / 2	75; 3-weekly	30; 5 / 1
Dose intensity, mg/m^2/wk	27	27	27	27	26.3	30	25	25
PSA response, % (95% CI)	46 (25-67)	41	40 (23-57)[4]	47 (33-61)	48	64 (51-76)	45 (40-51)	48 (42-54)
ORR, % (95% CI)	40	33	33 (0-66)[4]	29 (0-65)	0	17	12 (7-19)	8 (4-14)
Median overall survival, months (range, *95% CI*)	9.8 (4.5->22.3)	9.4 (1.6-18.2)	11.3[4] *(9-13.5)*	8.3 *(3.3-13.5)*	20 (4-41)	16.2	18.9 *(17.0-21.2)*	17.4 *(15.7-19.0)*
1-year survival rate, %		38				58.4		

If no value is specified then not available; Values between brackets are percentage of total number of patients unless otherwise specified; [1]PS ≥ 60 inclusion criterion, ≤ 13 % of patients PS ≤ 70; [2]Number of measurable lesions in both age groups; [3]Only estramustine allowed; [4]No statistically significant differences between age groups.

Table 5. Toxicity (%) in randomized trials comparing weekly and 3-weekly docetaxel

Reference – Cancer	**Tabernero [21] - Breast**		**Gridelli [27] - Lung**		**Gervais [28] - Lung**		**Tannock [15] - Prostate**	
N	42	41	110	110	62	63	335	334
Median age (range)	55 (33-72)	56 (25-75)	62 (26-74)	63 (28-75)	59 (37-72)	58 (26-74)	68 (42-92)	69 (36-92)
ECOG PS								
0-1	40 (95)	39 (95)	93 (85)	92 (84)	49 (79)	50 (79)	Karnfosky PS	≥ 60
≥ 2	1 (2)	2 (5)	17 (15)	18 (16)	13 (21)	13 (21)		
Dose, mg/m^2; schedule	100; 3-weekly	40; weekly	75; 3-weekly	33.3; weekly	75; 3-weekly	40; weekly	75; 3-weekly	30; weekly
Haematological gr 3-4								
Neutropenia	37	7	19	2	48	16	32	2
Anaemia	≤ 5	≤ 5	3	0	10	13	5	5
Thrombocytopenia	≤ 5	≤ 5	1	1			1	0
Febrile neutropenia	20	5	5	0	7	0	3	0
Non-Haematological gr 3-4								
Fatigue/asthenia	12	15	7	6	5	11	5	5
Nausea & vomiting	15	12	0	0	3	5		
Diarrhoea	7	2	3	3	2	3		
Stomatitis	17	7	1	0				
Skin	10	7	1	0				
Fluid retention	7	2						
Infection	2	7	0	4				
Neuro-motor /- sensor	17	2	1	4				
Non-Haematological gr 1-2								
Fatigue/asthenia	81	68	49	49			48	44
Nausea & vomiting			27	35			42	41
Diarrhoea			18	23			32	34
Fluid retention	33	33					19	12
Neuro-motor / -sensor			23	21			30	24
Alopecia, grade 2	87	78	20	7			65	50
Hyperlacrimation	39	53					10	21
Nail changes	56	56					30	37
Dose reductions	42	15			1	1	12	9
Treatment stopped[1]	37	46	21	11	5	13	11	16

If no value is specified then not available; [1]For reasons of toxicity

Haematological toxicity: As expected, weekly docetaxel is associated with mild haematological toxicity; severe neutropenia ranged from 2 % to 16 % for weekly docetaxel and febrile neutropenia was either absent or low (≤ 5 %) (Table 5). Only one non-randomized trial reported a higher incidence (22 %) of severe neutropenia which was attributed to baseline abnormalities in haematology parameters [22]. In the randomized trials, incidences of grade 3 to 4 neutropenia for 3-weekly treatment ranged from 19 % to 48 %, which is relatively low compared to previously reported values even though all patients had received some form of prior chemotherapy. Severe anaemia and thrombocytopenia are uncommon regardless of schedule. In two non-randomized trials higher incidences of severe anaemia (range, 13 - 17 %) were also reported, possibly explained by the fact that more than one third of all patients had a poor baseline performance status [23,24].

Non-haematological toxicity: Severe fatigue and asthenia were the most common complaints after weekly treatment. These side-effects usually occur at the end of the consecutive treatment weeks (6 weeks) and were partly reversible during the 2-week rest interval. In general, incidences range between 5 % and 20 %, which is actually not higher than observed after 3-weekly treatment. Higher incidences (33 %) were only incidentally observed (in patients with a poor performance status) [24]. Indeed, studies from Tannock and Gridelli [15,27] observed no difference in the incidence of fatigue/asthenia between the two schedules. In contrast, Tabernero and Gervais [21,28] have both reported that fatigue/asthenia were more common with the weekly regimen. Recommendations for management of severe fatigue include a dose reduction or a shorter schedule, for instance 2 or 3 consecutive weekly infusions, followed by a 1-week rest interval. However, it is advisable to exercise caution as it is unknown how these schedule changes will affect activity.

Weekly docetaxel is associated with an unexpected increase in the incidence of excessive tearing (incidences up to 52 % have been reported) [16]. Hyperlacrimation, due to canalicular/nasolacrimal duct stenosis, possibly a result of docetaxel secretion in tears [38], was initially classified as an unexpected and merely bothersome side-effect. However, currently it is recognized to be a complaint which, although mild (rarely classified ≥ grade 3), can be particularly persistent, leading to significant problems with reading, driving, and other daily activities requiring adequate visual function. Indeed, this recently underestimated side-effect has a substantial negative impact on a patient's QoL. Esmaeli and colleagues [39] specifically reported on the severity and management of excessive tearing in patients who received weekly (N = 71) or 3-weekly docetaxel (N = 72). After a median cumulative dose of 1080 mg (range, 168 – 2116 mg), corresponding to a median of 24 weeks (range, 11 – 48 weeks) after initiation of weekly treatment, a surgical intervention (temporary silicone intubation, dacryocystorhinostomy (DCR) with placement of a temporary silicone tube or a permanent Pyrex tube) was required in more than 40 % of patients' eyes, and successfully relieved symptoms. Indeed, in 21 patients with complaints in both eyes who received no surgical treatment, either due to patient refusal or because of other comorbidity, complaints were still

persistent 6 months after docetaxel discontinuation. Only 28 % of the patients had mild complaints and they were treated with topical steroids. In contrast, in the majority of patients given 3-weekly docetaxel complaints were adequately managed with topical treatment and only 3 patients required surgical intervention. Recommendations for the management of this common side-effect include a baseline ophthalmologic examination followed by regular monitoring so that early diagnosis is possible and appropriate treatment can be initiated [39,40]. Silicone intubation has been recommended as the safest approach in symptomatic patients if weekly treatment is to be continued [39].

In addition, cumulative nail toxicity can lead to severe discomfort, limitation of function and treatment discontinuation after repetitive dosing [18,22]. Although the incidence of low grade nail toxicity was not different for weekly or 3-weekly docetaxel in metastatic breast cancer patients, nail changes substantially affected the QoL in the weekly treatment arm and were the major reason for withdrawal, followed by excessive tearing, asthenia and infection [21]. Similarly, in the prostate cancer phase III trial [15], major reasons for withdrawal also included fatigue and nail changes. It should be noted that, in acknowledgement of the severe impact on a patient's QoL, the recent version of the Common Toxicity Criteria of the National Cancer Institute (CTC-NCI, version 3.0) includes grade 3 nail changes (interfering with daily life, severe adverse event), whereas version 2.0 only included rating up to grade 2 (partial/complete loss of nail(s), pain in nail beds). Most trials published to date, and certainly most of the larger randomized trials where accrual started several years ago, have used version 2.0 CTC-NCI for toxicity rating. It is likely that the reported severity of nail disorders is underestimated due to the lack of a more specific rating.

In general, in the randomized trials dose reductions were less often required after weekly treatment. Yet, in 3 out of 4 randomized trials, treatment discontinuations were more common after weekly treatment (Table 5). It is conceivable that persistent cumulative toxicities eventually lead to treatment discontinuation.

Prophylaxis schedules

Prophylaxis of cumulative fluid retention and hypersensitivity reactions is recommended when patients are treated with docetaxel. The most commonly used premedication schedule for 3-weekly treatment is dexamethasone 8 mg administered orally twice daily for 3 days, starting 1 day prior to treatment (range, 40 - 48 mg/3 weeks; dose intensity range, 13 - 16 mg/week). Although steroid premedication remains mandatory [41], at this point there is no universally recommended prophylactic regimen for weekly docetaxel. In most cases, an abbreviated course of 3 oral administrations of dexamethasone 8 mg given every 12 hours starting 12 hours prior to docetaxel infusion is chosen (24 mg/week; dose intensity 18 mg/week). This schedule adequately controls fluid retention and hypersensitivity reactions. It has been advised to monitor (elderly) patients more closely for signs of toxicity

after repetitive corticosteroid dosing. However, the incidence of corticosteroid-related complications (e.g. hyperglycaemia, peptic/duodenal ulcers) is low [34]. Indeed, dexamethasone dose intensity with weekly docetaxel is only marginally higher. Incidentally used schedules include (methyl)-prednisone containing regimens or a single dose of 8 mg dexamethasone 1 hour prior to infusion [15,18,19,22,28,36].

Conclusion

Since the landmark phase I trial [4], numerous clinical trials have evaluated weekly docetaxel treatment. However, due to considerably different patient populations enrolled in the various trials and the limited numbers, comparisons of weekly *vs* 3-weekly efficacy have been difficult. Hence, recommendations when or when not to consider weekly treatment were lacking. Recently, final results of the first comparative trials have become available. These randomized trials demonstrate, for the three approved indications, that the efficacy of weekly docetaxel is comparable to 3-weekly treatment, with the addition that activity in NSCLC is disappointingly low for both schedules. The toxicity profile of weekly docetaxel does however distinguish this schedule from the standard 3-weekly regimen. Acute toxicities, in particular myelosuppression, are, as expected, mild and never dose-limiting. However, cumulative side-effects were much more prominent and require increased awareness and early recognition for adequate management. The most common and dose-limiting toxicity is fatigue/asthenia. Other chronic toxicities include alopecia, excessive tearing and nail disorders. Despite the fact that the latter two side-effects are usually of low grade they were persistent and had a substantial negative impact on a patient's QoL, which has previously largely been underestimated. As of yet, there has not been any health economics assessment of the differences between the two schedules.

Given the similar efficacy observed for the two schedules and the above remarks on toxicity, it is reasonable to conclude that, at this point, 3-weekly docetaxel should still be considered the standard and most convenient schedule, and that treatment with weekly docetaxel should only be considered as an alternative for specific patient populations who are unlikely to tolerate the standard 3-weekly treatment. For patients with a poor performance status, multiple comorbidities, a history of extensive pretreatment and severe toxicity, decreased haematological reserves and elderly patients at high risk for myelotoxic complications, weekly docetaxel offers an additional treatment option. The place of elderly patients in this list deserves a more detailed discussion. Elderly patients form a heterogeneous group and therefore the interpretation of clinical trial results and subsequent implementation of treatment recommendations is especially difficult. Chronologic age itself is not a contra-indication for full-dose chemotherapy. However, ageing, a highly individual process, is frequently associated with a high prevalence of comorbidity, poor performance status and with a decline in functional reserves of organ systems, especially bone marrow. A patient's

physiologic age, determined through a comprehensive geriatric assessment has therefore been suggested to better predict the increased risk of chemotherapy induced (haematological) toxicity. In general, largely due to poor haematological reserves, elderly patients are considered to be more susceptible to drug-induced myelosuppression yet, if otherwise fit, not at an increased risk for other toxicities [42-45]. In our opinion, a decision to administer weekly or 3-weekly docetaxel to an elderly person should take into account all these aspects and result in a treatment tailored to the individual's tolerance. As increased exposure to docetaxel is a strong predictor of haematological toxicity regardless of schedule [46,47], altered pharmacokinetics have also been suggested to play a role. However, there are no differences in docetaxel pharmacokinetics between elderly and non-elderly patients [45,48] or for that matter, between the 3-weekly and weekly schedule [49]. Interestingly, in these trials, the majority of baseline patient characteristics did not differ between the age groups, however, elderly patients had statistically significant, albeit only slightly lower serum alpha–1 acid glycoprotein (AAG) levels ($P = 0.018$ and $P = 0.04$). Docetaxel is extensively bound to AAG and this protein is one of the main determinants of the fraction of unbound, pharmacologically active drug. Docetaxel-induced haematological toxicity is significantly better correlated with systemic exposure to unbound drug than with exposure to total drug [50]. Although unbound docetaxel pharmacokinetics have not been extensively investigated in elderly patients, it is possible that besides the increased susceptibility for myelosuppression due to a functional decline in haematological reserves, decreased AAG levels result in a higher exposure to the unbound fraction of docetaxel and contribute to the increased myelosuppression.

Several aspects of weekly docetaxel administration still remain to be further elucidated. The optimal duration of subsequent administrations, the timing of rest episodes and the optimal number of courses have not yet been established. Furthermore, it is unknown how frail patients selected for weekly treatment perceive more frequent visits to the hospital and the burden of travelling. The negative impact on QoL as reported is a particular concern in this respect. Finally, as indicated, there is as yet no comparative cost-effectiveness analysis including direct and indirect costs. For 3-weekly treatment the indirect costs associated with the management of adverse effects can significantly increase (up to 20 %) the total cost of docetaxel treatment [51].

For the time being, 3-weekly docetaxel remains the first schedule to be considered. However, there is no single schedule for any drug that offers an optimal balance between efficacy and toxicity in all patients. Instead, dose, schedule and overall toxicity should be considered against an individual's characteristics, including performance status, comorbidity, haematological reserves and prior treatment. As an individualized treatment, the weekly schedule of docetaxel appears to be an acceptable alternative.

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Chapter 3

Potential for Improvement of Docetaxel-based Chemotherapy: a Pharmacological Review

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Abstract

Since the introduction of docetaxel, research has focused on various approaches to overcome treatment limitations and to improve outcome. This review discusses the pharmacological attempts at treatment optimization, which include reducing interindividual pharmacokinetic and pharmacodynamic variability, optimizing schedule and route of administration, reversing drug resistance, and the development of structurally related second-generation taxanes.

Introduction

The anticancer drug docetaxel (Taxotere®) is approved for the treatment of patients with locally advanced or metastatic breast or non-small-cell lung cancer (NSCLC) and androgen-independent metastatic prostate cancer. The recommended dose ranges from 60 mg/m^2 to 100 mg/m^2 given as a 1-hour intravenous infusion once every 3 weeks. An important limitation associated with docetaxel use is the unpredictable interindividual variability in efficacy and toxicity. Since its clinical introduction, attempts to improve docetaxel treatment have covered various areas: reducing the interindividual pharmacokinetic (PK) and pharmacodynamic (PD) variability, optimizing schedule, route of administration and drug formulation, and reversing drug resistance. This review will discuss pharmacological strategies aimed to overcome the limitations of docetaxel therapy.

Alternative Schedules

When treated at a dose of 100 mg/m^2 once every 3 weeks, grade 4 neutropenia and febrile neutropenia occur in 75 %, respectively 11 %, of patients; a dose of 75 mg/m^2 only moderately reduces this incidence (http://www/taxotere.com). For patients with a poor performance status (PS), multiple comorbidities, decreased haematological reserves, a history of extensive pretreatment and severe toxicity, elderly patients and for patients for whom treatment is palliative, a less toxic schedule seemed desirable. Therefore, a schedule involving weekly administration was developed. Numerous trials have evaluated this schedule; however, due to considerably different study populations and small sample sizes, comparisons of weekly *vs* 3-weekly efficacy were difficult. Recent randomized trials although demonstrate, for the approved indications, that the efficacy of weekly docetaxel is comparable to 3-weekly treatment [1], the toxicity profiles are, however, distinctly different. With weekly docetaxel, acute toxicities, in particular myelosuppression, are mild and never dose-limiting. In contrast, cumulative side-effects are much more prominent. The most common and dose-limiting toxicity is fatigue/asthenia. These side-effects can only be managed by reducing the dose or by shortening the schedule, to 2 to 3 consecutive weekly

infusions, followed by a 1-week rest interval. Other cumulative toxicities include alopecia, excessive tearing and nail disorders. Although the latter two side-effects are usually mild, they are persistent, can lead to treatment discontinuation and have a substantial negative impact on a patient's quality of life. Given the similar efficacy observed for the two schedules and the remarks on toxicity, it is reasonable to conclude that, at this point, 3-weekly docetaxel is still the standard and most convenient schedule. Treatment with weekly docetaxel should only be considered as an alternative for specific patient populations.

Pharmacokinetic Optimization

The pharmacokinetics (PK) of total docetaxel are linear and independent of schedule. Nonetheless, there is large interpatient variability in exposure (AUC) and drug clearance [2-6]. In a large population PK/PD analysis variability in efficacy and toxicity was associated with variability in PK [3]; a 50 % decrease in docetaxel clearance increased the odds of developing grade 4 neutropenia and febrile neutropenia 4.3-fold and 3.0-fold, respectively. Subsequent studies have therefore focused on identifying factors, which most affect PK variability. Ultimately, reducing interpatient exposure variability should improve the risk-benefit ratio of docetaxel therapy.

Initially, the main predictors of total docetaxel clearance (variability) were body surface area (BSA), alpha-1 acid glycoprotein (AAG), hepatic function (elevated alkaline phosphatase (ALKPH) and transaminases levels) and age [7]. More recently, (hepatic) cytochrome P450 (CYP) isozyme 3A4 activity was also included [2]. The relevance of all these predictors to docetaxel dose optimization has been further (re-)evaluated.

Normalization of clearance for BSA reduces interindividual variability marginally (< 2 %), thus questioning the clinical relevance of BSA-based dosing [5]. Clearance was, however, significantly higher by 33 % (P = 0.0029) for patients with BSA values > 2.00 m^2 compared to values < 1.71 m^2. Flat-dosing, possibly differentiating for extremes of BSA (> 2.00 m^2), may be easier and just as precise and should be investigated prospectively.

In general, the unbound (i.e. free) fraction of any drug is pharmacologically active. In serum, docetaxel is extensively bound to albumin, lipoproteins and AAG; indeed the latter is the main determinant of docetaxel serum binding variability. Furthermore, AAG levels in cancer patients vary at least 4-fold [3,6,8]. High AAG levels have been associated with a decrease in the unbound fraction of docetaxel *in vitro*, and with reduced total docetaxel clearance [7,8] and lower response rate in man [3]. Yet, dosing recommendations based on individual AAG levels are not available. The formulation vehicle polysorbate 80, although rapidly degraded by serum esterases, also influences docetaxel protein binding, increasing the unbound drug fraction by on average 16 % to 24 % at peak polysorbate 80 concentrations [6,8]. Furthermore, higher polysorbate 80 exposure resulted in lower unbound docetaxel clearance. Most importantly, haematological toxicity is highly correlated with systemic exposure to

unbound docetaxel [6]. Thus, measuring unbound docetaxel concentrations should be considered in future PK/PD studies.

Mild hepatic impairment (total bilirubin < 1.5 x ULN, transaminases ≥ 1.5 to ≤ 3.5 x ULN concurrent with ALKPH ≥ 2.5 to ≤ 5 x ULN) decreases total docetaxel clearance by 27 % [3], and moderate (total bilirubin ≥ 1.5 to < 3.0 x ULN with any transaminase and ALKPH elevations) to severe impairment (total bilirubin ≥ 3.0 x ULN with any transaminase and ALKPH elevations) does so by 50 %. This should obviously have consequences, yet one is only warned that docetaxel should *generally* not be given to patients with total bilirubin > ULN, or with transaminases > 1.5 x ULN concomitant with ALKPH > 2.5 x ULN (http://www.taxotere.com). Dose adjustments therefore are left to the discretion of the physician, while they seem required.

Elderly age (≥ 65 years) does not alter total docetaxel PK [4]. Interestingly, the elderly patients' serum AAG levels were significantly ($P \leq 0.04$), albeit only slightly lower. Decreased AAG levels contribute to higher exposure to unbound docetaxel resulting in more myelosuppression [6]. Jointly with the increased susceptibility for myelosuppression due to a functional decline in haematological reserves at ageing, this could be a concern. Yet, toxicity did not occur more frequently in the elderly. Thus, age-related dose recommendations should predominantly be based on individual PS and comorbidity.

Docetaxel is primarily metabolized by (hepatic and intestinal) CYP3A, in particular by isoforms CYP3A4 and CYP3A5, the latter of which has a 10-fold lower affinity for docetaxel. Docetaxel metabolites are substantially less active than the parent drug; hence, CYP3A-mediated metabolism is the major route of inactivation. CYP3A activity in adults, and in patients treated with docetaxel, varies largely between individuals. This is believed to depend on environmental (CYP3A modulation), physiological (hepatic impairment) and genetic (CYP3A polymorphism) factors. Pretreatment CYP3A phenotyping has been suggested as a tool to individualize docetaxel dosing [9-12]. At present, the midazolam hydroxylation test and the erythromycin breath test (ERMBT) are the most widely applied phenotyping strategies, albeit that both have their limitations. The ERMBT did not consistently correlate with results from other CYP3A phenotypic probes [13] and may have limited value for CYP3A-phenotyping of docetaxel patients as erythromycin is preferentially metabolized by CYP3A4, whereas docetaxel is metabolized by CYP3A4 *and* CYP3A5. Yet, when compared to other variables (ALKPH, alanine aminotransferase and AAG), the ERMBT was the best single predictor of docetaxel clearance [2] and probe specificity issues are relevant only in individuals expressing significant CYP3A5 levels, which is rarely the case in Caucasians. In contrast, African-Americans have much higher CYP3A5 expression and in these patients midazolam, metabolized by both CYP3A4 and CYP3A5, may be more suitable. Clinical trials correlating phenotyping results to docetaxel PK demonstrate that midazolam, erythromycin and dexamethasone are predictors of docetaxel clearance [2,9,11]. As

dexamethasone is routinely used as premedication, it may be more attractive as a probe drug than midazolam or erythromycin. Recently, individualized dosing based on the 24-hour urinary metabolite of exogenous cortisol as phenotypic CYP3A probe was evaluated [12]. Individualized phenotypic dosing significantly reduced the interindividual PK variability compared to BSA-based dosing. Further larger studies, preferably comparing phenotyping strategies, are required to assess which probe is the best predictor of CYP3A activity. Nonetheless, phenotyping techniques have practical disadvantages (i.e. 24-hour urine collection, radioisotope administration) that may limit their applicability in common oncology practice.

The involvement of CYP3A in docetaxel elimination renders the drug potentially subject to a host of enzyme-mediated PK drug-interactions with conventional drugs, complementary and alternative medicine and food constituents that interfere with CYP3A function or expression. Docetaxel has a narrow therapeutic window. Therefore, the risk of a PK interaction resulting in under- or overexposure, thereby modifying treatment outcome, is high. For several coadministered cytotoxic agents, PK interactions with docetaxel are known and have led to dose or schedule recommendations. Interestingly, for the potent CYP3A inhibitor ketoconazole interaction data are inconsistent. Both trials observed large interindividual variability in reduction of docetaxel clearance [14,15]. Yet, in one this was highly significant [14] whereas in the other, although docetaxel clearance decreased 2 to 4-fold in 25 % of the patients, thus increasing the risk for severe neutropenia, it was not [15]. Efforts to reduce the interindividual PK variability through inhibition of CYP3A by ketoconazole have not been successful. No clinically relevant PK interaction has been observed between dexamethasone, a possible CYP3A inducer, and docetaxel [2,11]. Thus, there is no reason to abandon routine dexamethasone premedication. Clearly, the degree to which a PK interaction is clinically relevant, and requires an intervention, depends upon the CYP3A-inducing or -inhibiting properties of the coadministered agent. Since specific dose adjustment recommendations are not available, concomitant administration of potent CYP3A-modulating comedication should generally be avoided.

Docetaxel is also a substrate for the ATP-binding cassette transmembrane transporter protein ABCB1 (P-glycoprotein (P-gp); MDR-1). ABCB1 is expressed in tumours and in normal tissues including the blood-brain-barrier (BBB), biliary tract and intestinal epithelium. Although ABCB1 plays a (major) role in the intestinal absorption and biliary excretion of *orally* administered substrates, its influence on the plasma PK of *intravenously* administered drugs, including docetaxel, is minimal to absent [16]. ABCB1 inhibition does, however, significantly influence the faecal disposition of docetaxel, reducing the amount of excreted unchanged drug (approximately 18-fold) without affecting plasma PK [16], indicating that the effects of ABCB1 modulation on docetaxel PK cannot be evaluated when analysing only plasma.

Monitoring plasma levels and PK-guided dose adjustments is referred to as therapeutic drug monitoring (TDM). At present, the use of TDM in oncology is limited. A prerequisite for TDM is that *intra*individual PK variability is less than *inter*individual PK variability, which is the case for docetaxel. For reasons of patient convenience and practicality, validated limited sampling strategies (LDS), requiring only 2 to 4 samples to characterize an individual PK profile [3,7], should be used. LDS used in combination with a population PK model and Bayesian analysis, allows individual PK parameters to be estimated with adequate precision, while sampling and dosing times remain flexible. TDM could become an interesting strategy to docetaxel dose individualization provided a target concentration or exposure profile can be defined. It should be noted, however, that obtaining 2 to 4 samples from outpatients requires adequate planning and a good collaboration between pharmacy, outpatient clinic and the prescribing oncologist to assure that the sampling can be completed within the service hours of an oncology day unit.

Reversal of Drug Resistance

The most extensively studied mechanism of acquired or intrinsic resistance to taxanes is the overexpression of ABCB1. Numerous (pre)clinical investigations have evaluated coadministration of ABCB1 modulators (e.g. verapamil, cyclosporin A, valspodar), aiming to restore or enhance sensitivity to chemotherapy. However, the results were largely disappointing. To overcome the limitations of these first- and second-generation modulators (unacceptable toxicity and unpredictable PK interactions), highly specific and potent third-generation ABCB1 modulators, lacking interference with the plasma PK of cytotoxics, were developed.

In phase I trials, oral and intravenous R101933 (laniquidar) administered in combination with docetaxel, inhibited ABCB1 both in an *ex vivo* assay and *in vivo* (indicated by intestinal P-gp inhibition), and docetaxel plasma PK was not altered [16,17]. However, phase II studies were negative. Similar disappointing efficacy results were obtained in clinical trials of XR9576 (tariquidar) and docetaxel, and at present, there are no plans for further clinical development (http://www.qltinc.com). A phase I trial of docetaxel in combination with the orally administered agent LY335979 (zosuquidar) showed no PK interaction [18]. The limited cerebrospinal fluid penetration of docetaxel is also assumed to be due to ABCB1-mediated drug efflux and restricts treatment of brain tumours with docetaxel. Preclinical investigations with GF120918 (elacridar) suggested increased docetaxel brain concentrations without effect on plasma PK [19], but a phase I trial reported increased systemic exposure to docetaxel and reduced clearance [20]. This interaction will likely limit further clinical development.

Docetaxel is also a substrate of CYP1B1, a cytochrome isozyme not detected in human liver but (over)expressed in various tumours. In vitro docetaxel cytotoxicity in cells transfected with human CYP1B1 was decreased [21], but CYP1B1-mediated docetaxel

metabolism was not affected [22]. Meanwhile, the ability of CYP1B1 inhibitors to increase the cytotoxic effect of docetaxel has recently been demonstrated *in vitro*. Clinical studies have not yet been performed and the functional role of intratumoural CYP1B1(-mediated resistance) on docetaxel cytotoxicity remains to be elucidated.

Clearly the ultimate (multi)drug resistance reversal agent is not (yet) available. Moreover, one should realize that modulating one resistance mechanism will not yield important antitumour benefit, given the large number of resistance mechanisms in human tumour tissue.

Alternative Routes of Administration - Oral administration

In vitro increasing the duration of taxane exposure above a threshold level is more important than achieving high peak concentrations. Clinically, the duration of exposure to plasma levels greater than 0.080 μg/mL indeed predicted response [3]. Oral docetaxel treatment would be a patient-convenient way to achieve long-term drug exposure. However, development of a suitable oral formulation has been impeded by low (< 10 %) and highly variable oral bioavailability, due to the discussed extensive CYP3A-mediated first-pass metabolism and, to a lesser degree, to affinity for outward-directed transport by ABCB1 in the gastrointestinal tract. Modulating these elimination routes has therefore been a focus of research.

In wild-type mice, exposure to orally administered docetaxel was 6-fold lower compared to *Abcb1a/1b* knock-out mice [23]. More importantly, the relative bioavailability increased from 4 % to 183 % by coadministration of the potent CYP3A (and poor ABCB1) inhibitor ritonavir, increasing systemic exposure 50-fold. Subsequently, a small PK study, in which patients were given oral docetaxel (75 mg/m^2) with or without the ABCB1- and CYP3A inhibitor cyclosporin A (CsA), confirmed the observation [24]. In the presence of CsA, systemic exposure increased approximately 7-fold (from 0.37 mg·h/L ± 0.33 mg·h/L to 2.71 mg·h/L ± 1.81 mg·h/L). When given 100 mg/m^2 docetaxel *intravenously* (without CsA) the resulting systemic exposure was 4.27 mg·h/L ± 2.26 mg·h/L. Adjusted for the difference in dose, exposure following *oral* administration *with* concomitant CsA does not greatly differ from exposure after *intravenous* administration *without* CsA. The investigators performed a phase II trial with weekly oral docetaxel (100 mg) in combination with CsA [25]. Interpatient PK variability, haematological toxicity and antitumour activity seem to be in the same range as for intravenous docetaxel. Oral docetaxel (100 mg) was also combined with OC144-093, a potent and selective oral ABCB1 inhibitor, and compared to 100 mg intravenous docetaxel [26]. The relative oral bioavailability of docetaxel was 26 % ± 8 %, lower than previously observed after CsA coadministration, and systemic exposure after intravenous docetaxel was 3-fold higher compared to the oral application, despite the ABCB1 modulation. Thus indicating that CYP3A-mediated (first-pass) metabolism is the crucial process involved in the

poor oral bioavailability of docetaxel. Notwithstanding the fact that the oral bioavailability of docetaxel can be increased through pharmacologic modulation, the development of second-generation oral taxanes, is likely to prevail.

Second Generation Docetaxel-based Taxanes

Lately, structure activity relationship studies have focused on identifying novel structurally related docetaxel analogues with increased cytotoxicity in resistant tumours, increased penetration across the BBB, decreased toxicity, oral bioavailability and higher water solubility, the latter facilitating drug formulation. Chemical modification of the core structure of docetaxel has resulted in docetaxel-based second-generation taxanes, which are in different phases of clinical development (Table 1).

Docetaxel is synthesized from 10-deacetylbaccatin III, a non-cytotoxic precursor derived from the European yew tree. Research initially focused on modifications of this compound and yielded XRP9881 (RPR109881A) and XRP6258 (RPR116258A or TXD258). Both agents have comparable mechanism of action to docetaxel, and in tumour models sensitive to docetaxel, cytotoxic activity was similar to docetaxel (http://www.AventisOncology.com). Importantly, *in vitro* these agents are characterized by potent growth inhibitory activity in moderately and highly docetaxel-resistant cell lines, most probably based upon a substantially lower affinity for ABCB1. Furthermore, glioblastoma models proved to be sensitive to these agents, suggesting penetration of the BBB. Phase I trials and early phase II studies with XRP9881 in metastatic breast cancer patients suggest adequate activity [27]. A differentiating feature of XRP6258 is its antitumour activity following oral administration, yet initial development is as intravenous administration. Both agents demonstrate marked interpatient variability in drug clearance, similar to docetaxel. Short-lasting and manageable neutropenia, fatigue and diarrhoea are the dose-limiting toxicities.

Several cytotoxic analogues derived from 14-β-hydroxy-10-deacetylbaccatin III, a natural compound closely related to the core structure of docetaxel, have been evaluated. The most interesting is ortataxel (IDN5109, BAY59-8862). Ortataxel has adequate oral bioavailability and can modulate the function of various ABC transporter proteins, including ABCB1, MRP and BCRP [28]. The drug is not active in renal cancer patients, and phase II studies in taxane-resistant metastatic breast cancer and NCSLC patients are ongoing. MAC-321 or TL00139 exhibits a similar mechanism of cytotoxic activity as docetaxel [29], is highly effective both orally and intravenously administered and currently under investigation involving both administration routes. DJ-927 is a novel semi-synthetic taxane with high water solubility, lack of neurotoxicity, good oral bioavailability and superior antitumour activity compared to docetaxel in *in vitro* and *in vivo* models [30]. Preliminary results of a phase I trial of orally administered DJ-927 suggest that the agent may have favourable toxicological and pharmacological properties.

Table 1. Second-generation taxanes, structurally related to docetaxel

Drug	Nature of derivative	Cytotoxicity[1]	Cytotoxicity[2]	Development phase	Administration route(s) and recommended dose	Firm
XRP9881	10-DAB	Similar	Superior	Phase II	i.v. 90 mg/m^2, q 3wks	Aventis Pharma
XRP6258	10-DAB	Similar	Superior	Phase I	i.v. 30 mg/m^2, q 3wks also orally active	Aventis Pharma
Ortataxel	14-β-hydroxy-DAB	Similar	Superior[3]	Phase II	i.v. 75 mg/m^2, q 3wks also orally active	Bayer / Indena
MAC-321	10-deacetyl-7-propanoyl baccatin	Similar	Superior[4]	Phase II	i.v. 40 mg/m^2, q 3wks oral 60 mg/m^2, q 3wks	Wyeth-Ayerst
DJ-927	7-deoxy-9-β-dihydro-9,10,*O*-acetal taxane	Similar	Superior[5]	Phase I	oral 27 mg/m^2, q 3wks	Daiichi Pharmaceuticals

Abbreviations: 10-DAB, 10-deacetylbaccatin III; wks, weeks; i.v., intravenous. [1]Compared to docetaxel-sensitive cell lines and human xenografts; [2]Compared to docetaxel-(highly and moderately)-resistant cell lines and human xenografts (over)expressing *Abcb*-1; [3]More potent (20 to 30-fold) in human breast and colon cancer cell lines; [4]Drug resistance in KBV1 cells: 8-fold lower for MAC-321; [5]More potent (40 to 50-fold) in PC-6/VCR29-1 cell lines.

Conclusion

Continued research has offered us new and complementary insights on various aspects of docetaxel treatment, and yet, dose and schedule are still based on initial recommendations. Although this may sound disappointing, important steps forward have been made (Table 2) and research is ongoing. Besides the discussed areas of treatment optimization, future investigations will focus on further development of preclinically promising alternative formulations, on pharmacogenomic-based treatment optimization and on pharmacogenetic-based dose individualization strategies. However, given the large, ethnically diverse population studies required, introduction of the latter two strategies is not expected in the foreseeable future. On shorter term, it is likely that TDM will be explored as it provides a potential tool for rapidly achievable treatment optimization.

Table 2. Investigated areas of improvement of docetaxel-based chemotherapy

Area of improvement	Outcome
Weekly schedules	Alternative for patients at high risk for myelotoxic complications
Pharmacokinetics	Interindividual variability can be decreased by phenotypic individualized dosing Most predictive phenotyping probe controversial Practical disadvantages of phenotyping in oncology practice Therapeutic drug monitoring requires investigation
Reversal of resistance	ABCB1-modulating agents insufficiently reverse (multi)drug resistance due to multiple resistance mechanisms
Oral administration	Oral administration feasible upon pharmacologic modulation Second-generation oral taxanes likely to prevail
Second-generation taxanes	In clinical phase I/II development; also oral drugs
Alternative formulations	Alternative formulations in preclinical phase Introduction not foreseen in near future
Pharmacogenomics and Pharmacogenetics	Sufficiently powered trials necessary to determine clinical relevance

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Chapter 4

Alternative Drug Formulations of Docetaxel; a Review

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Anticancer Drugs, in press

Abstract

The anticancer drug docetaxel (Taxotere®) is formulated in the non-ionic surfactant polysorbate 80 (Tween®80). Early in the clinical development of docetaxel it became clear that docetaxel administration is associated with the occurrence of unpredictable (acute) hypersensitivity reactions and cumulative fluid retention. These side-effects have been attributed, in part, to the presence of polysorbate 80 and have consequently initiated research focused on the development of a less toxic, better-tolerated polysorbate 80-free formulation of docetaxel. More recently, there is increasing interest in developing a (polysorbate 80-free) docetaxel formulation that selectively targets malignant tissue, thereby increasing efficacy while decreasing the occurrence of side-effects related to wide and non-specific body distribution.

This review aims to discuss the preclinical and clinical results of pharmaceutical strategies (i.e. PEGylated (immuno)liposomal docetaxel, docetaxel-fibrinogen-coated olive oil droplets, docetaxel encapsulated nanoparticle-aptamer bioconjugates, submicronic dispersion formulation) to develop an alternative, solvent-free, delivery form for docetaxel characterized by increased efficacy and decreased toxicity.

Introduction

The anticancer drug docetaxel (Taxotere®, Sanofi-Aventis) demonstrates significant antitumour activity against various human malignancies and is approved for the treatment of patients with locally advanced or metastatic breast cancer, non-small-cell lung cancer, hormone refractory prostate cancer and advanced gastric cancer [1]. Docetaxel (Figure 1) is a semi-synthetic drug prepared by chemical modification of 10-deacetylbacattin III, an inactive precursor compound isolated from the needles of the European yew tree, *Taxus Baccata*. Due to its poor water solubility (3 μg/mL) docetaxel is dissolved in the non-ionic surfactant polysorbate 80 (Tween®80), the major component of which is polyoxyethylene-20-sorbitan monooleate (Figure 2), structurally similar to polyethyleneglycols. In early phase I clinical trials, docetaxel was supplied as a sterile solution containing 15 mg/mL docetaxel in 50 % polysorbate 80 and 50 % ethanol [2-6]. In order to decrease the amount of polysorbate 80 and ethanol administered to patients, this formulation was optimized and a new formulation was used in the phase II and III clinical trials, and subsequently marketed. The currently approved formulation contains 40 mg/mL docetaxel and 1040 mg/mL polysorbate 80 (i.e. 26 mg polysorbate 80 per mg docetaxel) and requires further dilution with 13 % ethanol prior to addition to the intravenous infusion solution.

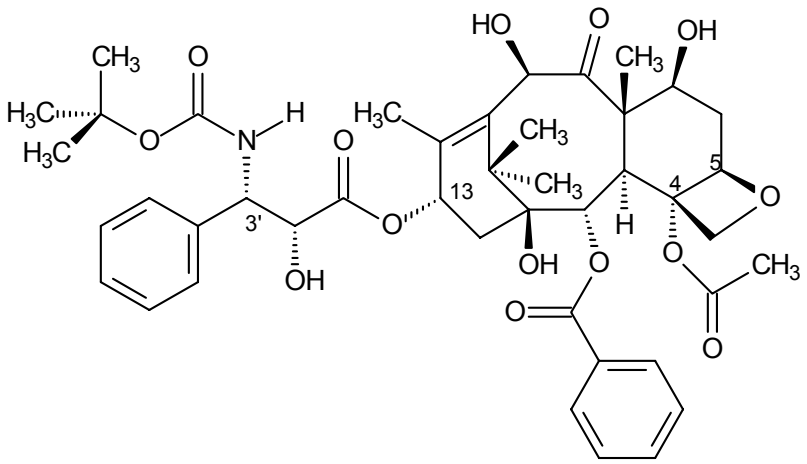

Figure 1. *Chemical structure of docetaxel.*

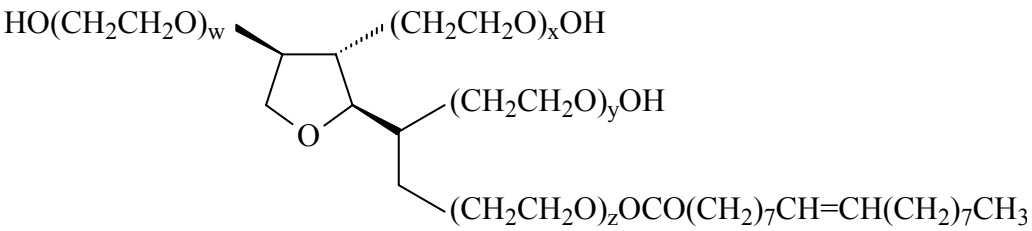

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Figure 2. *Chemical structure of the primary constituent of polysorbate 80: polyoxyethylene-20-sorbitan monooleate.*

Early in the clinical development of docetaxel it became clear that docetaxel administration is associated with the occurrence of unpredictable (acute) hypersensitivity reactions, widely ranging in incidence (range, 5 - 60 %) and severity (from mild pruritus to systemic anaphylaxis). In addition, fluid retention resulting in weight gain, peripheral oedema, and occasionally pleural or pericardial effusions, was reported with an incidence increasing above 50 % at cumulative docetaxel doses of ≥ 400 mg/m^2 [7-9]. The occurrence of hypersensitivity reactions has, in part, been attributed to intrinsic toxic effects of polysorbate 80, more specifically to oxidation products and oleic acid present in polysorbate 80, which are known to cause histamine release [10,11]. The role of histamine in the aetiology of polysorbate 80-induced hypersensitivity reactions is further supported by the fact that these side-effects are effectively ameliorated by premedication with corticosteroids and

antihistamines [12,13]. However, more recently, the allergenic origin of these hypersensitivity reactions has been opposed and a pathogenetic mechanism, involving the release of vasoactive substances, has been suggested [14]. The occurrence of cumulative fluid retention may, in part, be explained by the fact that the formulation vehicle has been shown to increase membrane permeability [15]. This finding is in line with reports of increased filtration of fluid to the interstitial space following a decrease in plasma colloid osmotic pressure, which occurs upon multiple docetaxel courses [16,17]. Moreover, the fact that corticosteroids significantly reduce and delay the onset of fluid retention, allowing for a higher cumulative dose to be administered [13], may also indicate an effect of vessel wall permeability. Although of minor importance, it should be noted that the beneficial effects of prophylactic corticosteroid administration may come at the cost of treatment-related morbidity (e.g. hyperglycaemia, peptic/duodenal ulcers). Finally, polysorbate 80 has been shown to increase plasma viscosity and produce changes in erythrocyte morphology, effects which have been suggested to contribute to mechanisms related to docetaxel-mediated cardiovascular side-effects [18].

The drawbacks associated with the presence of polysorbate 80 in the formulation of docetaxel initially initiated research focused on developing a formulation that enhances the solubilization of docetaxel while avoiding the use of polysorbate 80, thus resulting in a less toxic, better-tolerated polysorbate 80-free formulation. More recently, there is increasing interest in developing more advanced (solvent-free) formulations, which provide selective tumour delivery. Examples include targeted (immuno)liposomes and nanoparticles, which profoundly modify both the pharmacokinetics (PK) and pharmacodynamics (PD) of docetaxel, thereby increasing efficacy while decreasing the occurrence of side-effects related to wide and non-specific body distribution (e.g. neurotoxicity, musculo-skeletal toxicity, neutropenia).

This review aims to discuss the preclinical and clinical results of pharmaceutical strategies to develop an alternative, solvent-free delivery form for docetaxel. Related strategies, including the development of novel, structurally related docetaxel analogues, although also aimed at optimizing the risk-benefit ratio (i.e. balance between treatment-related toxicity and efficacy) for docetaxel treatment, have been discussed previously elsewhere [19].

Prerequisites for a polysorbate 80-free formulation

A polysorbate 80-free docetaxel formulation is only desirable if polysorbate 80 does not substantially contribute to docetaxel's antitumour efficacy. Several reports have suggested that polysorbate 80 possesses intrinsic tumour activity *in vitro* and in animals models [20,21], possibly attributable to oleic acid, rapidly cleaved from polysorbate 80 upon serum carboxylesterase-mediated hydrolysis [22], and known to interfere with malignant cell proliferation [23]. However, the exact contribution of polysorbate 80 and/or oleic acid to

clinically observed antitumour efficacy has not (yet) been clarified. Furthermore, due to its extremely low volume of distribution [24,25], delivery of polysorbate 80 outside the central compartment to the tumour is believed to be insignificant, consequently excluding any substantial contribution to cytotoxicity. In addition, docetaxel is a well known substrate for the transmembrane transporter ABCB1 (P-glycoprotein; MDR-1) [26]. ABCB1, which is expressed in tumours and normal tissues, acts as a drug/xenobiotic-efflux pump [27] and its overexpression is implicated in the occurrence of (multi)drug resistance (MDR). Polysorbate 80-mediated inhibition of ABCB1 has been observed *in vitro* [28,29], although not confirmed *in vivo* [30]. Moreover, to date, clinical investigations evaluating docetaxel administration in combination with ABCB1 inhibitors (e.g. laniquidar, tariquidar, zosuquidar, elacridar) [19], in the hope of restoring or enhancing chemosensitivity, have been disappointing and have not (yet) yielded an adequate MDR-reversal agent. Furthermore, although ABCB1 plays a (major) role in the faecal disposition of docetaxel [31], its influence on docetaxel plasma PK is minimal to absent [32]. Overall, it is unlikely that a polysorbate 80-free docetaxel formulation will significantly affect docetaxel's cytotoxic properties.

Recent *in vitro* and *in vivo* data have demonstrated that low doses of docetaxel [33,34] and clinically achievable concentrations of polysorbate 80 [35] both exhibit antiangiogenic properties. However, clinically relevant concentrations of polysorbate 80 (i.e. concentrations achieved at the end of docetaxel infusion) nullify docetaxel-mediated inhibition of angiogenesis [35], suggesting that a polysorbate 80-free docetaxel formulation may potentially have a positive effect on the antiangiogenic capacity of docetaxel.

In vitro studies have demonstrated that docetaxel is extensively bound to albumin and alpha-1 acid glycoprotein (AAG) and that the latter is the main determinant of variability in docetaxel serum binding [36]. Lately, the influence of polysorbate 80 on the PK of total and unbound docetaxel (the latter being the pharmacologically active fraction) has been extensively investigated [37]. At clinically relevant concentrations, polysorbate 80 significantly increased the fraction of unbound docetaxel *in vitro*, a finding that was confirmed *in vivo*. In addition, Baker *et al.* demonstrated that there is a significant relationship between systemic exposure to polysorbate 80 and the clearance rate of unbound docetaxel; higher polysorbate 80 exposure is associated with reduced clearance of unbound docetaxel, consequently resulting in increased unbound drug exposure [25]. Moreover, exposure to *unbound* docetaxel was more closely related to drug-induced severe haematological toxicity than *total* docetaxel exposure. The exact mechanistic basis for the decreased protein binding of docetaxel in the presence of polysorbate 80 is unknown, yet is presumably the result of micellar complexes formed by polysorbate 80 with serum proteins (albumin and AAG) [38], thus leading to saturable protein binding of docetaxel [36] and/or the result of displacement of protein-bound docetaxel by rapidly generated polysorbate 80 degradation products (e.g. oleic acid) [39]. Overall, it is clear that the degree of docetaxel plasma binding and consequently the fraction of, and exposure to unbound drug is, in part, influenced by the formulation vehicle. A

polysorbate 80-free formulation, may thus, through a decrease in the fraction of unbound drug, reduce the incidence and severity of unpredictable neutropenia, thereby improving the risk-benefit ratio for docetaxel treatment. One could argue that such a formulation might compromise drug efficacy due to decreased concentration of the pharmacologically active drug fraction in plasma and, more importantly, at the tumour site. However, to date, population PK/PD studies have identified systemic docetaxel exposure as a significant predictor of (haematological) toxicity, yet the correlation between any measure of docetaxel exposure (i.e. total clearance, total systemic exposure, peak plasma-level, duration of plasma-levels greater than a certain value) and antitumour response is much less clear [9]. Furthermore, data on intratumoural docetaxel PK are lacking and an improvement in the risk-benefit ratio, through a decrease in toxicity, may outweigh a decrease, to a yet unknown degree, in antitumour efficacy.

In conclusion, based on the biological and pharmacological properties of polysorbate 80, it seems unlikely that a polysorbate 80-free docetaxel formulation will compromise docetaxel antitumour efficacy.

Alternative formulations – Preclinical data

Avoiding the use of polysorbate 80 while at the same time developing a drug formulation that targets malignant tissue, has received substantial interest recently and has led to several alternative, solvent-free docetaxel formulations with varying potential to selectively deliver docetaxel to the tumour, thereby potentially enhancing efficacy while decreasing the occurrence of undesirable side-effects. One approach to avoid polysorbate 80 administration and selectively target the tumour is the use of fibrinogen microspheres as delivery vehicle, as previously investigated for other anticancer drugs [40,41]. Local fibrin(ogen) deposition occurs within the stroma of the majority of solid tumours and is associated with tumour angiogenesis, growth and metastatic potential [42]. In addition, thrombin-mediated accumulation and retention of intravenously administered fibrinogen-coated olive oil droplets, at fibrin(ogen)-rich sites, has been demonstrated [43]. These features initiated the preparation of murine-fibrinogen-coated micronized olive oil droplets loaded with docetaxel [44] and subsequently, evaluation of this formulation's antitumour activity upon intraperitoneal (i.p.) administration to mice bearing a fibrin(ogen)-rich ascites tumour [45]. Upon i.p. treatment with the docetaxel-fibrinogen-coated olive oil droplet formulation (docetaxel dose ~ 20 mg/kg; mean olive oil droplet size ~ 12 μm), median survival increased approximately 2-fold compared to treatment with docetaxel solubilized in polysorbate 80. A preliminary toxicity assessment based on the change in weight of healthy, tumour-free mice 15 days following i.p. injection of either normal saline, docetaxel solubilized in polysorbate 80 or docetaxel-loaded-fibrinogen-coated olive oil droplets demonstrated no significant differences. The association of docetaxel with tumour cells was monitored by administering tumour-bearing mice either

docetaxel solubilized in polysorbate 80 or docetaxel-loaded-fibrinogen-coated olive oil droplets, both spiked with [^{3}H]-docetaxel. Docetaxel association with tumour cells, measured by liquid scintillation counting 48 hours after treatment, was at least 10-fold increased upon i.p. administration of docetaxel-loaded olive oil droplets compared to docetaxel solubilized in polysorbate 80. These findings suggest potential to improve the therapeutic efficacy of docetaxel treatment. However, several issues require to be further addressed, including the feasibility of intravenous administration, which requires smaller droplet size, the influence of anticoagulants or fibrinolytic agents, which may potentially reduce the therapeutic efficacy of the fibrinogen-coated olive oil formulation and toxicity aspects related to the observed significant antibody response (i.e. droplet-induced production of antifibrinogen antibodies), of which the long-term effects on effectiveness are yet unclear.

Recently, research has increasingly focused on nanotechnological devices for the development of (biomarker)-targeted delivery systems for multiple therapeutic agents [46]. Nanotechnology is a multidisciplinary field, which covers a diverse array of devices derived from engineering, biology, physics and chemistry. These nanotechnology devices (nanotherapeutics) include nanovectors aimed at improving the tumour-targeting efficacy of anticancer drugs [47]. An injectable drug-delivery nanovector is defined as a hollow or solid structure with a diameter in the 1 - 1000 nm range. It can be filled with anticancer drugs and targeting moieties can be attached to its surface resulting in specific and differential uptake by the targeted cells, in order to deliver a constant dose of chemotherapy over an extended period of time. Probably the most well known, simplest and earliest examples of nanovectors applied in cancer treatment are liposomes, which are a hollow type of nanovector, whereas nanoparticles are considered solid nanovectors. Liposomes are spherical particles (vesicles) consisting of one or more lipid bilayer membranes, which encapsulate an internal space where notably hydrophilic agents can be entrapped; the lipid bilayer membrane of the liposome may serve as a reservoir for hydrophobic drugs. PEGylated liposomes (STEALTH® [sterically-stabilized] liposomes) differ from conventional liposomes by a polymer (polyethylene glycol, PEG) surface coating. These modified liposomes are characterized by reduced uptake by the reticulo-endothelial system, favourable PK (long circulating time, slow clearance rate, small volume of distribution), reduced accumulation in healthy tissues and, most importantly, by increased, preferential tumour uptake due to their ability to extravasate through the hyperpermeable tumour vasculature, a tumour-targeting mechanism known as enhanced permeation and retention [48,49]. These distinct features make PEGylated liposomes an attractive drug carrier. Indeed, for anticancer drugs, the advantages of PEGylated liposomes are best illustrated by PEGylated liposomal doxorubicin (Caelyx®, Doxil®, Myocet®). The wish to circumvent the use of polysorbate 80 and to improve the therapeutic index for docetaxel-based therapy through specific tumour targeting has led to the successful preparation of PEGylated liposomal docetaxel [50] without compromising cytotoxicity. Indeed,

in vitro cytotoxic activity of the PEGylated docetaxel formulation was almost equipotent to the non-liposomal docetaxel formulation. PK profiles for docetaxel solubilized in polysorbate 80 and docetaxel encapsulated in the PEGylated liposomes, assessed after a single intravenous bolus dose to mice, were both best described by a two-compartment model. However, the PK parameters differed significantly; docetaxel terminal half-life was increased nearly 13-fold upon liposomal encapsulation and clearance and volume of distribution were decreased more than 100-fold and 6-fold, respectively, compared to docetaxel solubilized in polysorbate 80. Further increase of the docetaxel concentration inside the PEGylated liposomes (currently 0.7 ± 0.2 mg/mL) is required before clinical trials can be initiated to determine if the improved PK features result in selective and efficient tumour uptake and reduced toxicity. Interestingly, in rats and mice [51,52], the PK of a second dose of PEGylated liposomes (devoid of encapsulated drug) was dramatically altered compared to the first dose in a time-interval dependant manner. The most prominent difference was a major increase in clearance, hence the observation is referred to as the 'accelerated blood clearance'(ABC)-phenomenon. Initially, the ABC-effect was suggested to be caused by a considerable increase in hepatic accumulation, possibly involving certain serum factor(s) secreted into the blood after the first dose of PEGylated liposomes. Most recently, evaluations have demonstrated that IgM is the major serum protein, which selectively binds to PEGylated liposomes upon repeated injection, and that these IgM-bound PEGylated liposomes can then activate the complement system [53], thus leading to accelerated clearance and enhanced hepatic uptake. Theoretically, the ABC-phenomenon can potentially compromise therapeutic efficacy and the strongly increased drug uptake in the liver may cause severe undesirable liver toxicity. Moreover, repeated administration of PEGylated liposomes may lead to the occurrence of unexpected immune reactions. However, in clinical practice the occurrence of immune reactions after repeated doses of PEGylated liposomal doxorubicin is rare (1 - 5 %), suggesting that the observed ABC-phenomenon for PEGylated docetaxel may have only a minor impact. Nevertheless, future research in the design and clinical use of PEGylated liposomal docetaxel, should determine the implications of these findings.

Covalent attachment of targeting ligands, such as monoclonal antibodies specific for antigens expressed on the surface of cancer cells, is another modification of the conventional liposome with the aim to improve selective tumour delivery. Docetaxel has been shown to enhance tumour response upon irradiation [54], however, clinical application of this radiosensitizing potential is limited due to side-effects associated with the drug's poor tumour selectivity. To increase tumour delivery and to evaluate the radiosensitizing properties of docetaxel, human colon adenocarcinoma cell lines expressing carcinoembryonic antigen (CEA), were treated with irradiation and PEGylated docetaxel 'immunoliposomes', i.e. immunoliposomes prepared by coupling monoclonal antibodies against CEA to the PEG-coating of the lipid membrane [55]. Specifically, cells were incubated (2 h, 37 °C) with different concentrations of immunoliposomal docetaxel or liposomal docetaxel (range, 1 –

1000 nmol/L docetaxel) after which the cells were washed and further incubated (24 - 48 h, 37 °C). Non-incubated cells received a series of test radiation doses ranging from 0 Gy to 8 Gy to determine the degree of radiotoxicity; radiotoxicity was most pronounced at a dose of 2 Gy. Consequently, this radiation dose was used to irradiate the cells incubated with immunoliposomal- and liposomal docetaxel. Cytotoxicity, assessed using the colourimetric MTT assay, was induced by immunoliposomal docetaxel in a dose and time-dependant manner. Similar evaluation of the cytotoxic efficacy of the multimodality treatment demonstrated that the effects of immunoliposomal docetaxel were potentiated upon radiation compared to liposomal docetaxel with irradiation or only irradiation ($P < 0.05$). Furthermore, flow cytometric analysis demonstrated that upon treatment with immunoliposomal docetaxel combined with irradiation, apoptosis was significantly increased compared to the multimodality treatment for liposomal docetaxel. Further research should determine if this specific immunoliposomal docetaxel formulation offers potential to improve local radiotherapy in the treatment of colon cancer.

As mentioned, an expanding number of nanovectors are currently under development for novel, optimized drug-delivery modalities [46,47,56]. Approaches include molecular targeting of nanovectors through conjugation of active recognition moieties to the surface of the nanovector (an approach characterized by potential advantages above conventional antibody-targeted therapy), intracellular targeting of nanoparticles by folate, dendritic polymers as multifunctional nanodevices, silicon and silica materials as materials for injectable nanovectors, metal-(e.g. gold) based nanovectors and polymer-based nanovectors of which the latter seem to be the most promising for clinical translation. Most recently, docetaxel-encapsulated nanoparticles formulated with biocompatible and biodegradable poly(D,L-lactic-*co*-glycolic acid)-block PEG-copolymer and surface functionalized with A10 2'-fluoropyrimidine aptamers (i.e. RNA oligonucleotides; nucleic acid ligands) [57] that bind to the extracellular domain of the transmembrane prostate-specific membrane antigen (PSMA), a well characterized antigen expressed with high specificity on the surface of prostate cancer cells, have been successfully developed *in vitro* (Figure 3) and their cytotoxicity evaluated using a xenograft nude mouse prostate cancer model [58]. Due to the surface functionalization with the specific PSMA aptamers, these docetaxel-encapsulated nanoparticle-aptamer bioconjugates (Doc-Np-Apt) exert significantly enhanced cellular cytotoxicity *in vitro* resulting from targeted delivery and enhanced cell-specific uptake compared to non-targeted docetaxel-encapsulated nanoparticles (lacking the PSMA aptamer). A single intratumoural injection of Doc-Np-Apt (40 mg/kg) *in vivo* was significantly more efficacious regarding tumour size reduction and survival time compared to an equivalent dose of non-targeted docetaxel-encapsulated nanoparticles. The enhanced efficacy was attributed to delayed clearance from the target site due to preferential binding to the PSMA proteins, leading to internalization and subsequent intracellular drug release. Mean body weight loss at nadir was significantly decreased (2-fold) for Doc-Np-Apt compared to non-targeted docetaxel-

encapsulated nanoparticles, suggesting reduced treatment toxicity. Furthermore, there was no evidence of persistent haematological toxicity. Several aspects of this approach have the potential to facilitate translation into clinical practice, including the fact the poly(D,L-lactic-*co*-glycolic acid) is a component the FDA has approved for clinical use, and the fact that the targeting molecules (aptamers) are small, relatively stable, non-immunogenic and easy to produce on a large scale. However, before clinical application is possible several aspects, including potential sensitization reactions, biological/biophysical barriers impeding targeted delivery, and the tailoring of dosing and administration schedules remain to be examined.

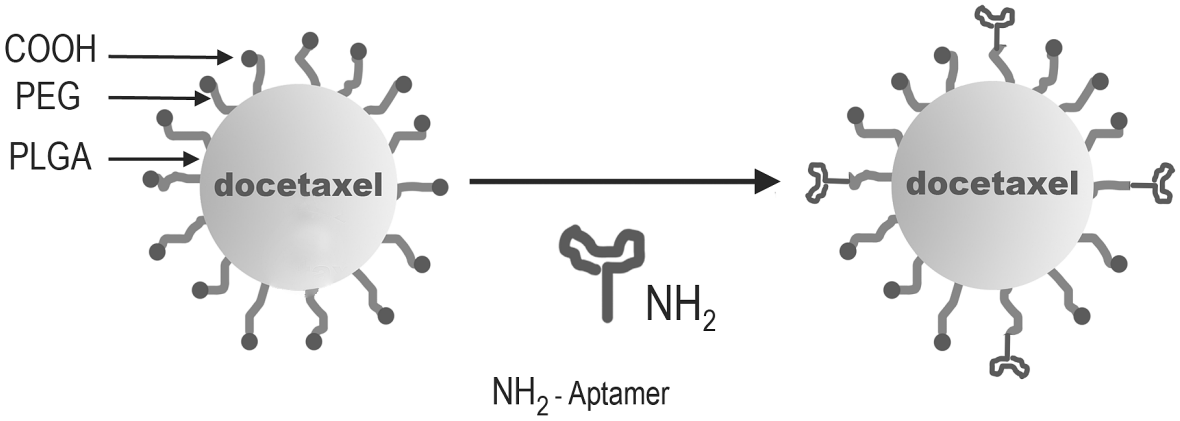

Figure 3. *Schematic representation of the coupling of amine-functionalized A10 PSMA aptamers to a docetaxel-encapsulated, PEGylated nanoparticle by carbodiimide chemistry, thus resulting in a docetaxel-encapsulated-PEGylated-poly(D,L-lactic-co-glycolic-acid)-nanoparticle-aptamer-bioconjugate.*

Abbreviations: PEG, polyethyleneglycol; COOH, carboxylic acid; PLGA, poly(D,L-lactic-co-glycolic acid); NH_2-aptamer, amine-functionalized A10 prostate specific membrane antigen aptamer; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; NHS, N-hydroxysuccinimide.

Alternative formulations – Clinical data

Clinical studies evaluating the efficacy and safety profile of a polysorbate 80-free docetaxel formulation are limited to two phase I studies [59,60]. Both trials evaluated a polysorbate 80-free submicronic dispersion formulation (Sanofi-Aventis) administered once every three weeks, as a 1-hour intravenous infusion without corticosteroid or antihistamine premedication. In patients with advanced solid tumours (N = 41), the maximum tolerated dose (MTD) was 145 mg/m^2 (without granulocyte colony-stimulating factor support), setting the recommended dose level at 130 mg/m^2, which is significantly higher than 100 mg/m^2, the highest recommended single agent dose (http://www.taxotere.com). Despite the lack of premedication, no hypersensitivity reactions were observed and fluid retention occurred in 49 % of the patients at a relatively high median cumulative dose (575 mg/m^2), yet was severe in less than 5 % of the cases. Dose-limiting toxicities (DLT) did not differ from those seen with the current formulation of docetaxel (severe neutropenia and febrile neutropenia), nor did the spectrum of haematological and non-haematological toxicities. Interestingly, a retrospective analysis concluded that at equimolar doses the solvent-free formulation appeared to result in a lower incidence and severity of infections, mucositis, diarrhoea and neuropathy, the latter finding supporting the notion that polysorbate 80, capable of producing vesicular degeneration [61], contributes, in part, to the predominantly mild sensory neuropathy observed in a high proportion of patients treated with docetaxel. The overall response rate (ORR) among thirty one breast cancer patients who had received a dose ≥ 100 mg/m^2 was 48.4 % (95 % confidence interval (CI), 30.2 – 66.9 %), which is comparable to the results of the pivotal trial leading to the approval of docetaxel for locally advanced or metastatic breast cancer (ORR 47.8 %; 95 % CI, 40.1 – 55.5 %) [62]. A PK evaluation revealed that clearance was independent of dose and similar to previously observed values [9]. The polysorbate 80-free formulation was also administered in combination with doxorubicin as first-line chemotherapy to patients with metastatic breast cancer (N = 38). In this study, antitumour activity was observed at all dose levels and the MTD was reached at 100 mg/m^2 polysorbate 80-free docetaxel and 60 mg/m^2 doxorubicin. Febrile neutropenia was again the DLT. The recommended dose for phase II evaluation was set at 85 mg/m^2 polysorbate 80-free docetaxel and 60 mg/m^2 doxorubicin, which is higher than the doses applied in a randomized phase III study evaluating docetaxel (75 mg/m^2) and doxorubicin (50 mg/m^2) treatment for the same indication [63]. In order to continue the development of this well tolerated and active formulation the manufacturing process was modified to allow for large-scale (i.e. industrial) production. However, this upscaling process led to some slight modifications of the formulation's physico-chemical properties, which may have been the cause of toxicities (oedema, hand and foot skin reactions) observed at lower doses and in earlier cycles compared to the initial solvent-free formulation. Although these toxicities were not severe enough to be classified as DLTs, they did often lead to treatment withdrawal and were the

reason to discontinue further clinical development of this polysorbate 80-free formulation (personal communication Sanofi-Aventis).

Discussion

Pharmaceutical excipients have an essential role in drug formulations. However, in contrast to earlier views, these excipients, including the non-ionic surfactant polysorbate 80 used to solubilize the hydrophobic anticancer drug docetaxel, are not inert vehicles, but are able to affect drug disposition and toxicity patterns [61]. Consequently, acknowledging that a less toxic (i.e. polysorbate 80-free) formulation of docetaxel is desirable, recent research has focused on developing alternative (solvent-free) drug delivery forms while continuing to maximize the drug's antitumour efficacy through preferential uptake of the drug at the site of action, the tumour. This pharmaceutical strategy has lead to several alternative drug formulations including fibrinogen-coated olive oil droplets loaded with docetaxel, PEGylated docetaxel liposomes, docetaxel immunoliposomes, and docetaxel-encapsulated nanoparticle-aptamer bioconjugates, all currently in different preclinical stages of development and with different advantages and disadvantages (Table 1). Whether the observed advantages (improved PK, selective tumour uptake, increased survival) over the current docetaxel formulation translate to clinical benefits remains to be seen. Potential drawbacks, which require to be overcome include the ABC-effect observed with PEGylated liposomes, the occurrence of (unexpected) immune reactions, also seen with docetaxel-fibrinogen-coated olive oil droplets and, most importantly, overcoming the pharmaceutical challenge of achieving encapsulation of a therapeutically meaningful amount of drug in the liposomes [64]. It would seem that the use of monoclonal antibodies or the more recently introduced aptamers (DNA or RNA oligonucleotides) as targeting ligand has much potential given the increasing number of well defined biomarkers (e.g. antigens) expressed on the cancer cell surface. Which of these two targeting approaches proves to be the more suitable remains to be examined. Initial problems related to specificity, purity, immunogenicity, relatively long development times and batch-to-batch variability upon large-scale biological production of (monoclonal) antibodies, although largely overcome, could still complicate their general application. On the other hand, aptamer synthesis does not rely on biological systems and is an entirely chemical process, which can easily be scaled up. Indeed, aptamer drug-targeting could potentially provide an adequate alternative to antibody-based drug-targeting techniques. Moreover, although the aptamer-based targeting approach is considered highly promising, it is expected that the greatest gain in optimizing therapeutic selectivity will be achieved by synergistic combinations of different nanotechnological targeting strategies.

Table 1. Advantages & disadvantages of the alternative formulations of docetaxel currently in development compared to the current formulation

Formulation	Advantages	Disadvantages
Taxotere® (Sanofi-Aventis)	FDA approved (since 1996) Substantial clinical experience Well established PK/PD relationships	Docetaxel solubilized in polysorbate 80 Polysorbate 80 associated with: - hypersensitivity reactions - cumulative fluid retention
PEGylated liposomal docetaxel	Potential for selective tumour delivery Tumour targeting via EPR-phenomenon Equipotent *in vitro* cytotoxicity[1] Improved PK features[1]: ↑ $t_{1/2}$, ↓ CL, ↓ Vd	Preclinical development stage ↑ docetaxel concentration in liposomes required Observed ABC-phenomenon may: - compromise efficacy - lead to adverse (immune) reactions
PEGylated docetaxel immunoliposomes	Potential for selective tumour delivery Tumour targeting via monoclonal antibodies Increased radiosensitizing potential[1] Potential to improve local radiotherapy	*In vitro* development stage Problems related to use & production of Mabs
Docetaxel-fibrinogen-coated olive oil droplets	Potential for selective tumour delivery Tumour targeting via fibrinogen microspheres ↑ median survival[1] (i.p.) ↑ docetaxel tumour concentration[1] (i.p)	Preclinical development stage i.v. dosing not yet evaluated Anti-fibrinogen antibodies observed Influence of anticoagulants to be determined
Docetaxel-encapsulated-nanoparticle-aptamer	Potential for selective tumour delivery Tumour targeting via aptamers Aptamers: small, non-immunogenic, stable, large-scale production possible Enhanced cytotoxicity *in vitro*[2], enhanced efficacy *in vivo*[2], reduced toxicity *in vivo*[2]	Preclinical development stage Problems related to use of PEG-copolymer

Abbreviations: FDA, US Food and Drug Administration; PK/PD, pharmacokinetic/pharmacodynamic; EPR, enhanced permeation and retention; $t_{1/2}$, terminal half-life; CL, clearance, V_d, volume of distribution; ABC, accelerated blood clearance; i.p., intraperitoneal administration; i.v., intravenous administration; Mabs, monoclonal antibodies; [1]Compared to docetaxel solubilized in polysorbate 80; [2]Compared to non-targeted docetaxel-encapsulated nanoparticles.

Currently, only one polysorbate 80-free docetaxel formulation has been evaluated clinically in two phase I trials. Although this formulation demonstrated uncompromised cytotoxic activity, the occurrence of toxicities leading to treatment withdrawal resulted in the discontinuation of further clinical development of this solvent-free docetaxel formulation.

To date, attempts to improve taxane-based treatment have largely focused on, and have been most successful for paclitaxel [65,66], the first taxane to be introduced (FDA approval 1992). Indeed, most recently paclitaxel nanoparticles conjugated to albumin molecules (Abraxane®, Abraxis Oncology; FDA approval 2005), the latter enhancing the transport of the nanoprticles across the vascular endothelium, demonstrated an improved therapeutic index compared to paclitaxel (Taxol®) in the treatment of patients with metastatic breast cancer [67]. Several reasons may explain why the majority of research has focused on improvement of the paclitaxel drug formulation and include the earlier introduction and thus larger body of clinical experience, and the fact that a greater improvement may be made for current paclitaxel-based treatment with less effort, as the formulation vehicle Cremophor EL used to solubilize paclitaxel presents more PK and PD drawbacks [61] than polysorbate 80. However, docetaxel is also a highly suitable candidate to concentrate on, given its superiority above paclitaxel in overall survival in patients with metastatic breast cancer [68], linear PK [69], single enzyme-mediated metabolism [70,71] and the existing extensive knowledge on PK/PD relationships [9]. The success of Abraxane® suggests that developing merely a solvent-free formulation is not enough therapeutic improvement to warrant extensive clinical evaluation. The combination of a solvent-free docetaxel formulation with tumour targeting characteristics ultimately shows the most promise of future therapeutic gain for this highly active drug.

In conclusion, preclinical research aimed at optimizing the risk-benefit ratio for docetaxel-based therapy through development of a less toxic, solvent-free (polysorbate 80-free) formulation with tumour targeting properties is ongoing and encouraging. However, it is unlikely that an alternative formulation will be available for clinical use in the near future. Although this may sound disappointing it is clear that one is convinced of the need to optimize the risk-benefit ratio for docetaxel treatment and any advances which can be made in this area, however modest they may be, are worth further research.

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Chapter 5

Effect of Cytochrome P450 3A4 Inhibition on the Pharmacokinetics of Docetaxel

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Abstract

Objective: In vitro studies indicate that the anticancer drug docetaxel is primarily eliminated by cytochrome P450 (CYP) 3A4-mediated metabolism. Coadministration of drugs that modulate the activity of CYP3A4 is, therefore, likely to have undesirable clinical consequences. 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 cross-over 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 analysed using noncompartmental analysis.

Results: Ketoconazole coadministration resulted in a 49 % decrease in clearance of docetaxel (P = 0.018). The mean (± SD) clearance values were 35.0 L/h ± 11.8 L/h (95 % confidence interval, 24.1 – 45.9 L/h) for docetaxel alone and 18.2 L/h ± 9.68 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 plasma concentration-time curve of ketoconazole (R^2 = 0.529; P = 0.064).

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 docetaxel 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 (CYP) 3A 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 predominantly 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 faeces, whereas total urinary excretion is also less than 10 % [8]. The metabolites demonstrate substantially reduced cytotoxic activity as compared with 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 [10-12]. Although the fact that docetaxel is predominantly 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 this trial was to assess the effect of CYP3A inhibition on the pharmacokinetics of docetaxel in cancer patients, by the use of the model inhibitor ketoconazole [13].

Methods

Patient 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 the following: (1) a life expectancy of at least 12 weeks; (2) a World Health Organization performance status of 1 or less; (3) no chemotherapy, hormonal therapy, radiotherapy, or major surgery within 4 weeks prior to treatment; (4) age more than 18 years; (5) adequate contraception for women of child-bearing potential; and (6) adequate bone marrow function (absolute neutrophil count $> 1.5 \times 10^9$/L; platelet count $> 100 \times 10^9$/L), renal function (serum creatinine level $\leq 1.5 \times$ the upper limit of normal), and hepatic function (serum bilirubin $\leq 1 \times$ the upper limit of normal, alanine aminotransferase and aspartate aminotransferase levels $< 2.5 \times$ the upper limit of normal, and alkaline phosphatase level $\leq 5 \times$ the upper limit of normal 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, Hoevelaken, The Netherlands), administered 3 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 was given 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 area under the plasma concentration-time curve of docetaxel is dose-proportional over a large dose range (5 mg/m^2 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 behaviour (reviewed by Clarke and Rivory [8]). Therefore, values for clearance of docetaxel between the treatment courses with and without ketoconazole coadministration were compared directly without any correction.

The first ketoconazole dose was administered 1 hour before the docetaxel infusion was started, 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 being maintained above the therapeutic threshold level. The allocation sequence of the courses for each patient was determined at study entry by use of a restricted-block randomization procedure. Premedication consisted of dexamethasone (8 mg oral dose) given twice daily for 3 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) [16]. Patients benefiting from docetaxel treatment were offered continuation of treatment beyond cycle two 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 (Sparreboom A, unpublished data, 2002). In this group of patients, the standard deviation (SD) of the expected differences of the 2 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. On the basis of a pair-wise (2-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) [17].

Pharmacokinetic analysis

Blood samples were collected in glass tubes containing lithium heparin as anticoagulant and immediately centrifuged (4000 *g* at 4 ºC for 10 minutes) to separate plasma, which was stored at –80 ºC until analysis. Samples were taken at the following time points: immediately before 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 ultraviolet detection, respectively, according to published procedures [18,19]. This assay for docetaxel has a lower limit of quantitation of 0.0004 μg/mL (0.5 nmol/L), which is sufficiently sensitive to allow quantitation of docetaxel in samples (within the collection time period tested) obtained from patients treated with low drug doses. Determination of the fraction of unbound docetaxel was performed by use of equilibrium dialysis with a tritiated docetaxel tracer (Moravek Biochemicals, Brea, CA, USA) [20].

Pharmacokinetic parameters for docetaxel and ketoconazole were calculated by non-compartmental analysis as implemented in the software package WinNonlin version 4.0 (Pharsight, CA, USA). For docetaxel, the parameters of interest included the peak plasma concentration (C_{max}), area under the plasma concentration-time curve extrapolated to infinity (AUC), clearance (defined as dose divided by AUC), and volume of distribution at steady state (Vss), as well as the half-life of the terminal phase. The latter parameter was calculated as ln(2)/*k*, in which *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}, 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 (CIs), unless stated otherwise. The effect of ketoconazole coadministration on the pharmacokinetic parameters of docetaxel was evaluated statistically by use of a nonparametric, 2-sided, Wilcoxon signed rank test for paired observations. The relationship between the exposure to ketoconazole and reduction of docetaxel clearance was evaluated by a least-squares linear regression analysis. The cut-off for statistical significance was set at $P < 0.05$. All statistical calculations were performed using NCSS 2001 (Number Cruncher Statistical System, Kaysville, UT, USA).

Results

Patients and toxicity profiles

To determine the influence of ketoconazole coadministration on the pharmacokinetics of docetaxel, a total of 7 patients entered the study (Table 1). All patients completed the study within the scheduled time. Uncomplicated grade 4 neutropenia was observed in 3 patients and grade 3 leukocytopenia was observed in another 2 patients during the single agent cycle with docetaxel. During the combination course with ketoconazole administration, only minimal toxicity was noted.

Table 1. Baseline patient characteristics [1]

Characteristic	No. of patients	Median	Range
Age (years)		40	36 - 59
Sex			
Male	4		
Female	3		
Body-surface area (m^2)		1.8	1.6 – 2.1
WHO performance		1	0 – 1
0	3		
1	4		
Tumour type			
Head and neck	2		
Cervix	1		
Sarcoma	1		
Melanoma	1		
ACUP	1		
Rectum	1		
Chemistry			
ASAT (U/L)		24	17 – 79
ALAT (U/L)		18	6 – 30
Alk Phos (U/L)		73	62 – 241
Total bilirubin (μmol/L)		6	4 – 11
WBC (x 10^9/L)		8.4	6.7 – 12.9
ANC (x 10^9/L)		7.1	2.6 – 15.1

Abbreviations: WHO, World Health Organization; ACUP, adenocarcinoma of unknown primary; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; Alk Phos, alkaline phosphatase; WBC, white blood cell count; ANC, absolute neutrophil count. [1]The upper limit of institutional normal for the pretherapy clinical chemistry parameters are as follows: ASAT, < 93 U/L for men and < 78 U/L for women, ALAT, < 103 U/L for men and < 78 U/L for women; Alk Phos, ≤ 600 U/L; total bilirubin, < 16 μmol/L.

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; these are similar to previous findings. The mean time to peak concentration on day 1 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 interindividual and intraindividual pharmacokinetic variation after the same oral dose has been reported. This is partly a result of differences in gastric acidity, because 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 1 patient this could be explained by administered comedication (see below).

Docetaxel analysis

The observed plasma concentrations of docetaxel for both treatments are shown in Figure 1. When ketoconazole was coadministered, 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 % ($P = 0.018$) (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 with the corresponding AUC of ketoconazole (Figure 2), as determined by a linear regression analysis ($R^2 = 0.529$; $P = 0.064$). A similar relationship was not observed with the time to peak concentration of ketoconazole ($R^2 = 0.047$; $P = 0.639$), suggesting that the rate of absorption was unrelated to variability in effect. For 1 patient, the fractional change was only 0.68, which was attributable to a very low exposure to ketoconazole resulting from concomitant administration of ranitidine, which is known to alter the gastrointestinal absorption of ketoconazole [21].

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 %), which is consistent with previous estimates [20]. The fraction unbound docetaxel was not significantly different in courses with and without ketoconazole (5.74 % ± 1.96 % *vs* 5.81 % ± 1.45 %; $P = 0.74$), indicating that protein binding of docetaxel is not significantly affected by ketoconazole.

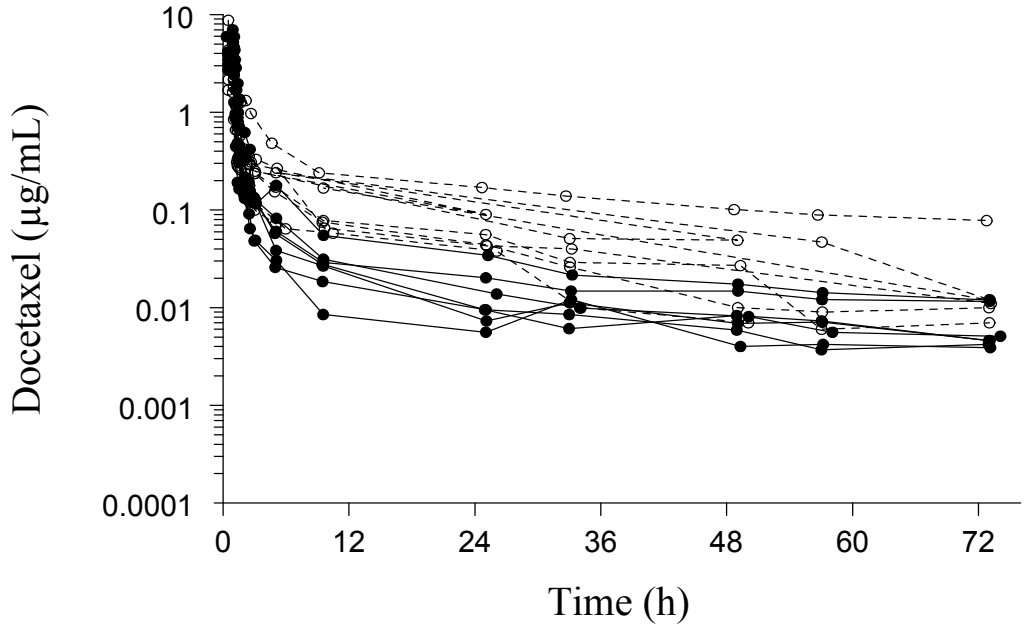

Figure 1. *Observed plasma concentrations of docetaxel in the absence (closed circles, solid lines; dose, 100 mg/m^2) and presence of ketoconazole coadministration (open circles, dashed lines; dose, 10 mg/m^2; data normalized to 100 mg/m^2).*

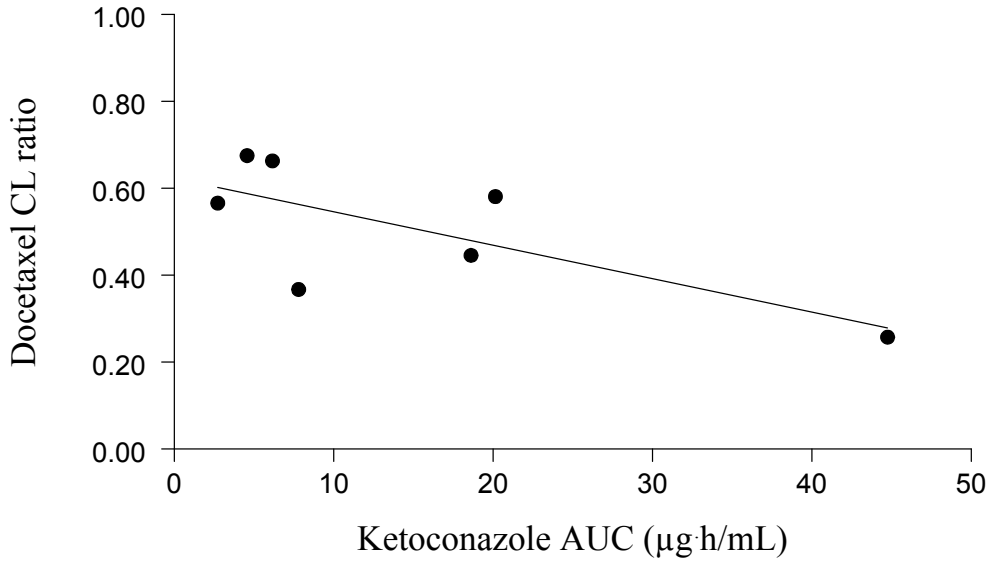

Figure 2. *Relationship between ketoconazole area under the curve (AUC, µg·h/mL) and the fractional change in docetaxel clearance (CL), defined as the ratio of CL in the presence and absence of ketoconazole coadministration. The change is described by the following equation: (0.623 ± 0.0660) – (0.0077 ± 0.0033) × (ketoconazole AUC in µg·h/mL); R^2 = 0.529; P = 0.064).*

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

Parameter	**Docetaxel (mean ± SD and 95 % CI or median and range)**	**Docetaxel/ketoconazole (mean ± SD and 95 % CI or median and range)**	**Ratio and 95 % CI**	***P-value***
Dose (mg)	190 (160 – 210)	19 (16 – 21)	NA	NA
Dose (mg/m^2)	100	10	NA	NA
T_{inf} (h)	1.05 (0.95 – 1.07)	1.03 (0.59 – 1.15)	NA	NA
C_{max} (µg/mL)	4.42 ± 1.32 (3.19 – 5.64)	0.543 ± 0.221 (0.339 – 0.748)	NA	NA
AUC (µg·h/mL)	5.90 ± 2.56 (3.53 – 8.27)	1.28 ± 0.628 (0.699 – 1.86)	NA	NA
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)	0.31
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)	0.018
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)	0.018
V_{ss} (L)	477 ± 265 (232 – 722)	388 ± 283 (126 – 649)	0.87 (0.52 – 1.22)	0.50
$T_{1/2}$ (h)	41.0 ± 10.9 (30.9 – 51.0)	28.3 ± 7.89 (21.0 – 24.4)	0.73 (0.45 – 1.02)	0.043

Abbreviations: CI, confidence interval; NA, 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; V_{ss}, volume of distribution at steady state; $T_{1/2}$, half-life of the terminal phase.

Discussion

This study shows that the clearance of docetaxel is significantly reduced by 49 % on coadministration with ketoconazole, albeit with 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 to 4 in approximately 90 % of patients (http://www.taxotere.com). Bruno *et al*. [22] 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. In this 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 (i.e. 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 [23], 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 [24]. However, the polymorphic distribution of CYP3A5 indicates that metabolically active CYP3A5 is expressed in approximately 30 % of Caucasians and in 50 % to 73 % of African Americans [25,26]. 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 inhibition constant values [27]. Furthermore, the percentage of inhibition by ketoconazole is inversely related to the fractional percentage of CYP3A5 in total CYP3A [27]. 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, coadministration 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 is 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 [28,29]. 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 6

Influence of High-Dose Ketoconazole on the Pharmacokinetics of Docetaxel

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Abstract

Objective: The pharmacokinetics (PK) of docetaxel are characterized by large interindividual variability in systemic drug exposure (AUC) and drug clearance. The PK variability is thought to be largely related to differences in the catalytic function of CYP3A, involved in docetaxel metabolism and elimination. As variability in efficacy and toxicity is associated with variability in docetaxel AUC and clearance, reducing interindividual PK variability may help improve the risk-benefit ratio of docetaxel therapy. We investigated if high-dose ketoconazole, a potent CYP3A inhibitor, could result in a uniform reduction of docetaxel clearance and reduce the interindividual variability in docetaxel AUC and clearance.

Methods: Seven patients were treated in a randomized cross-over design with intravenous docetaxel (100 mg/m^2) followed 3 weeks later by docetaxel (15 mg/m^2) given in combination with orally administered ketoconazole (400 mg 3 times daily, up to 47 hours after docetaxel infusion) or vice versa. Docetaxel plasma concentration-time data were described by a three-compartment PK model. Ketoconazole plasma concentration-time data were described by a one-compartment PK model.

Results: Docetaxel clearance was reduced by 50 % (P = 0.018) from 32.8 L/h ± 13.7 L/h to 16.5 L/h ± 8.15 L/h upon ketoconazole coadministration, albeit with large interindividual variability (fractional change in clearance, range 0.31 – 0.66). In the presence of ketoconazole, interindividual variability in docetaxel clearance and AUC, expressed as coefficient of variation, was increased from 41.6 % to 49.5 % and from 28.0 % to 35.1 %, respectively, and not, as we had hypothesized, reduced.

Conclusion: Inhibition of CYP3A by concomitant high-dose ketoconazole administration does not result in a uniform reduction of docetaxel clearance and does not reduce the interindividual variability in docetaxel AUC or clearance. This approach is unsuitable as method to achieve a uniform docetaxel PK profile.

Introduction

Since the introduction of docetaxel (Taxotere®) the metabolism, elimination pathways and clinical pharmacokinetics (PK) of this anticancer drug have been subject of thorough investigation. After intravenous (i.v.) administration, the drug is extensively metabolized to four pharmacologically inactive metabolites [1] by hepatic and intestinal cytochrome P450 (CYP) isozymes 3A [2-4], the catalytic activity of which is represented by two major isoforms, CYP3A4 and CYP3A5. CYP3A4 is regarded as the most active enzyme, possesses the highest affinity for docetaxel [4] and is predominant in Caucasians individuals [5-7]. Docetaxel is also a substrate for the ATP-binding cassette transmembrane protein ABCB1 (P-glycoprotein; MDR-1), which is known to act as a (drug/xenobiotic-)efflux pump [8].

Docetaxel plasma PK, although linear and independent of dose and schedule [9,10], is characterized by large interpatient variability notably in the PK parameters systemic drug exposure (expressed as area under the plasma concentration-time curve, AUC) and drug clearance. Indeed, for these two parameters typical values for interpatient variability (expressed as coefficient of variation) range between 30 % to 45 % [11-16]. Bruno *et al*. [14] reported that docetaxel AUC is a significant predictor of time to progression and that docetaxel clearance is a strong and independent predictor of severe neutropenia and febrile neutropenia. Theoretically, reducing the interindividual variability in AUC and clearance could aid in optimizing the risk-benefit ratio for docetaxel therapy. A potential approach to reduce the interindividual variability in docetaxel PK parameters is by eliminating the source (i.e. cause) of variability. Phenotypic expression of CYP3A activity has been identified as a strong predictor of docetaxel clearance [11,17-19] and observed variation in (intrinsic) CYP3A catalytic activity has been suggested to account to a large extent for the significant interindividual variability in docetaxel PK [2]. Moreover, the involvement of CYP3A, the activity of which can be readily induced or inhibited, in docetaxel elimination, renders the drug subject to a host of enzyme-mediated PK drug-interactions.

We recently conducted a randomized cross-over trial to evaluate the effect of concomitant orally administered standard-dose ketoconazole, a potent CYP3A inhibitor [20], on the plasma PK of docetaxel in cancer patients [12]. The reduction of docetaxel clearance was highly significant (average decrease 49 %, P = 0.018). However, we observed wide interindividual variability in the decrease of docetaxel clearance (range, 32 – 74 % reduction) as also previously reported by others [21]. Moreover, the decrease in docetaxel clearance was weakly correlated with corresponding systemic exposure to ketoconazole. Furthermore, interindividual variability in clearance was increased upon concomitant ketoconazole administration compared to single agent treatment (33.7 % *vs* 53.2 %). With the aim to eliminate the observed variability in the extent to which docetaxel clearance is reduced, we subsequently treated patients with a higher ketoconazole dose, the dose currently under investigation for the treatment of androgen-independent prostate cancer patients in combination with docetaxel [22]. We hypothesized that an increase in ketoconazole dose would result in sufficiently high exposure to ketoconazole in each patient and thus in maximum CYP3A inhibition overall, thereby eliminating or largely reducing the variability in (the reduction of) docetaxel clearance. Furthermore, we wished to assess if we could reduce the interindividual variability in docetaxel clearance and AUC by temporarily suppressing the source of variability, i.e. CYP3A catalytic activity, as such resulting in a uniform PK profile.

Part of the variation in total CYP3A activity, which is reflected by significant interindividual variability in the PK of CYP3A substrates [23-25] is suggested to be related to genetic diversity in the genes encoding the CYP3A4 and CYP3A5 proteins [26]. To date, most evidence infers that it is unlikely that CYP3A4 single nucleotide polymorphisms (SNPs) contribute substantially to the interindividual variability in CYP3A4 activity *in vivo*, due to

their limited functional significance and/or low allele frequency [27-30]. Furthermore, more than 80 % of Caucasian individuals is deficient in CYP3A5 metabolic activity due to the inactive CYP3A5*3C/*3C variant genotype [5,6]. However, for individuals with at least one CYP3A5*1 allele, CYP3A5 may account for at least 50 % of total CYP3A content, resulting in approximately 2- to 3-fold higher CYP3A activity *in vitro* [31]. Moreover, CYP3A4 and CYP3A5 show distinct differences in susceptibility to inhibition by ketoconazole [32]. Accordingly, part of the resulting interindividual variability in decrease of docetaxel clearance after ketoconazole treatment may be dependent on functional CYP3A4 and/or CYP3A5 genetic variants. We therefore included CYP3A4 and CYP3A5 genotyping in the study.

We here report on the plasma PK of docetaxel in cancer patients, after i.v. administration of the drug with and without concomitant oral high-dose ketoconazole administration.

Patients and methods

Patient selection and study design

Eligibility criteria, study design and sample calculation have been reported previously for the standard-dose ketoconazole study [12], and were the same for the current study. Briefly, cancer patients for whom no other treatment option was available were treated according to a randomized cross-over design (in order to exclude sequence bias) with two courses of docetaxel administered as a 1-hour i.v. infusion once every 3-weeks. One course was given at a dose of 100 mg/m^2 and the other at a dose of 15 mg/m^2 in combination with seven 400 mg doses of ketoconazole, orally administered once every 8 hours, starting 1 hour before docetaxel infusion, up to 47 hours after the start of docetaxel infusion. Concomitant use of CYP3A inducing or inhibiting medication, dietary supplements or other compounds was not allowed. Administration of medication known to inhibit gastric acid secretion (e.g. proton pump inhibitors, H2-receptor antagonists), thereby increasing stomach pH and interfering with the gastrointestinal absorption of ketoconazole [33], was also prohibited. The Erasmus MC Investigational Review Committee approved the study protocol and written informed consent for participation was obtained from all patients prior to study entry.

Sample collection

Blood samples were collected and processed as previously reported [12]; samples were taken at the following time points: immediately before docetaxel infusion, at 30 and 55 minutes after the start of docetaxel infusion, and at 10, 20 and 30 minutes and 1, 1.5, 2, 4, 5, 8.5, 24, 32.5 and 48 hours after the end of docetaxel infusion.

Ketoconazole analysis

Determination of ketoconazole concentrations in plasma was performed by reversed-phase high performance liquid chromatography (HPLC) with ultraviolet detection (206 nm) according to previously published procedures [34]. Briefly, prior to extraction and quantitation samples were diluted 5- or 10-fold (depending on the sampling time point) with phosphate buffered saline solution (Oxoid, Basingstoke, United Kingdom). Quantitative extraction was achieved by a single solvent extraction using a mixture of acteonitrile-n-butyl chloride (1 : 4 v/v, 5 mL) after adding 100 µL methanol 0.05 M NaOH (2 : 3 v/v) and 100 µL internal standard solution (0.01 mg/mL clotrimazole in 50 % acetonitrile) to the prediluted samples (500 µL). Ketoconazole and clotrimazole were separated on a column packed with Inertsil ODS-80A (150 x 4.6 mm, 5 µm particle size, Alltech Applied Science, Breda, The Netherlands) and a mobile phase composed of water-acetonitrile-tetrahydrofuran-ammonium hydroxide-triethylamine (45 : 65 : 2.5 : 0.1 : 0.1, v/v/v/v/v). Ketoconazole plasma concentrations were quantitated over the range of 20.0 – 2000 ng/mL. Quality control (QC) samples at ketoconazole concentrations of 75 ng/mL, 750 ng/mL, 1500 ng/mL, and 15,000 ng/mL, the latter QC diluted 10-fold prior to processing, were assayed in duplicate and distributed among the calibrators and patient samples in the analytical run.

Docetaxel analysis

Docetaxel was quantitated using HPLC with tandem mass-spectrometric detection (LC-MS/MS). Briefly, to 100 µL plasma aliquots, up to 20-fold diluted, 200 µL internal standard solution (5 ng/mL paclitaxel in acetonitrile) was added. Analyte and internal standard drug were extracted by liquid-liquid extraction with 1 mL n-butylchloride. Samples were vigorously mixed for 5 minutes, followed by centrifugation at 18,000 *g* also for 5 minutes. Subsequently, 1 mL of the clear supernatant was evaporated under a stream of nitrogen at 60 °C and reconstituted with 150 µL of acetonitrile/water/formic acid (40 : 60 : 0.1, v/v/v). Aliquots of 50 µL were injected into the LC-MS/MS (Model 2795 XC chromatograph, Waters Alliance, Mildford, MA, USA). Chromatographic separations were achieved on a Alltima HP C18 HL 3 µm column (50 x 2.1 mm internal diameter, Alltech Applied Science, Breda, The Netherlands). The mobile phase was composed of acetonitrile and water containing formic acid (0.1 % v/v) and delivered using a linear gradient setting at a flow rate of 0.2 mL/min where the composition changed from 50 % to 100 % acetonitrile in 1 minute with an overall run-time of 5 minutes. Detection was performed with a MicroMass Quatro Micro triple-quadropole mass spectrometer (Cary, NC, USA) in the positive ion mode. The electrospray ionization operated at 3.2 kV and at a cone voltage of 25 V. The detector was programmed to allow the $[MH]^+$ ions of docetaxel (m/z 808.40) and paclitaxel (m/z 854.40) to pass through the first quadropole and into the collision cell. The collision energy for collision-induced dissociation of docetaxel and paclitaxel was set at 12 eV and 20

eV, respectively, with argon used as collision gas at a pressure of 0.0023 mbar. The daughter ions of docetaxel (m/z 527.20) and paclitaxel (m/z 286.20) were monitored through the third quadropole. The dwell time per channel for data collection was 0.150 seconds. Docetaxel plasma concentrations were quantitated over the range of 5.00 ng/mL to 500 ng/mL. QC samples at docetaxel concentrations of 15.0 ng/mL, 225 ng/mL, 400 ng/mL and 6000 ng/mL, the latter QC diluted 20-fold prior to processing, were assayed in duplicate and distributed among the calibrators and patient samples in the analytical run.

Pharmacokinetic data analysis - Ketoconazole

For ketoconazole, a PK population model was fitted to the plasma-concentration time data from all patients simultaneously, using the NONMEM software program (double precision, version V; level 1.1) [35]. For modelling purposes, we included the data from the patients previously treated with standard-dose ketoconazole. The PK population model did not include data from patients who were (erroneously) administered comedication known to limit ketoconazole gastrointestinal absorption. The NONMEM model accounts for both fixed and random effects, the latter corresponding to interpatient and residual variability. The first-order conditional estimation (FOCE) method was used throughout the analysis, taking into account the interaction between interpatient and residual variability. Based on the derived population PK model and the observed individual plasma concentrations, individual PK parameter estimates were obtained by Bayesian (POSTHOC) analysis. Ketoconazole plasma concentration-time data were described by a one-compartment model with first order absorption and elimination (ADVAN 2 TRANS 2). The following PK parameters were estimated: K_a, first order rate absorption constant (h^{-1}) describing absorption from the gut; V/F, central volume of distribution (L), CL/F, oral clearance (L/h) and lag time (h), the delay between drug administration and the beginning of absorption. Individual systemic exposure (AUC) was calculated by dividing total ketoconazole dose by apparent oral clearance (CL/F). Simultaneous analysis of the data from all patients requires statistical models for interpatient and residual variances. For interpatient variability, a log-normal parameter distribution was assumed. For instance, the variability in clearance was estimated using:

$$CL_i = CL_{pop}^{\eta}$$

where CL_i is the clearance of the "ith" individual, CL_{pop} is the typical value in the population and η is the random interpatient variable with mean of zero and variance of ω^2 [36]. For a non-linear mixed effects model, the residual variance corresponds to the difference between the observed concentration (C_{obs}) and predicted concentration (C_{pred}). The latter is predicted based on the observed individual parameters (e.g. CL_i).

Residual variance was modelled with a proportional error (ε) as follows:

$$C_{obs} = C_{pred} + C_{pred}*\varepsilon$$

where ε is an independent random variable with mean of zero and variance of σ^2. At each step during the population model building, a specific assumption was tested and evaluated using the likelihood ratio test [37].

Pharmacokinetic data analysis - Docetaxel

Individual docetaxel PK parameters were estimated using model dependant methods implemented in WinNonLin 4.0 (Pharsight, CA, USA). Concentration-time data were fit with a three-compartment model with reciprocal squared prediction weighting. Model adequacy was guided by inspection of the coefficient of variation of the fitted PK parameters, and by the Akaike information criteria [38]. Maximum plasma concentrations (C_{max}) were obtained from the model-estimated plasma concentration at the end of docetaxel infusion. Calculated secondary parameters included half-life during the terminal phase of the disposition curve ($t_{1/2,\gamma}$), AUC and total systemic clearance (CL, defined as docetaxel dose divided by AUC).

CYP3A4 and CYP3A5 genotype analysis

Polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis for CYP3A5*3C, CYP3A5*6, CYP3A4*1B, CYP3A4*3, CYP3A4*17 and CYP3A4*18A variant alleles was performed as described previously [6,39,40]. Based on allele frequencies in the Caucasian population, in which the CYP3A5*3C allele is the predominant allele, CYP3A5*6 genotyping was only performed for individuals with no, or only one CYP3A5*3C allele (i.e. with apparently at least one CYP3A5*1 allele).

Study Objectives

The primary objectives of this (pilot) study were to evaluate if concomitant oral high-dose ketoconazole administration results in a uniform reduction of docetaxel clearance and can significantly reduce the interindividual variability, expressed as the coefficient of variation (CV, the result of expressing the standard deviation (SD) as percentage of the mean), in docetaxel clearance and AUC by 20 - 25 percentage points compared to single agent treatment. The aspired reduction in interindividual variability was based on our aim to achieve a clinically meaningful reduction of the typical interindividual variability values previously reported [11-16].

Statistical considerations

PK parameter estimates for docetaxel and ketoconazole are reported as mean ± SD with range in parenthesis unless stated otherwise. The difference in docetaxel PK parameters between the two courses was evaluated statistically using a non-parametric two-tailed, Wilcoxon signed rank test for paired observations after testing for period effects.

The difference in interindividual PK variability between the two courses was evaluated statistically after expressing the interindividual variability as the SD of the log-transformed (ln) values for CL (SD lnCL) and AUC (SD lnAUC). The SD of lnCL and lnAUC is a one-parameter estimate that approximates the CV for CL and AUC. We tested whether the SD for lnCL or lnAUC would be significantly reduced upon concomitant ketoconazole administration. Statistical evaluation was performed using Pitman's test which says that the null hypothesis of equal SDs of two paired measurements is equivalent to a zero Pearson correlation between sum and difference of the two paired measurements assuming a normal (Gaussian) distribution [41]. In addition, as our sample size was limited, 95 % confidence intervals for the CV and the change in CV upon concomitant ketoconazole administration were estimated for the parameters CL and AUC using bootstrap methods. For this purpose, we drew 10,000 bootstrap samples of (seven) paired observations, with replacement, from our data-set of (seven) measurement pairs and calculated the CVs and change in CV in each bootstrap sample. The 2.5 and 97.5 percentiles of the obtained distribution of 10,000 CV- and CV-change-values are the estimated 95 % confidence limits of the CV and CV-change.

For all tests the significance level was set at $P < 0.05$. Statistical calculations were performed with SPSS, version 11.5 (Chicago, IL, USA).

Results

Baseline patient, toxicity and genetic profiles

A total of eight Caucasian patients were enrolled in the study. One patient was erroneously administered the proton pump inhibitor pantoprazole concomitant with ketoconazole. Thus, a total of seven patients were included in the final analysis. Table 1 lists the baseline patient characteristics.

Only minimal docetaxel-related toxicity was observed in the course in combination with ketoconazole, and all patients tolerated the high-dose ketoconazole treatment well. In the single agent course three patients developed uncomplicated grade 4 neutropenia concomitant with grade 3 leucocytopenia, all other toxicities were not severe, and manageable.

All patients were CYP3A5*3C variant allele homozygotes. The lack of CYP3A5*1 allele carriers indicates that for all evaluated patients total CYP3A activity is solely attributable to CYP3A4. No patients were identified carrying variant alleles for CYP3A4*1B, CYP3A4*3, CYP3A4*17 or CYP3A4*18A.

Table 1. Baseline patient characteristics (N = 7)

Characteristic	Value
Age (years)	56 (44 – 69)
Sex	
Male	6
Female	1
Body-surface area (m^2)	1.88 (1.61 – 2.19)
WHO performance status	1 (0 – 1)
Tumour type	
Breast	1
Prostate	1
Head & Neck	3
Other	2
Haematology	
WBC (x 10^9/L)	9.1 (5.7 – 17.2)
ANC (x 10^9/L)	6.8 (3.2 – 12.3)
Platelets (x 10^9/L)	282 (156 – 480)
Haemoglobin (mmol/L)	8.3 (5.8 – 9.2)
Clinical chemistry	
ASAT (U/L)	25 (20 – 68)
ALAT (U/L)	20 (9 – 28)
Alk Phos (U/L)	100 (73 – 135)
Total Bilirubin (μmol/L)	6 (5 –15)
Total Protein (g/L)	74 (59 – 85)
Serum Albumin (g/L)	40 (28 – 46)
Serum AAG (g/L)	1.48 (0.86 – 2.62)

Abbreviations: WHO, World Health Organization; WBC, white blood cell count; ANC, absolute neutrophil count; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; Alk Phos, alkaline phosphatase; AAG, alpha-1 acid-glycoprotein. Values are given as median with range in parentheses (except for sex and tumour type).

Ketoconazole analysis

Ketoconazole PK was best described by a one-compartment model with first-order absorption and elimination. The population values for K_a, V/F, CL/F and lag time were 1.34 h^{-1}, 71.7 L, 7.76 L/h and 0.395 h, respectively. The corresponding values for interpatient variability were 122 %, 84 % and 67 %. Interpatient variability for lag time was not modelled. The residual variance error was 31.4 %. Based on the individual Bayesian PK parameter estimates (Table 2) the mean (± SD) AUC for ketoconazole was 542 mg·h/L (± 185 mg·h/L; CV = 34 %) which is in line with previous data indicating substantial interindividual PK variability after the same oral dose (CV for T_{max}, C_{max}, AUC and $T_{1/2} \geq 50$ %) [42-44]. In our patient group the observed wide interindividual variability in systemic

exposure was largely due to one patient for whom systemic exposure was extremely high (943 mg·h/L). Indeed, if we exclude this patient, mean systemic exposure is 476 mg·h/L (range, 378 – 536 mg·h/L) and interindividual variability is reduced to 12 %.

Table 2. Individual Bayesian pharmacokinetic parameters of ketoconazole (N = 7)

Parameter	Value
Ka (h^{-1})	2.29 ± 2.93 (0.141 – 8.69)
V/F (L)	109 ± 45.2 (33.1 – 162)
CL/F (L/h)	5.54 ± 1.35 (2.97 – 7.40)
AUC (mg·h/L)	542 ± 185 (378 – 943)

Abbreviations: Ka, absorption rate constant; V/F, central volume of distribution; CL/F, oral clearance; AUC, area under the plasma concentration-time curve. Values are reported as mean ± SD with range between parentheses.

Docetaxel analysis

In the absence of ketoconazole all patients were administered docetaxel (100 mg/m^2) according to protocol (median 190 mg, range, 160 - 220 mg); in the presence of ketoconazole median dose was 30 mg (range, 20 - 30 mg). Observed docetaxel plasma concentration-time curves for both treatments are shown in Figure 1. Table 3 lists a summary of the PK parameters of docetaxel with and without concomitant ketoconazole administration and includes the corresponding values for interindividual PK variability. As expected clearance was significantly reduced upon ketoconazole coadministration from 32.8 L/h ± 13.7 L/h to 16.5 L/h ± 8.15 L/h (P = 0.018) and AUC, adjusted for difference in dose (thus enabling comparison) was increased from 34.5 ng·h·mL^{-1}·mg^{-1} ± 12.2 ng·h·mL^{-1}·mg^{-1} to 71.9 ng·h·mL^{-1}·mg^{-1} ± 27.2 ng·h·mL^{-1}·mg^{-1} (P = 0.018). The mean fractional change in docetaxel clearance (ratio of clearance in the presence of ketoconazole to clearance in the absence of ketoconazole, $CL_{keto=1}$: $CL_{keto=0}$) was 0.50 (median 0.53, range, 0.31 – 0.66), indicating that concomitant ketoconazole administration reduces docetaxel clearance on average by 50 % which is similar to our previous data [12] despite an almost five-fold increase in total ketoconazole dose. Furthermore, the range for the fractional change in docetaxel clearance (0.31 – 0.66) is only marginally narrower than we previously reported after standard-dose ketoconazole treatment (0.26 – 0.68). For both treatment courses, the degree of interindividual variability for clearance and (absolute) AUC is not reduced by ketoconazole administration (41.6 % *vs* 49.5 % and 28.0 % *vs* 35.1 %, respectively (P = 0.546 and P = 0.513). The 95 % confidence intervals for the change in the CV-values for clearance (-4.85 to +19.8 percentage points) and (absolute) AUC (-6.40 to +18.6 percentage points), generated

Table 3. Docetaxel pharmacokinetic parameters (N = 7)

Parameter	Ketoconazole = 0	Ketoconazole = 1	*P*-value	CV % Ketoconazole = 0	CV % Ketoconazole = 1	Difference in CV, percent points
CL (L/h)	32.8 ± 13.7 (19.2 – 60.2)	16.5 ± 8.15 (10.1 – 31.9)	0.018	41.6 (16.1 – 52.9)	49.5 (24.3 – 59.9)	+7.90 (-4.85 to +19.8)
AUC (μg·h/mL)	6.43 ± 1.80 (3.66 – 8.85)	1.94 ± 0.680 (0.939 – 2.76)	NA	28.0 (13.1 – 38.0)	35.1 (16.8 – 47.3)	+7.10 (-6.40 to +18.6)
AUC / dose (ng·h·mL^{-1}·mg^{-1})	34.5 ± 12.2 (16.6 – 52.1)	71.9 ± 27.2 (31.3 – 98.8)	0.018	35.4 (17.6 – 48.4)	37.9 (17.1 – 51.2)	+2.5 (-10.5 to +18.5)
Cmax (μg/mL)	4.29 ± 1.05 (2.67 – 5.42)	0.633 ± 0.171 (0.356 – 0.842)	NA	24.4	27.0	
Cmax / dose (ng/mL·mg^{-1})	23.0 ± 7.51 (12.1 – 33.3)	23.4 ± 7.51 (11.9 – 32.7)	0.446	32.6	32.0	
$T_{1/2,\gamma}$	17.9 ± 3.66 (14.0 – 24.2)	24.7 ± 4.61 (20.0 – 30.4)	0.043	20.5	18.7	

Abbreviations: Ketoconazole = 0, ketoconazole absent; Ketoconazole = 1, ketoconazole present; CL, clearance; AUC, area under the plasma concentration-time curve; C_{max}, peak plasma concentration; $T_{1/2,\gamma}$, terminal elimination half-life; CV, coefficient of variation; NA, not applicable. Values are reported as mean ± SD with range in parentheses. The 95 % confidence intervals for the CV values for CL, AUC, AUC / dose and the corresponding difference in CV values between the two courses, are reported in parentheses.

using 10,000 bootstrap samples, rules out the possibility that the interindividual variability is reduced by 20 - 25 percentage points.

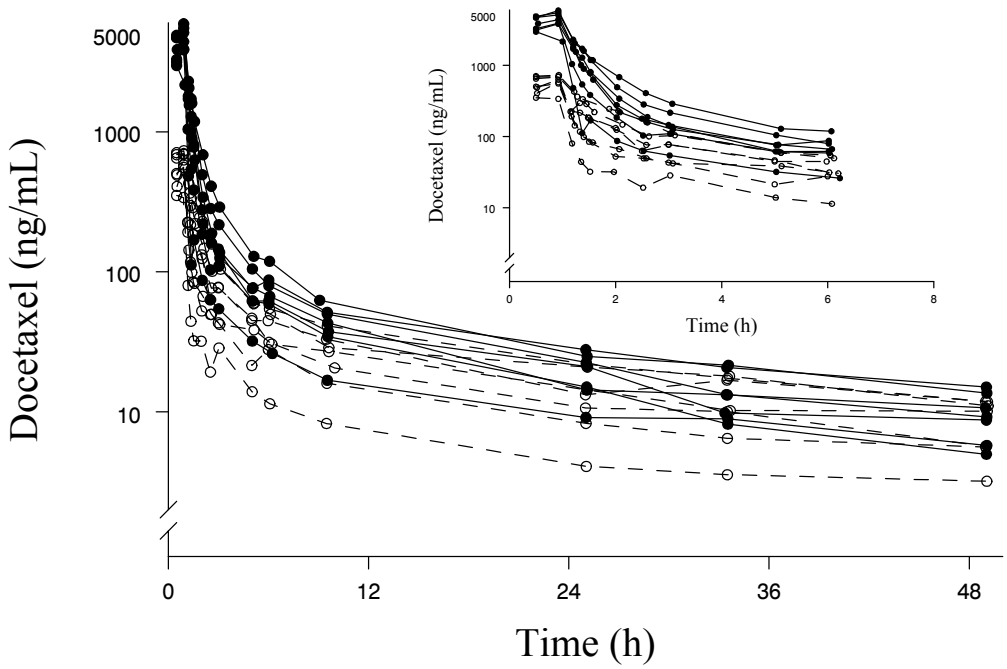

Figure 1. *Observed plasma concentrations of docetaxel in the absence (solid line, closed symbols, dose 100 mg/m^2) and presence (dashed line, open symbols, dose 15 mg/m^2) of ketoconazole. Insert: observed docetaxel plasma concentrations up to 6 hours after start of docetaxel infusion.*

In contrast to our previous finding for the patients treated with standard-dose ketoconazole, where the fractional change in docetaxel clearance was weakly correlated (R^2 = 0.529, P = 0.064) with corresponding ketoconazole AUC (for the first dosing interval), we now observed no clear relationship between fractional change in docetaxel clearance and total ketoconazole AUC (Figure 2). Indeed, figure 2 suggests that upon achieving a sufficiently high systemic ketoconazole exposure (i.e. above a certain threshold ketoconazole exposure level), the observed variability in the fractional change in docetaxel clearance occurs independent of (increasing) ketoconazole AUC.

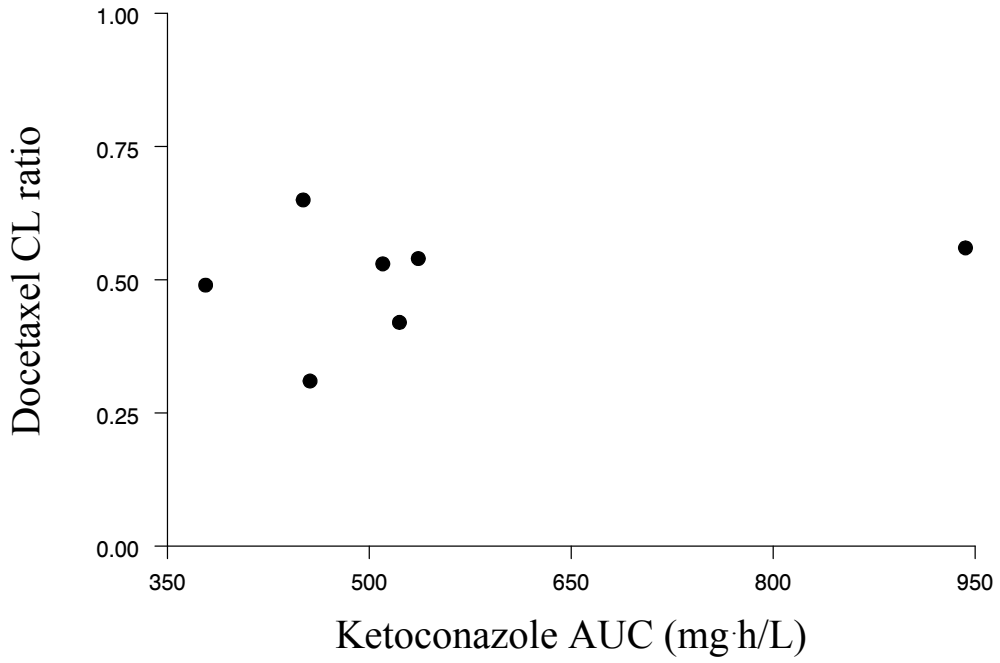

Figure 2. *Relationship between ketoconazole area under the plasma concentration-time curve (AUC) and fractional change in docetaxel clearance (CL) defined as the ratio of CL in the presence and absence of ketoconazole.*

Discussion

Previously we reported that docetaxel clearance is significantly reduced by 49 % after coadministration of standard-dose ketoconazole (total dose 600 mg) albeit with large interindividual variability (fractional change in clearance range, 0.26 - 0.68) [12]. Furthermore, the interindividual variability for docetaxel clearance (expressed as CV) was increased from 33.7 % to 53.2 % in the presence of ketoconazole. As the observed variability in clearance reduction was correlated to corresponding ketoconazole exposure, we hypothesized that an increase in ketoconazole dose would result in sufficiently high exposure to ketoconazole in each patient thereby achieving maximum CYP3A inhibition overall. However, the here reported study results show that, despite an almost five-fold increase in ketoconazole dose (total dose 2800 mg), variability in the fractional change in docetaxel clearance remains (range, 0.31 - 0.66) and is only marginally less than we previously reported. Furthermore, interindividual variability in docetaxel clearance and AUC after high-dose ketoconazole coadministration was not reduced (Table 3).

Our findings are in line with preliminary observations where docetaxel clearance also showed increased variability upon ketoconazole coadministration both across and within different docetaxel dose levels (range, 10 mg/m^2 to 30 mg/m^2) [45]. Furthermore, Van

Veldhuizen *et al*. [21] also reported inconsistent effects of concomitant ketoconazole on docetaxel systemic exposure. We therefore conclude that strong inhibition of CYP3A activity, through concomitant oral ketoconazole administration, does not reduce interindividual variability in docetaxel PK and is thus not suitable as a method to achieve a uniform PK profile.

Several reasons may explain why our approach has not been successful. The choice for ketoconazole as CYP3A inhibitor, although highly potent, may, in retrospection, not be ideal. Ketoconazole is subject to large inter- and intraindividual variability in absorption [33,42], (largely) based on differences in intrinsic gastric acid secretion, which can be further increased through comedication. Indeed, the patient excluded due to erroneous pantoprazole use had the lowest exposure to ketoconazole (AUC 217 mg·h/L). Interestingly, despite the lowest ketoconazole exposure, docetaxel clearance was reduced from 45.4 L/h to 20.2 L/h, indicating that for this patient the fractional change in docetaxel clearance (0.45) is within the range reported for the other seven patients (0.31 – 0.66). In addition, if we include this patient in the PK analysis interindividual variability values for clearance in the absence and presence of ketoconazole are marginally affected and then correspond to 39.0 % and 45.3 %, respectively (95 % confidence interval for change in CV, -4.40 to +16.3 percentage points).

In the here reported high-dose ketoconazole cohort no clear relationship was detected between ketoconazole exposure and decrease in docetaxel clearance (Figure 2). A possible explanation for this finding is that although sufficiently high systemic ketoconazole exposure was achieved, thus maximally inhibiting docetaxel metabolism and maximally reducing clearance in each individual patient, other factors also play a role in the (observed) variability in the fractional change in docetaxel clearance. Most importantly, we must conclude that a further increase in ketoconazole dose is pointless. Moreover, higher ketoconazole doses are not feasible due to the associated unacceptable (gastrointestinal) toxicity [46].

All patients were CYP3A5*3C/*3C (variant allele) homozygotes, thus deficient in CYP3A5 catalytic activity. Accordingly, (part of) the interindividual variability in the decrease of docetaxel clearance cannot be explained for by interindividual variability in CYP3A5 activity (i.e. variable contribution of CYP3A5 to overall CYP3A activity) and/or by the differences in susceptibility to inhibition by ketoconazole for CYP3A4 and CYP3A5. In this study, the observed effects of ketoconazole are solely attributed to the effect on CYP3A4 activity. No patient carried any of the, currently known and, based upon effect on activity and/or allele frequency, potentially clinically relevant CYP3A4 SNPs (CYP3A4*1B, CYP3A4*3, CYP3A4*17 or CYP3A4*18A). This indicates that it is highly unlikely that genetic differences contributed to the observed interindividual variability in the decrease of docetaxel clearance. Indeed, to date genetic variants in CYP3A4 and CYP3A5 have not been shown to significantly affect docetaxel clearance or the PK of frequently used CYP3A phenotyping probes such as midazolam, dexamethasone and erythromycin [18,19,47,48].

In vitro experiments have indicated that the quantitative contribution of CYP3A4/5 to the hepatic metabolism of docetaxel ranges from 64 % to 93 %, suggesting that less than 7 % to 36 % of the metabolism may be caused by other CYP-enzymes, not inhibited by ketoconazole [4]. For instance, although reports are inconsistent, the predominantly extra-hepatic (e.g. intestinal) expressed isozyme CYP1B1 has been associated with docetaxel metabolism [49,50].

Although ketoconazole is also a (weak to modest) inhibitor of ABCB1 [51], preclinical observations [52,53] and clinical observations [54-56] indicate that the influence of (inhibition of) ABCB1 function on the plasma PK of docetaxel is minimal to absent. Thus, we conclude that (variable) involvement of ABCB1 can be ruled out as explanation for the observed variability in reduction of docetaxel clearance.

Despite the fact that this study is limited by small sample size, our current findings, lead us to conclude that purposely manipulating the PK profile of docetaxel through drug-induced modulation (i.e. inhibition) of the major route of metabolism, in order to reduce interindividual PK variability, is not feasible with orally administered high-dose ketoconazole. It would seem that, given the large number of mechanisms contributing to the variable plasma disposition of docetaxel, attempting to control one factor, albeit the most important factor in the route of elimination, does not yield the anticipated effect, a uniform PK profile.

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Chapter 7

Quantification of [^{3}H]-Docetaxel in Faeces and Urine: Development and Validation of a Novel Combustion Method

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Abstract

Most radiolabelled biological samples require extensive sample preparation to reduce quenching interference before quantification of radioactivity is possible. Clearly, a more rapid and simple method ensuring a constant count rate and optimal counting efficiency has important advantages. We report on the development and analytical method validation of a rapid and simple combustion method to quantify [^{3}H]-docetaxel excreted in human faeces and urine. A 3-day validation procedure was performed; quality control (QC) samples, prepared in blank faeces and urine, were combusted 5 times and aliquots of the produced tritiated combustion water were counted in a liquid scintillation counter. The validation runs demonstrated adequate precision (< 7.6 %) across all QC levels. Sensitivity at the lowest QC level was excellent and recovery of radioactivity constant ranging from 85 % to 91.8 %. Clinical applicability of the method was tested in a cancer patient receiving docetaxel and a tracer amount of [^{3}H]-docetaxel; during the first 72-hours after [^{3}H]-docetaxel infusion, 60 % of total radioactivity was excreted in the collected faeces and urine, which is within the expected range. Combustion of tritiated faeces and urine samples is a simple, rapid, sensitive, precise and reproducible method with high recovery. It can be applied to quantify [^{3}H]-docetaxel excretion after intravenous administration.

Introduction

An important reason to administer a non-toxic tracer amount of radiolabelled drug to a patient is to quantify the drug in biological samples (e.g. faeces, urine, ascites) at detection levels below the lower limit of quantification for routinely used analytical techniques (e.g. high-performance liquid chromatography, HPLC). Radiochemicals used in (clinical) research include [^{3}H]- or [^{14}C]-radiolabelled compounds. Liquid scintillation counting (LSC) is the most efficient method to quantify these low energy beta-emitters. However, most radiolabelled biological samples cannot be directly subjected to LSC and first require a sample preparation procedure for example to reduce colour or chemical quenching interference. Indeed, with LSC the detection and quantification limit is affected by sample size, sample preparation method and counting efficiency. Ideally, the sample size is as large as possible, and pretreatment is simple, rapid and results in a clear, homogeneous and stable mixture of radioactive sample and appropriate liquid scintillation cocktail, thus allowing for a constant count rate and optimal counting efficiency. This ultimately gives the most accurate and reproducible counting results. Various sample preparation procedures to solubilize a biological matrix are applied and include acid or alkaline digestion. Indeed, in preclinical mass balance studies with [^{3}H]-radiolabelled paclitaxel and docetaxel, faeces sample pretreatment involved initial homogenization with 4 % bovine serum albumin in water, followed by a 1.5-hour incubation with a (tissue) solubilizer (Soluene®) and a 2-hour

incubation with 2-propanol [1,2]. Finally, 30 % hydrogen peroxide, necessary to bleach the samples, was added before mixing with a compatible liquid scintillation cocktail (Hionic-Fluor). Combustion of biological samples is an alternative sample preparation method [3-6] and has several practical advantages. Combustion of a [^{3}H]-radiolabelled sample produces a colourless amount of tritiated (combustion) water, which can be counted immediately after homogeneously mixing with liquid scintillation cocktail. It is a rapid method, quenching interference is avoided and sample size can be increased compared to acid or alkaline digestion, as such positively influencing the detection and quantification limit.

The anticancer drug docetaxel (Taxotere®) is approved for the treatment of patients with locally advanced or metastatic breast cancer, non-small-cell lung cancer and androgen-independent metastatic prostate cancer (http://www.taxotere.com). After intravenous (i.v.) administration the drug is extensively and predominantly metabolized by hepatic and intestinal cytochrome P450 (CYP) isozymes 3A [7,8]. Indeed, the major metabolites of docetaxel and less than 10 % of the parent drug are excreted into the faeces, whereas total urinary excretion is less than 10 % [9,10]. Preclinical metabolism and excretion studies with [^{14}C]-radiolabelled docetaxel have demonstrated that the elimination of radioactivity in mice, dogs and rats was rapid and almost complete (range, 85 - 95%) within 7 days after administration [11,12]. Clinical studies assessing excretion and metabolism of [^{14}C]-radiolabelled docetaxel are limited [13]. In patients (N = 3) approximately 80 % of total administered radioactivity was excreted in the 7-day faeces, with the majority of excretion occurring during the first 48 hours and only 5 % of total radioactivity was retrieved in urine during the first 6 hours after administration. The catalytic activity of the isozyme CYP3A is readily modulated by inducing or inhibiting compounds such as drugs, dietary supplements and complementary and alternative medicines [14,15]. Although docetaxel is subject to a host of CYP3A-mediated interactions that can affect treatment outcome, data on potential interactions are lacking. We therefore recently conducted a study to evaluate the effect of coadministration of the potent CYP3A inhibitor ketoconazole (Nizoral®) on the pharmacokinetics of docetaxel in cancer patients [14]. A secondary objective of this project was to assess the influence of ketoconazole on the excretion profile and mass balance of docetaxel and its metabolites in faeces and urine samples using a radiolabelled tracer amount of [^{3}H]-docetaxel. In the context of this secondary objective we now report on the development and analytical method validation of a combustion method to quantify [^{3}H]-docetaxel excreted in faeces and urine samples according to international guidelines for bioanalytical method validation [16].

Materials and Methods

Chemicals and Solutions

Tritiated docetaxel ([^{3}H]-docetaxel; 1.0 mCi/mL in ethanol, specific activity 7.2 Ci/mmol) was manufactured by Moravek Biochemicals Inc. (Brea, CA, USA) and 5 mL aliquots (working stock solution) of a 200-fold dilution in ethanol absolute (> 99.9 %; Merck, Darmstadt, Germany) were stored at –80 °C. Purity of the [^{3}H]-docetaxel was analysed at two intervals (directly after purchase and prior to patient administration) by a reversed-phase HPLC method with ultraviolet detection at 230 nm [17]. Briefly, to 10 µL docetaxel stock solution (1.0 mg/mL) prepared by dissolving the appropriate amount of drug (purity 99.3 %; Aventis Pharma, Hoevelaken, The Netherlands) in dimethylsulfoxide (purity 99.9 %; Sigma, St. Louis, MO, USA), 100 µL of [^{3}H]-docetaxel working stock solution and 390 µL of purified water was added and mixed; a 100 µL aliquot of this solution was injected on the HPLC system and the eluted mobile phase collected in polyethylene vials every 30 seconds up to 15 minutes after injection; 150 µL of each fraction of collected eluted mobile phase was mixed with 2 mL liquid scintillation cocktail (Emulsifier Scintillator 299; Packard Bioscience, Groningen, The Netherlands). The collected fractions and a corresponding blank were counted in a liquid scintillator counter (Wallac 1409 Liquid Scintillation Counter; Perkin Elmer, Boston MA, USA). To assess purity of the [^{3}H]-docetaxel lot, the time course (i.e. elution profile) of the recovered radioactivity was compared to the chromatographic profile of (unlabelled) docetaxel. Faeces and urine were obtained from healthy volunteers. Faeces was homogenized in 3 volumes (1 : 3 w/v) of phosphate buffered saline solution (PBS; Oxoid, Basingstoke, UK) using an Ultra-TurraxT25 homogenizer (Janke & Kunkel, IKA Labortechnik, Staufen, Germany) and stored at –20 °C. Pooled urine samples did not undergo any processing and were immediately stored at –20 °C. Water purified and deionized by the Milli-Q UF Plus system (Millipore®; Bedford, MA, USA) was used in all aqueous solutions.

Quality control sample preparation

Three levels of quality control (QC) samples (QC-low; QC-medium; QC-high) were prepared in homogenized blank faeces and two levels in pooled blank urine (QC-low; QC-high). For this purpose a 1 : 10 and a 1 : 100 dilution (in PBS) of [^{3}H]-docetaxel working stock solution were prepared. Weighted aliquots of 50 g of blank homogenized faeces were spiked with 800 µL or 300 µL of the 1 : 10 dilution to prepare the QC-high and QC-medium samples, respectively; 50 µL of the same dilution was used to spike 50 mL of blank urine (QC-high). Similarly, weighted aliquots of 50 g of homogenized blank faeces and 50 mL of blank urine were both spiked with 50 µL 1 : 100 dilution to produce QC-low samples. The chosen levels of radioactivity were based on preliminary data of measurements in patient

samples at time points when maximum and minimum excretion was expected. All QC-samples were stored at –20 °C until analysis. For recovery calculation the nominal level of radioactivity in the amounts of [^{3}H]-docetaxel used for spiking was measured in duplicate by LSC.

Measurement of radioactivity levels

The radionuclide fraction of [^{3}H] was obtained after complete combustion of an aliquot of each QC-sample in a closed-type combustion flask (Parr; flask volume 300 mL), thus reducing the sample to ash and yielding tritiated (combustion) water, [$^3H_2$]O. Briefly, after accurately weighing the combustion flask a known amount (approximately 1.0 g to 1.5 g) of sample was placed in the sample basket (1.5 cm high; 3 cm diameter) with a copper ignition wire connected to it. Additionally, for urine samples a piece of filter paper was added to absorb the sample and prevent it from spilling out of the basket. The combustion flask was then hermetically closed and filled with 25 atm oxygen using an oxygen cylinder with a pressure regulator. Combustion was started by applying an electric current (12 V) to the ignition wire. After combustion, the flask was cooled in an ice-water bath for approximately 5 minutes. The combustion flask was then weighed again to determine the total produced amount of tritiated combustion water. Finally, to an exact (i.e. known) amount (approximately 2 mL) of tritiated combustion water 18 mL liquid scintillation cocktail (Emulsifier Scintillator 299) was added and the solution manually mixed in glass counting vials (Econo glass vial – 20 mL; Packard Bioscience) until homogeneous. Samples were counted in a liquid scintillator counter (Wallac 1409; Perkin Elmer). Counting time was set at 1 hour per sample and in each run a corresponding combustion blank and a [^{3}H]-standard were included to correct for background radioactivity and to check stability of the counting instrument, respectively. Using the known amounts of combusted sample and produced and counted tritiated combustion water, the total amount of radioactivity in the QC-samples could be calculated.

Validation of precision and recovery

The validation procedure was performed on three separate days (i.e. runs) and each faeces or urine QC sample was processed (i.e. combusted) 5- or 6 times, respectively. The precision of the assay was assessed by between-run precision (reproducibility) and within-run precision (repeatability). Estimates of the between-run precision and within-run precision were obtained for each (radioactivity) level by one-way analysis of variance (ANOVA) using the run day as classification variable. The between-groups mean square (MS_{betw}), the within-groups mean square (MS_{with}), and the grand mean (GM) of the observed radioactivity levels across run days were calculated in SPSS for Windows, version 9.0 (SPSS; Chicago, IL, USA).

The between-run precision (BRP) was defined as:

$$BRP\ (\%) = \{[(MS_{betw} - MS_{with}) / N]^{0.5} / GM\} \times 100$$

where N represents the number of replicate measurements within each validation run. The within-run precision (WRP) was calculated as:

$$WRP\ (\%) = \{(MS_{with})^{0.5} / GM\} \times 100$$

In cases where the MS_{with} is greater than the MS_{betw}, the resulting variance estimate is negative, implying that no additional variance was observed as a result of performing the assay in different runs. The average recovery (REC) for each QC sample was calculated as:

$$REC\ (\%) = (GM / \text{nominal radioactivity level}) \times 100$$

The bioanalytical method validation is accepted when the WRP and BRP at each level of QC samples are 15 % or less and at least 80 % of the QC samples at each level have an absolute deviation of 15 % or less of their nominal concentration [16].

Lower Limit of Quantification

In general, for a concentration (i.e. level of radioactivity) to be acceptable as the lowest level of quantification the percentage of deviation from the nominal value of at least 80 % of the samples assayed should be 20 % or less, with a resulting WRP of 20 % or less [16]. Although no samples were spiked with levels of radioactivity lower than the QC-low nominal values, calculation of the statistical error (standard deviation, SD and coefficient of variation, CV) for the accumulated counts for the QC-low samples gives an impression of the level of quantification (i.e. sensitivity), which can be achieved. The SD in the net count rate after correcting the gross count rate (R_a) for the blank (R_b), was calculated using the following error formula:

$$SD = \sqrt{(R_a / t_a + R_b / t_b)}$$

where the counting time for both sample and blank (t_a and t_b) was 60 minutes.
The CV (%) was calculated as:

$$CV\ (\%) = \sqrt{\{1 / (R_a * t_a) + 1 / (R_b * t_b)\}} \times 100$$

Clinical applicability

Complete 24-hour urine and faeces were collected up to 72 hours after a 1-hour i.v. infusion of 210 mg docetaxel (dose 100 mg/m^2) and a tracer amount (approximately 50 μCi) of [^{3}H]-docetaxel to a cancer patient participating in the previously mentioned study [14]. Samples were stored, processed and analysed (in duplicate) as described for the QC-samples. The observed radioactivity levels in the samples were used to calculate the total amount of radioactivity recovered in each collected faeces and urine sample and the percentage of recovered radioactivity (i.e. relative to the amount which was added to the docetaxel infusion), was determined.

Potential loss of added [^{3}H]-docetaxel due to adhesion to the infusion bag (NaCl 0.9 % wt/vol, 250 mL Viaflex; Baxter, Utrecht, The Netherlands), i.v. administration set (ref: 591.078J; Graseby, Watford, UK) or connect set (ref. 76.4440; Codan, Lensahn, Germany) was assessed. For this purpose the clinical practice of infusion was simulated using two docetaxel infusions (dose 20 mg and 160 mg docetaxel), which both contained the same amount of [^{3}H]-docetaxel used in the clinical trial [14]. After the simulated infusion duplicate specimens of the infusion bags and lines were combusted, the combustion water distillated to produce a clear sample, mixed with liquid scintillation cocktail, and followed by LSC to determine the level of radioactivity.

Results

Method Validation

No signs of degradation or decreased purity of the lot of [^{3}H]-docetaxel, used for validation of the combustion method and throughout the clinical trial were observed, even after a period of prolonged storage at –80 °C; more than 90 % of the radioactivity recovered in the collected fractions of eluted mobile phase after chromatographic analysis of [^{3}H]-docetaxel was detected at the retention time of docetaxel (Figure 1).

At each of the three or two, respectively, QC levels of [^{3}H]-docetaxel in homogenized faeces and urine the within-run precision and between-run precision was less than 7.6 % (Table 1). The ratio of the volumes of [^{3}H]-docetaxel 1 : 10 dilution used to spike the faeces QC samples (QC-medium 300 μL : QC-high 800 μL = 1.0 : 2.7) could also be calculated for the observed levels (grand mean) in the corresponding samples (QC-medium : QC-high = 1.0 : 2.6), indicating linearity in the counting results. Similar results for linearity were seen in the urine samples. Furthermore, the ratio of the observed (grand mean) levels in the QC-low samples for the two matrices both spiked with 50 μL 1 : 100 dilution were as expected (QC-low-urine : QC-low-faeces = 1.0 : 1.1). These results indicate that matrix interference can be excluded. Recovery of radioactivity in faeces and urine was constant and ranged from 85.0 % to 91.8 % (Table 1). At the lowest levels of radioactivity, calculation of the statistical error

demonstrated that the SD was less than 1 cpm and the CV was 5.2 % and 4.9 % for faeces and urine samples, respectively.

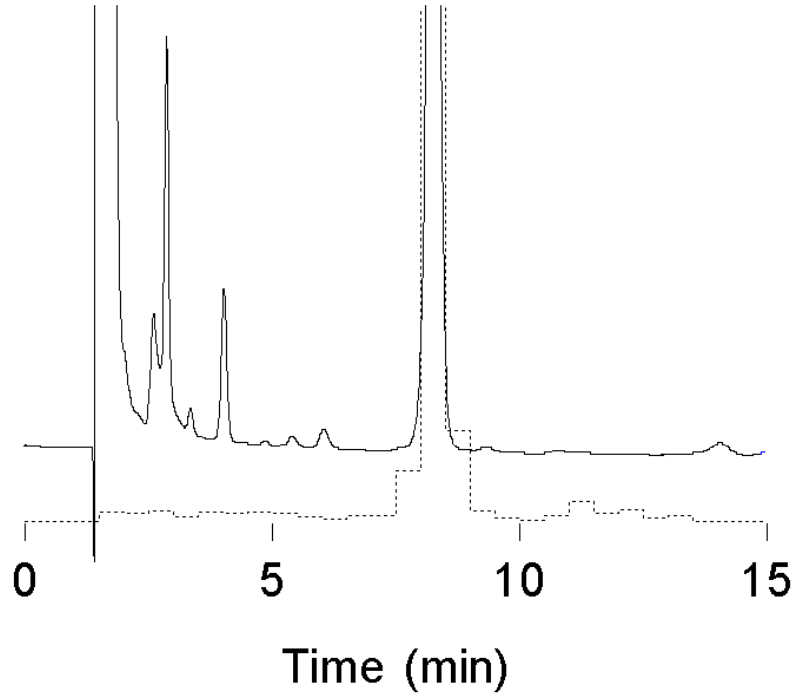


Figure 1. *Chromatographic tracing with ultraviolet detection (solid line), and off-line liquid scintillation counting (dashed line) after injection of docetaxel and an added tracer amount of [^{3}H]-docetaxel*.

Table 1. Within-run precision, between-run precision and recovery of [^{3}H]-docetaxel in human faeces and urine

QC sample	Nominal (dpm / g)	Observed (dpm / g)	WRP (%)	BRP (%)	N	REC[1] (%)
Faeces						
QC-low	190	170	5.2	#	5	89.2 (4.4)
QC-medium	13722	12425	1.8	#	5	90.5 (1.5)
QC-high	35344	32457	2.0	1.5	5	91.8 (2.2)
Urine						
QC-low	186	158	7.6	#	6	85.0 (6.4)
QC-high	2147	1967	2.2	#	6	91.6 (2.0)

Abbreviations: QC, quality control; WRP, within-run precision; BRP, between-run precision; N number of replicate observations within each validation run; REC, recovery; #, no additional variation was observed by performing the assay in different runs. [1]mean value for all observations with standard deviation between parentheses.

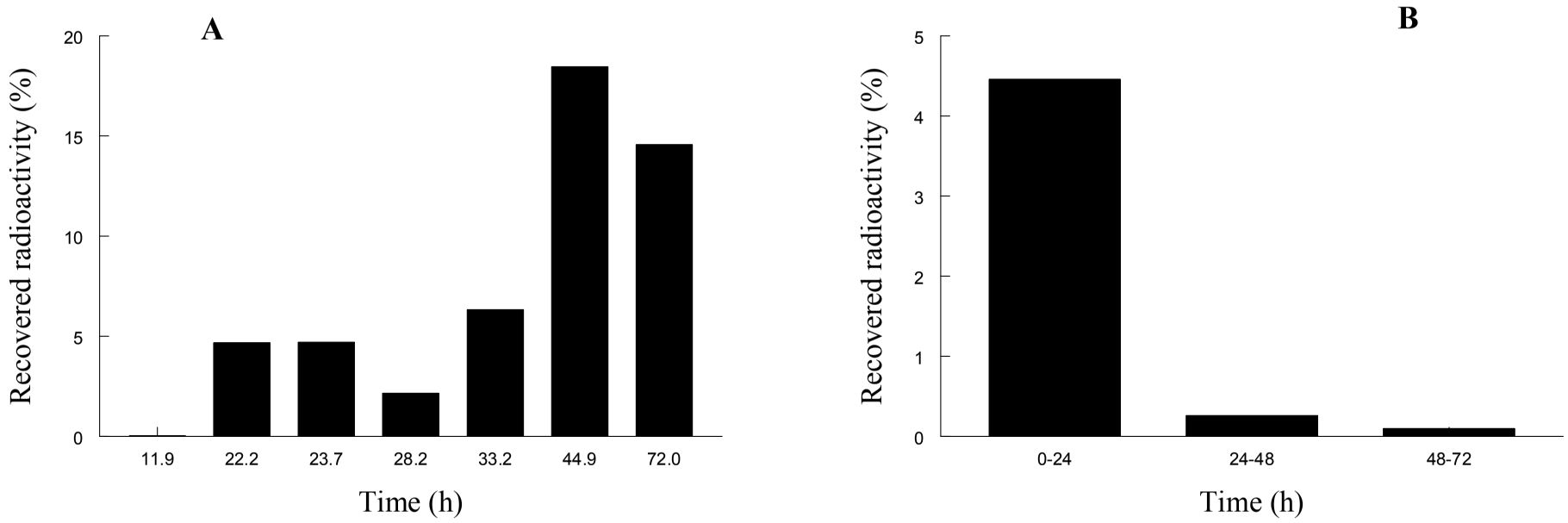
Clinical applicability

The radioactivity excretion profiles in faeces and urine after docetaxel infusion (210 mg) and an added tracer amount of [^{3}H]-docetaxel are displayed in Figures 2A and 2B, respectively. During the first 72 hours after i.v. administration (i.e. the period during which the patient was admitted in the hospital) the total percentage of recovered radioactivity was 60.41 % (55.79 % in faeces and 4.62 % in urine), which is within the expected range considering the time interval of sample collection. Loss of added [^{3}H]-docetaxel due to adhesion to the infusion bag or administration set was neglectable for both high (100 mg/m^2) and low (10 mg/m^2) doses of docetaxel (≤ 0.3 %).

Discussion

We have here described the validation of a combustion method to quantify radioactivity levels in human faeces and urine collected after i.v. administration of a tracer amount of [^{3}H]-docetaxel added to a docetaxel infusion. The described method is rapid, specific, precise, has a recovery of at least 85 % and allows sample aliquots of approximately 1.5 g (1 : 3 w/v homogenized faeces or 2 mL urine) to be processed, which is more than previously described for analytical procedures using a non-combustion sample pretreatment method (i.e. 200 µL faeces homogenate 1 : 10; 20 mg dried faeces; http://www.packardbioscience.com) [2], as such lower levels of radioactivity can be detected. Indeed, when we analysed our QC samples according to the previously described non-combustion method [2] the lowest QC level (for both matrices) could not be quantitated [data not shown]; recovery for the other two QC levels was in the same range as we found for the combustion method. Furthermore, these samples were clear, yet never completely colourless after sample preparation and chemiluminescence correction was required.

The described combustion method was applied to samples collected from a cancer patient who received a docetaxel infusion with a tracer amount of [^{3}H]-docetaxel added. Recovered radioactivity was within the expected range taking into account the period of sample collection (i.e. 72 hours). The method can therefore be applied to assess the influence of ketoconazole on the excretion profile and mass balance of docetaxel and its metabolites in faeces and urine samples.



Figures 2A and 2B. *Radioactivity recovery (%) in faeces samples (A) and urine samples (B) collected up to 72 hours after intravenous administration of 210 mg docetaxel and an added amount tracer amount of [^{3}H]-docetaxel.*

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Chapter 8

Influence of Ketoconazole on the Faecal and Urinary Disposition of Docetaxel

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Submitted

Abstract

Objective: The anticancer drug docetaxel is extensively metabolized by cytochrome P450 (CYP) 3A isozymes. Furthermore, docetaxel is also a substrate for the transmembrane ATP-binding cassette efflux transporter protein ABCB1. CYP3A-inhibition significantly reduces docetaxel total systemic clearance, on average by 50 %. However, data on the effect of CYP3A-inhibition on the faecal and urinary excretion of docetaxel are lacking. To further elucidate the role of CYP3A- and ABCB1-mediated elimination pathways for docetaxel we investigated the effect of the potent CYP3A-inhibitor, and also ABCB1-inhibitor, ketoconazole on the faecal and urinary disposition of docetaxel in cancer patients.

Methods: Fifteen patients were treated with docetaxel (100 mg/m^2), followed 3 weeks later by a reduced dose in combination with orally administered ketoconazole, or vice versa. Six patients were also administered [^{3}H]-radiolabelled docetaxel. Faecal and urinary specimens, collected up to 72 hours post-infusion, were analysed for cumulative parent drug and radioactivity excretion.

Results: Ketoconazole coadministration increased faecal parent drug excretion 2-fold from 2.6 % ± 2.8 % to 5.2 % ± 5.4% (mean ± SD, $P = 0.03$) but did not affect urinary parent drug excretion ($P = 0.69$). The sum of faecal and urinary parent drug excretion was 5.3 % ± 3.0 % for docetaxel alone and 7.8 % ± 5.6 % in the presence of ketoconazole, respectively ($P = 0.04$). Total recovered radioactivity values were 45.8 % ± 19.1 % and 32.4 % ± 19.7 %, respectively ($P = 0.23$).

Conclusion: CYP3A-inhibition by ketoconazole increases faecal parent drug excretion 2-fold in cancer patients. A more pronounced increase was not achieved, most likely due to concomitant intestinal ABCB1-inhibition.

Introduction

In recent years, the clinical pharmacokinetics (PK) of the anticancer drug docetaxel have been subject of thorough investigation. After intravenous administration the drug is extensively metabolized by hepatic and intestinal cytochrome P450 (CYP) isozymes 3A [1,2], the catalytic activity of which is represented by two major isoforms, CYP3A4 and CYP3A5, albeit that CYP3A4 is the most active, possesses the highest affinity for docetaxel [3] and is predominant in Caucasians [4]. Moreover, CYP3A activity is readily induced or inhibited by a broad range of compounds. Docetaxel is also a substrate for the ATP-binding cassette membrane-localized transporter, ABCB1 (P-glycoprotein; MDR-1) which acts as a (drug/xenobiotic-)efflux pump [5,6]. ABCB1 is expressed in several tissues including the renal tubule, biliary tract and intestinal epithelium [7] and plays a prominent role in the faecal elimination of docetaxel [8,9].

Preclinical studies with [^{14}C]-radiolabelled docetaxel have demonstrated that administered radioactivity is predominantly (range, 85 – 95 %) excreted in the faeces within 7 days and that urinary excretion accounts for less than 5 % [10-12]. Furthermore, the administered dose is primarily (≥ 75 %) excreted as four pharmacologically inactive metabolites [13] and less than 10 % is excreted unchanged [14]. Clinical studies assessing excretion of [^{14}C]-radiolabelled docetaxel are limited; in cancer patients (N = 3) approximately 80 % of total administered radioactivity was excreted in faeces collected up to 7 days post-infusion and 5 % of total radioactivity was recovered in urine [15].

The faecal disposition of docetaxel in the presence of ABCB1-inhibition has been studied previously [8], however, the effect of inhibition of CYP3A, the drug's major route of inactivation, on docetaxel faecal and urinary excretion, has never yet been evaluated. We previously evaluated the effect of concomitant orally administered ketoconazole, a potent CYP3A-inhibitor [16] and also weak to modest ABCB1-inhibitor [7,17,18], on docetaxel plasma PK in cancer patients [19,20]. Subsequently, the current study was undertaken to evaluate the effect of both CYP3A- and ABCB1-inhibition on docetaxel faecal and urinary excretion. The objectives of the current study were therefore to evaluate the influence of ketoconazole on (1) the faecal and urinary excretion of unchanged docetaxel (i.e. parent drug), on (2) the total faecal and urinary excretion of docetaxel using [^{3}H]-radiolabelled docetaxel, and on (3) the mass balance of ([^{3}H]-radiolabelled-)docetaxel.

Wide variation in total CYP3A activity exists, as reflected by substantial interindividual PK variability for CYP3A substrates [21]. Part of this variability has been related to genetic diversity in the genes encoding CYP3A4 and CYP3A5 proteins [22]. However, currently, most evidence infers that it is unlikely that CYP3A4 single nucleotide polymorphisms (SNPs) contribute substantially to variability in CYP3A4 activity *in vivo* [23-25] due to the low frequency of genetic polymorphisms. On the other hand, more than 80 % of Caucasians is deficient in CYP3A5 activity due to the CYP3A5*3C/*3C variant genotype [4]. In individuals with at least one CYP3A5*1 allele, CYP3A5 may account for at least 50 % of total CYP3A content, resulting in 2- to 3-fold higher CYP3A activity *in vitro* [26]. Moreover, CYP3A4 and CYP3A5 show distinct differences in susceptibility to inhibition by ketoconazole [27]. ABCB1 polymorphisms have not been shown to affect docetaxel plasma PK [28,29], yet their influence on faecal and urinary excretion is unknown. We included CYP3A5 and ABCB1 genotyping in our study to gain insight in the effect of the various SNPs on docetaxel faecal and urinary excretion. Genotyping for CYP3A4 rare polymorphisms was performed to exclude genetic diversity in CYP3A4 activity.

We here report on the disposition of docetaxel in the faeces and urine of cancer patients, after intravenous administration of the drug with and without concomitant oral administration of ketoconazole, a potent inhibitor of the drug's major route of inactivation.

Methods

Patient selection and study design

Eligibility criteria and study design have been reported previously [19,20]. Briefly, cancer patients for whom no other treatment was available were treated with two courses of docetaxel administered as a 1-hour intravenous infusion once every 3-weeks. Initially, one course was given at a 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 ketoconazole, orally administered once every 24 hours up to 47 hours after docetaxel infusion (standard-dose ketoconazole) [30]. Seven patients were treated accordingly followed by plasma PK analysis. We observed wide variability in the reduction of docetaxel total clearance [19], which was attributed to variability in systemic ketoconazole exposure. We subsequently treated patients with a higher ketoconazole dose (seven 400 mg doses every 8 hours, up to 47 hours after docetaxel infusion; high-dose ketoconazole [31]) in combination with docetaxel (15 mg/m^2) [20]. We hypothesized that an increase in ketoconazole dose would result in sufficiently high ketoconazole exposure in each patient and thus in maximum CYP3A-inhibition overall, thereby reducing the variability in the extent to which docetaxel clearance is reduced. In addition, we administered a tracer-dose of [^{3}H]-radiolabelled docetaxel to each patient for radioactivity measurements in faeces and urine, in the absence and presence of high-dose ketoconazole. The study protocol was approved by the Erasmus MC Investigational Review Committee and all patients gave written informed consent for participation.

Chemicals

Tritiated docetaxel ([^{3}H]-docetaxel; 1.0 mCi/mL in ethanol, specific activity 7.2 Ci/mmol; Moravek Biochemicals, Brea, CA, USA) and 5 mL aliquots of a 200-fold dilution in ethanol absolute (> 99.9 %; Merck, Darmstadt, Germany) were stored at –80 °C prior to adding to unlabelled docetaxel diluted in 250 mL 0.9 % (w/v) sodium chloride solution. Purity of [^{3}H]-docetaxel was analysed by a reversed-phase high performance liquid chromatography (HPLC) method with ultraviolet detection at 230 nm [32]. The elution profile of the recovered radioactivity was compared to the chromatographic profile of unlabelled docetaxel; the product showed no signs of degradation or decreased purity [33].

Sample Collection

During both courses, complete stool collections were obtained up to 72 hours post-infusion and stored at –80 ºC until processing. Subsequently, weighted faeces samples were homogenized in 3 volumes (1 : 3 w/v) of phosphate buffered saline solution (PBS; Oxoid, Basingstoke, United Kingdom) using an Ultra-TurraxT25 homogenizer (Janke & Kunkel, IKA Labortechnik, Staufen, Germany) operating at 20,500 rpm and then stored at –80 °C prior to radioactivity measurements. Aliquots of faeces homogenates were further diluted (up

to 5-fold) with PBS and stored at –80 °C prior to chromatographic analysis. All voided urine was collected quantitatively during three intervals post-infusion (0-24 h, 24-48 h, 48-72 h) and two portions from each interval were stored at –80 °C prior to radioactivity measurements and chromatographic analysis.

Docetaxel analysis

Docetaxel was quantitated in faeces and urine samples using HPLC with tandem mass-spectrometric detection (LC-MS/MS). Prior to extraction all samples were diluted at volume ratios of at least 1 : 1 up to 1 : 50 with analyte-free lithium-heparinized human plasma. Hereafter, 100 μL aliquots of the diluted faeces or urine sample were processed and analysed as described previously [20]. Docetaxel faeces and urine concentrations were quantitated over the range of 5.00 ng/mL to 500 ng/mL. For quantitation of docetaxel in the patient samples, quality control (QC) samples at docetaxel concentrations of 5.00 ng/mL, 15.0 ng/mL, 225 ng/mL, 400 ng/mL and 6000 ng/mL, the latter QC diluted 20-fold prior to processing, were assayed in duplicate and distributed among the calibrators and samples in the analytical run. Finally, given the volume of collected urine and the weight of the collected faeces samples, the absolute amount of docetaxel excreted in each urine and faeces specimen was calculated assuming that 1 gram unhomogenized faeces equals 1 mL.

Radioactivity measurements

Details of the development and validation of the analytical method used to quantitate radioactivity levels in faeces and urine have been reported elsewhere [33]. Briefly, the radionuclide fraction of [^{3}H] was obtained after complete combustion of a known amount of sample aliquot (undiluted urine and 1 : 3 w/v diluted faeces) in a closed-type combustion flask of known weight, thus yielding a known amount of tritiated combustion water, [^{3}H]$_2$O. Subsequently, liquid scintillation (LS) cocktail was added to a quantitatively taken amount of [^{3}H]$_2$O, the solution then mixed until homogeneous and the samples counted in a LS-counter. Finally, based on the amount of radioactivity in the produced [^{3}H]$_2$O, the excreted radioactivity in the combusted samples was determined and thus the total amount of recovered radioactivity in the collected faeces and urine specimens. As [^{3}H]-radiolabelled docetaxel is metabolized and eliminated identically to unlabelled drug, the recovered radioactivity reflects the sum of radioactivity from excreted parent drug *and* all excreted metabolites.

Genotype analysis

Polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis for CYP3A5*3C, CYP3A5*6, CYP3A4*1B, CYP3A4*3, CYP3A4*17, CYP3A4*18A and ABCB1 3435C>T variant alleles was performed as described previously [4,34,35]. Based on

allele frequencies in the Caucasian population, in which the CYP3A5*3C allele is the predominant allele, the rare CYP3A5*6 variant allele, which also results in completely or severely decreased CYP3A5 activity was only determined for individuals with no, or only one CYP3A5*3C allele (i.e. with apparently at least one CYP3A5*1 allele).

Statistical considerations

Excretion parameters are reported as mean values ± standard deviation (SD) unless stated otherwise. The difference in parameters between the two courses was evaluated statistically using a non-parametric two-tailed, Wilcoxon signed rank test for paired observations after testing for period effects. Comparison of the mean urinary and faecal parent drug excretion (in the presence of ketoconazole) between the high- and standard-dose ketoconazole patient-groups showed no significant differences (P = 0.17 and P = 0.95 respectively, Mann-Whitney U test). Furthermore, the extent to which docetaxel total clearance was reduced was almost identical for high- and standard-dose ketoconazole treatment [19,20] suggesting that the increase in ketoconazole dose did not augment the extent of CYP3A-inhibition. Therefore, the excretion data of all patients were evaluated together, irrespective of ketoconazole dose. The significance level was set at $P < 0.05$. Statistical calculations were performed with SPSS version 11.5 (Chicago, IL, USA).

Results

Baseline patient profiles

A total of fifteen Caucasian patients were included (standard-dose ketoconazole, N = 7; high-dose ketoconazole, N = 8). For all patients urine samples were collected during both courses. For one patient (high-dose ketoconazole group) no faeces samples were collected, thus faeces samples were available for fourteen patients during both courses. None of the patients were administered CYP3A- inducing or inhibiting comedication, however two patients were administered medication known to reduce ketoconazole gastrointestinal absorption (pantoprazole and ranitidine), thus potentially decreasing systemic ketoconazole exposure [36]. For the patient administered pantoprazole (and high-dose ketoconazole) ketoconazole exposure (area under the concentration-time curve, AUC) was approximately 4-fold higher than median exposure (AUC = 60.1 mg·h/L; range, 19.6 – 81.3 mg·h/L) for patients treated with standard-dose ketoconazole (without interfering medication). As previously discussed, we did not observe a significant difference in docetaxel urinary and faecal excretion in the presence of ketoconazole between standard- or high-dose ketoconazole, therefore this patient was included in our analysis. Of note, this was also the patient for whom no faeces samples were available. For the patient administered ranitidine (and standard-dose ketoconazole), ketoconazole exposure was indeed the lowest (AUC = 12.8 mg·h/L), yet differed only marginally from the exposure (AUC = 19.6 mg·h/L) for

another patient not administered interfering comedication. Furthermore, for this patient, faecal parent drug excretion in the presence of ketoconazole was increased by 44 % suggesting an inhibitory effect of ketoconazole. We therefore also included this patient in our analysis. Thus, fifteen patients were evaluable for cumulative urinary parent drug excretion-analysis and fourteen for cumulative faecal parent drug excretion-analysis. Urine and faeces samples for radioactivity measurements were available for six and five patients, respectively, in the absence and presence of high-dose ketoconazole. Table 1 summarizes the baseline characteristics for the evaluable patients.

Table 1. Baseline patient characteristics

Characteristic	**Value**
Number	15
Age (years)	50 (36 – 69)
Sex (male / female)	11 / 4
Body-surface area (m^2)	1.89 (1.55 – 2.19)
WHO performance status	1 (0 - 1)
Tumour type	
Head & Neck	6
Melanoma	2
Breast	1
Prostate	1
Other	5
Clinical Chemistry	
WBC (x 10^9/L)	8.2 (5.7 – 14.9)
ANC (x 10^9/L)	6.8 (3.2 – 12.3)
Platelets (x 10^9/L)	264 (156 – 930)
Haemoglobin (mmol/L)	8.3 (5.8 – 9.2)
ASAT (U/L)	25 (17 – 79)
ALAT (U/L)	20 (6 – 30)
Alk Phos (U/L)	77 (58 – 241)
Total Bilirubin (μmol//L)	6 (4 – 15)
Serum Albumin (g/L)	41 (28 – 46)
Serum AAG (g/L)	1.03 (0.86 – 1.67)

Abbreviations: WHO, World Health Organization; WBC, white blood cell count; ANC, absolute neutrophil count; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; Alk Phos, alkaline phosphatase; AAG, alpha-1 acid glycoprotein. Values are given as median with range in parentheses (except for sex and tumour type).

All patients were genotyped for CYP3A5*3C, CYP3A4*1B, CYP3A4*3, CYP3A4*17 and CYP3A4*18A variant alleles. Fourteen patients were homozygous for the CYP3A5*3C variant allele, and consequently deficient in CYP3A5 activity, indicating that

for these patients total hepatic and intestinal CYP3A activity is solely attributable to CYP3A4 activity. Only CYP3A5 (not CYP3A4) is expressed to any significant degree in renal tubular epithelial cells [37]. Thus for the CYP3A5*3C homozygous individuals, any effect of ketoconazole on urinary parent drug excretion cannot be attributed to CYP3A(5)-inhibition. One patient carried a single CYP3A5*1 allele, yet not the CYP3A5*6 variant allele, indicating that in this patient CYP3A5 is (most probably) active. This same patient was the only heterozygous carrier of the CYP3A4*1B variant allele (*1A/*1B). All other patients were homozygous CYP3A4*1A/*1A. No patients carried variant alleles for CYP3A4*3, CYP3A4*17 or CYP3A4*18A. ABCB1 3435C>T genotyping results were available for thirteen patients. The genotype distribution was one homozygous wild-type patient (C/C), eight heterozygous variant patients (C/T) and four homozygous variant patients (T/T).

Faecal and urinary parent drug excretion

A summary of the cumulative excretion of parent drug in faeces and urine collected up to 72 hours post-infusion during both courses is listed in Tables 2 and 3. Data are reported as total fraction (i.e. percentage) of the administered absolute docetaxel dose, which is excreted unchanged in the faeces (fe_f, %) or urine (fe_u, %).

After single agent dosing a minor fraction of the administered docetaxel dose was recovered in the faeces unchanged (2.6 % ± 2.8 %). Total faecal parent drug excretion was approximately 2-fold higher in the presence of ketoconazole (5.2 % ± 5.4 %, $P = 0.03$). We observed a trend towards statistical significance when the faecal parent drug excretion in the absence and presence of ketoconazole was evaluated for each 24-hour interval (0 - 24 h, $P = 0.87$, N = 7; 24 – 48 h, $P = 0.52$, N = 9; 48 – 72 h, $P = 0.03$, N = 6). Faecal specimens were not available for every patient in each 24-hour interval, explaining why the number of patients included in these sub-analyses is less than the fourteen evaluable patients. Furthermore, this also explains why the sum of the average fraction of parent drug excreted during each 24-hour period does not equal the total cumulative faecal parent drug excretion (0 – 72 h).

Figure 1 shows the cumulative faecal parent drug excretion in the absence of ketoconazole as a function of CYP3A4*1B, CYP3A5*3C and ABCB1 3435C>T genotype. Mean faecal parent drug excretion for the CYP3A5*3C and CYP3A4*1A homozygous patients was 2.6 % and 2.4 % for the single CYP3A5*3C and CYP3A4*1B heterozygous patient. The genotype distribution (13 *vs* 1) does not allow statistical evaluation, however, these data do not suggest a substantial difference in faecal excretion for the evaluated genotypes. Mean faecal parent drug excretion for the C/T and T/T ABCB1 genotypes was 2.6 % and 4.2 %, respectively ($P = 0.85$). Faecal excretion for the patient with C/C genotype was 1.3 %.

Table 2. Cumulative faecal excretion of parent drug

Parameter	Ketoconazole = 0	Ketoconazole = 1	*P*-value
fe_f (%) 0 - 72 h, N = 14	2.6 ± 2.8 (0.02 – 10.0)	5.2 ± 5.4 (0.04 – 14.3)	< 0.05
fe_f (%) 0 - 24 h, N = 7	1.7 ± 3.1 (0.03 – 8.7)	1.7 ± 2.4 (0.08 – 6.7)	0.87
fe_f (%) 24 - 48 h, N = 9	1.8 ± 1.4 (0.01 – 3.91)	2.7 ± 2.2 (0.37 – 6.6)	0.52
fe_f (%) 48 - 72 h, N = 6	0.74 ± 0.41 (0.01 – 1.1)	3.0 ± 1.9 (1.1 – 5.5)	< 0.05

Abbreviations: Ketoconazole = 0, ketoconazole absent; Ketoconazole = 1, ketoconazole present; fe_f, cumulative faecal parent drug excretion expressed as percentage of the absolute docetaxel dose administered; Values are given as mean ± SD with range in parentheses.

The cumulative urinary parent drug excretion did not differ significantly between the two courses (P = 0.69) and renal parent drug excretion predominantly (> 80 %) took place during the first 24 hours post-infusion regardless of ketoconazole administration (Table 3). The ratio of the fraction of parent drug excreted during the first 24 hours to the total fraction of excreted parent drug (fe_u 0 – 24 h : fe_u 0 – 72 h) was significantly lower in the presence of ketoconazole (P = 0.02, N = 15). This was also the case when we evaluated this ratio for patients administered high-dose ketoconazole separately (P = 0.01), yet not for patients administered standard-dose ketoconazole (P = 0.93).

Table 3. Cumulative urinary excretion of parent drug

Parameter	Ketoconazole = 0	Ketoconazole = 1	*P*-value
fe_u (%) 0-72 h, N = 15	2.6 ± 1.4 (1.3 – 5.7)	2.6 ± 1.1 (1.1 – 5.0)	0.69
fe_u0-24 h : fe_u0-72 h, N = 15	0.94 ± 0.03 (0.86 – 0.97)	0.88 ± 0.07 (0.78 – 1.00)	< 0.05
fe_u0-24 h : fe_u0-72 h, N = 7[1]	0.93 ± 0.04 (0.86 – 0.97)	0.93 ± 0.07 (0.84 – 1.00)	0.93
fe_u0-24 h : fe_u0-72 h, N = 8[2]	0.95 ± 0.02 (0.91 – 0.97)	0.83 ± 0.04 (0.78 – 0.89)	< 0.05

Abbreviations: Ketoconazole = 0, ketoconazole absent; Ketoconazole = 1, ketoconazole present; fe_u, cumulative urinary parent drug excretion expressed as percentage of the absolute docetaxel dose administered; ratio fe_u0-24 h : fe_u0-72 h, ratio of the urinary excretion of parent drug during the first 24 hours to the total urinary excretion of parent drug up to 72 hours post-infusion. [1]Standard-dose ketoconazole; [2]High-dose ketoconazole. Values are given as mean ± SD with range in parentheses.

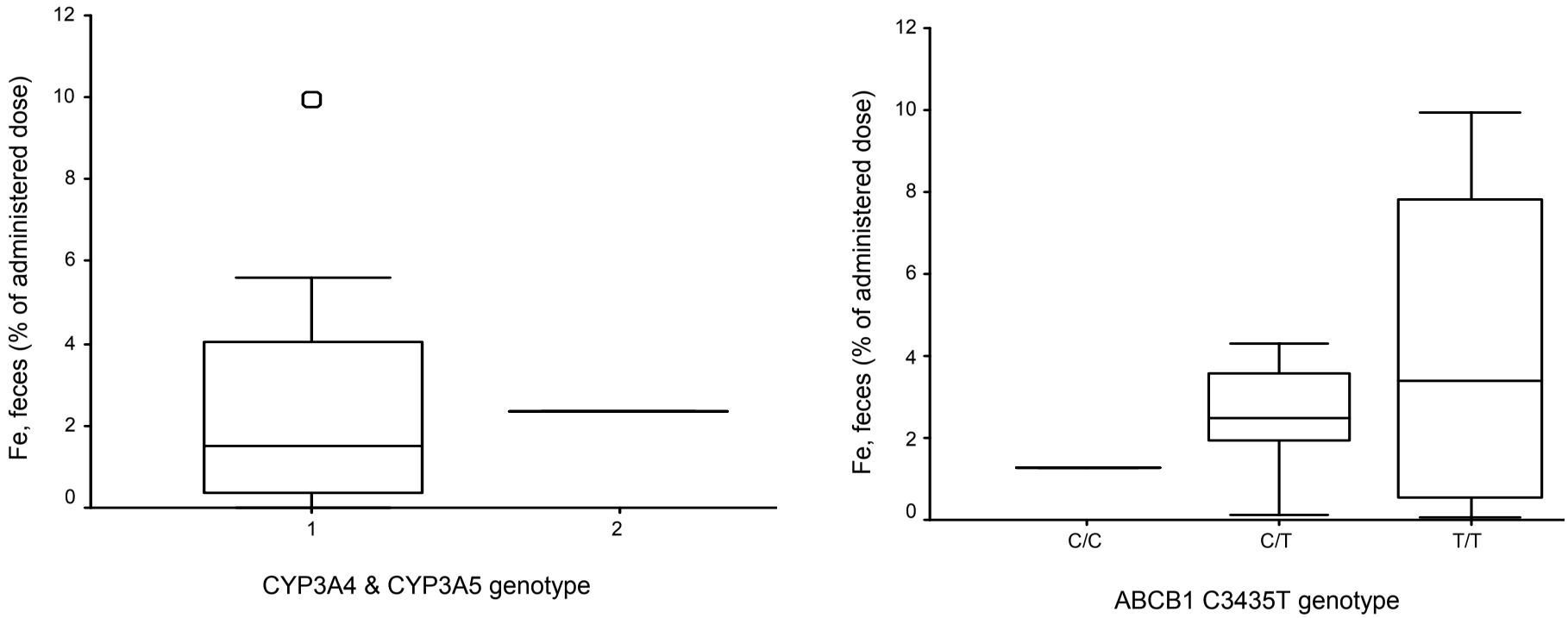

Figure 1. *Box plots of the percentage cumulative faecal parent drug excretion as a function of CYP3A5*3C, CYP3A4*1B and ABCB1 C3435T genotype; (1) denotes CYP3A5*3C homozygous variant allele carriers and patients carrying no CYP3A4*1B variant allele (i.e. CYP3A4*1A/*1A; N = 14); (2) denotes CYP3A5*3C heterozygous variant allele carriers and CYP3A4*1B heterozygous variant allele carriers (N = 1); C/C denotes ABCB1 homozygous wild-type allele carriers (N = 1), C/T denotes patients carrying one variant allele (N = 8) and T/T denotes homozygous variant allele carriers (N = 4). The box represents the difference between the 25th and 75th percentiles, whereas the horizontal line inside the box represents the median. Open circles are defined as outliers. Whiskers are drawn from the ends of the box to the largest and smallest values that are not outliers.*

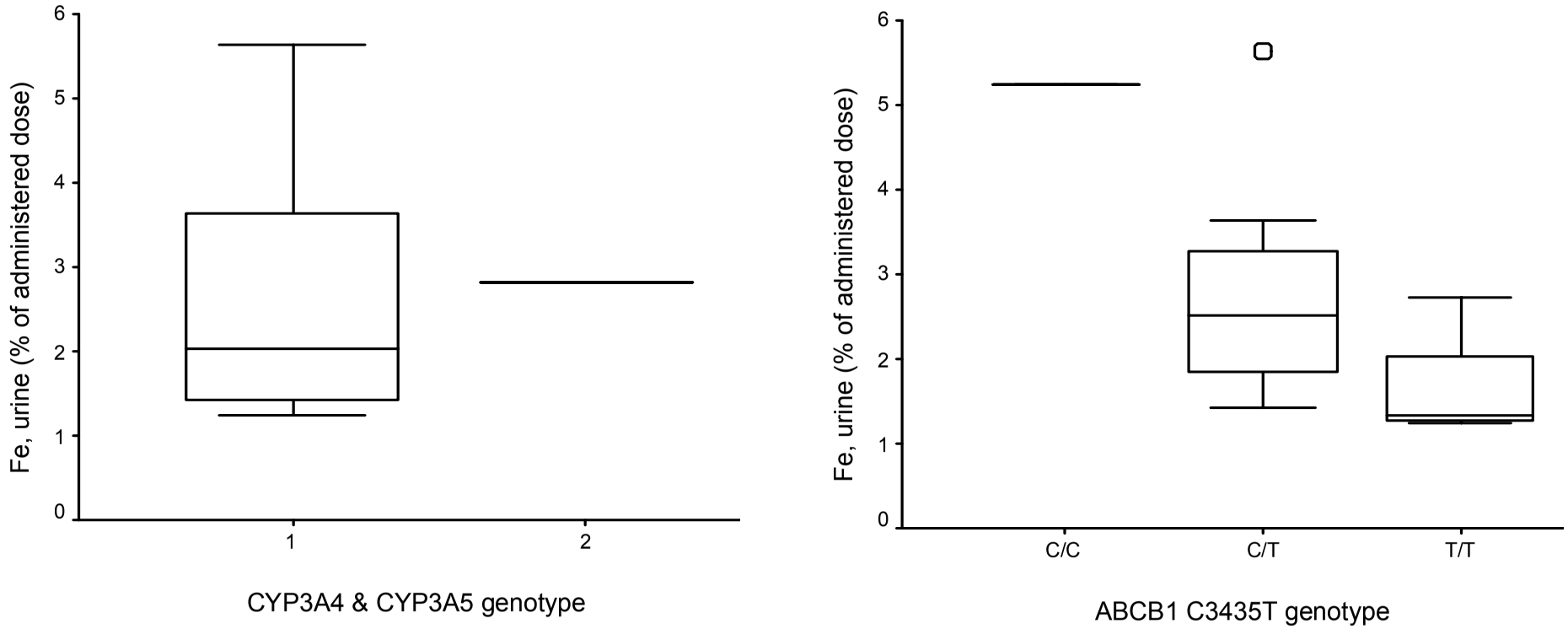

Figure 2. *Box plots of the percentage cumulative urinary parent drug excretion as a function of CYP3A5*3C, CYP3A4*1B and ABCB1 C3435T genotype; (1) denotes CYP3A5*3C homozygous variant allele carriers and patients carrying no CYP3A4*1B variant allele (i.e. CYP3A4*1A/*1A; N = 14); (2) denotes CYP3A5*3C heterozygous variant allele carriers and CYP3A4*1B heterozygous variant allele carriers (N = 1); C/C denotes ABCB1 homozygous wild-type allele carriers (N = 1), C/T denotes patients carrying one variant allele (N = 8) and T/T denotes homozygous variant allele carriers (N = 4). The box represents the difference between the 25th and 75th percentiles, whereas the horizontal line inside the box represents the median. Open circles are defined as outliers. Whiskers are drawn from the ends of the box to the largest and smallest values that are not outliers.*

Figure 2 shows the cumulative urinary parent drug excretion in the absence of ketoconazole as a function of CYP3A4*1B, CYP3A5*3C and ABCB1 3435C>T genotype. Mean urinary parent drug excretion for the CYP3A5*3C and CYP3A4*1A homozygous patients was 2.6 % and 2.8 % for the CYP3A5*3C and CYP3A4*1B heterozygous individual, again not suggesting a substantial difference between the evaluated genotypes. Mean urinary parent drug excretion for the C/T and T/T ABCB1 genotypes was 2.8 % and 1.7 %, respectively, (P = 0.04). Urinary excretion for the patient with C/C genotype was 5.2 %.

Radioactivity recovered in faeces and urine

A summary of the cumulative radioactivity recovered in faeces (Ra_f, %) and urine (Ra_u, %) samples collected up to 72 hours post-infusion during both treatments is listed in Table 4.

Table 4. Cumulative radioactivity recovered in faeces and urine

Parameter	Ketoconazole = 0	Ketoconazole = 1	*P*-value
Ra_f (%) 0-72 h, N = 5	40.4 ± 19.9 (7.37 – 55.8)	27.7 ± 20.3 (1.17 – 45.5)	0.23
fe_f : Ra_f, N = 5	0.10 ± 0.05 (0.05 – 0.17)	0.26 ± 0.09 (0.12 – 0.35)	< 0.05
Ra_u (%) 0-72 h, N = 6	5.13 ± 1.23 (3.76 – 6.94)	4.52 ± 1.38 (3.48 – 7.14)	0.17
Ra_u0-24 h : Ra_u0-72 h, N = 6	0.89 ± 0.04 (0.82 – 0.93)	0.78 ± 0.05 (0.72 – 0.86)	< 0.05
fe_u: Ra_u, N = 6	0.59 ± 0.22 (0.28 – 0.83)	0.66 ± 0.11 (0.52 – 0.81)	0.27

Abbreviations: Ketoconazole = 0, ketoconazole absent; Ketoconazole = 1, ketoconazole present; Ra_f, Ra_u, cumulative radioactivity recovered in faeces and urine, respectively, expressed as percentage of the absolute amount of added radioactivity; fe_f : Ra_f, ratio of total faecal excretion of parent drug to total faecal excretion of parent drug *and* all metabolites; fe_u : Ra_u, ratio of total urinary excretion of parent drug to total urinary excretion of parent drug *and* all metabolites; Ra_u0-24 h : Ra_u0-72 h, ratio of recovered urinary radioactivity during the first 24 hours to total recovered urinary radioactivity up to 72 hours post-infusion. Values are given as mean ± SD with range in parentheses.

Mean cumulative faecal excretion of radioactivity was lower in the presence of ketoconazole (27.7 % *vs* 40.4 %), but this difference was not significant (P = 0.23). Mean faecal parent drug excretion in the presence and absence of ketoconazole was 6.7 % and 3.3 % (for these five patients), respectively, suggesting that the fraction of excreted metabolites in the presence and absence of ketoconazole was 21.0 % and 37.1 %, respectively (P = 0.14).

We evaluated the influence of ketoconazole on the ratio of faecal parent drug excretion to faecal excretion of parent drug *and* all metabolites, the latter reflected by cumulative radioactivity excretion. This ratio (fe_f : Ra_f) was significantly higher (P = 0.04) in the presence of ketoconazole (0.26 ± 0.09) compared to single agent docetaxel (0.10 ± 0.05), confirming the observed increased parent drug excretion.

The total fraction of radioactivity recovered in urine did not differ significantly between the two courses (P = 0.17). For both courses, the percentage of urinary recovered radioactivity is approximately 2 % higher than cumulative urinary parent drug excretion, suggesting that approximately 2 % of the administered docetaxel dose is eliminated renally as metabolite(s). The ratio of the cumulative fraction of radioactivity recovered in urine during the first 24 hours to the total fraction of urinary excreted radioactivity (ratio Ra_u 0 - 24 h : Ra_u 0 – 72 h) was significantly lower in the course with ketoconazole (P = 0.03). In contrast to the faecal excretion data, ketoconazole did not influence the ratio of urinary parent drug excretion to urinary excretion of parent drug *and* all metabolites (fe_u : Ra_u; P = 0.27).

Mass balance

The mass balances of excreted parent drug and recovered radioactivity (determined by adding the cumulative faecal and urinary excretion data) for each course are summarized in Table 5. For parent drug total cumulative excretion is significantly higher in the presence of ketoconazole (P = 0.04). The mass balance of recovered radioactivity was lower in the course with ketoconazole, yet the difference was not significant (P = 0.23).

Table 5. Mass balance parent drug excretion and Mass balance recovered radioactivity

Parameter	Ketoconazole = 0	Ketoconazole = 1	*P*-value
Mass balance (%) 0 – 72 h, N = 14 (fe_f + fe_u)	5.3 ± 3.0 (1.3 – 11.3)	7.8 ± 5.6 (1.1 – 18.0)	< 0.05
Mass balance (%) 0 – 72 h, N = 5 (Ra_f + Ra_u)	45.8 ± 19.1 (13.7 – 60.4)	32.4 ± 19.7 (5.4 – 49.5)	0.23

Abbreviations: Ketoconazole = 0, ketoconazole absent; Ketoconazole = 1, ketoconazole present; fe_f, fe_u, cumulative excretion of parent drug in faeces and urine, respectively, expressed as percentage of the absolute docetaxel dose administered; Ra_f, Ra_u cumulative radioactivity recovered in faeces and urine, respectively, expressed as percentage of the absolute amount of added radioactivity. Values are given as mean ± SD with range in parentheses.

Mean cumulative total parent drug excretion for the CYP3A5*3C and CYP3A4*1A homozygous patients was 5.3 % and 5.2 % for the CYP3A5*3C and CYP3A4*1B heterozygous individual. Mean cumulative total parent drug excretion for the C/T and T/T

ABCB1 genotypes was 5.5 % and 5.8 %, respectively (P = 1.0). Cumulative total parent drug excretion for the patient with C/C genotype was 6.5 %.

Discussion

Although docetaxel plasma PK in cancer patients is well characterized, data on the faecal and urinary excretion of the drug are limited. In this study we evaluated the faecal and urinary excretion of docetaxel parent drug and [^{3}H]-radiolabelled docetaxel in cancer patients, after intravenous docetaxel administration with and without concomitant orally administered ketoconazole, a potent inhibitor of CYP3A-mediated metabolism, the drug's major route of inactivation, and also a (weak to modest) inhibitor of ABCB1, which plays an important role in the faecal disposition of docetaxel [8,9]. Assessing purposeful modulation of docetaxel faecal and urinary disposition can contribute to a better overall understanding of the mechanistic aspects involved in docetaxel metabolism. Recently, hepatic CYP3A-induction *in vitro* led to CYP2C8-mediated formation of a previously unidentified docetaxel metabolite [38]. Furthermore, preliminary data indicate that renal function is the most significant predictor of docetaxel clearance in the presence of ketoconazole [39], which is in contrast with single agent treatment where hepatic function is a significant predictor of docetaxel clearance [40,41].

In the absence of ketoconazole, faecal parent drug excretion was consistent with previous findings [9] and approximately 2-fold increased in the presence of ketoconazole. The relative contribution of ABCB1-inhibition is quantitatively less significant than the contribution of CYP3A-inhibition to the overall effect of ketoconazole-mediated drug-interactions [7,17,18]. Thus, the here observed drug-interaction should primarily be attributed to CYP3A-inhibition, however the influence of concomitant ABCB1-inhibition should not be overlooked. In the presence of a selective ABCB1-inhibitor re-absorption of docetaxel parent drug from the intestinal lumen (following biliary secretion) is a very efficient process and subsequent CYP3A-mediated metabolism in the liver and intestinal mucosa consequently leads to a marked reduction in faecal parent drug excretion [8,9]. If theoretically, ketoconazole were to act only as a selective CYP3A-inhibitor, adequate ABCB1-mediated efflux of parent drug into the intestinal lumen would not be impaired. Consequently, due to ketoconazole-mediated selective CYP3A-inhibition, one would expect the administered docetaxel dose to be excreted predominantly as parent drug. Concomitant ketoconazole-mediated ABCB1-inhibition most likely explains why we did not observe a more marked increase in faecal parent drug excretion in the presence of ketoconazole (Figure 3: a schematic representation of the role of hepatic CYP3A and intestinal ABCB1 in docetaxel metabolism and excretion and the inhibitory effect of ketoconazole). Interestingly, we observed a trend towards a statistically significant difference in faecal parent drug excretion in the presence and absence of ketoconazole as the time interval post-infusion increased. The effect of ABCB1-inhibition

is expected to be most pronounced shortly after ketoconazole administration due to high local drug concentrations in the gut, and to decrease over time, such that in the 48 - 72 h interval, when no ketoconazole was administered, the (relative) contribution of ketoconazole-mediated CYP3A-inhibition is more pronounced than in the 0 – 24 h interval.

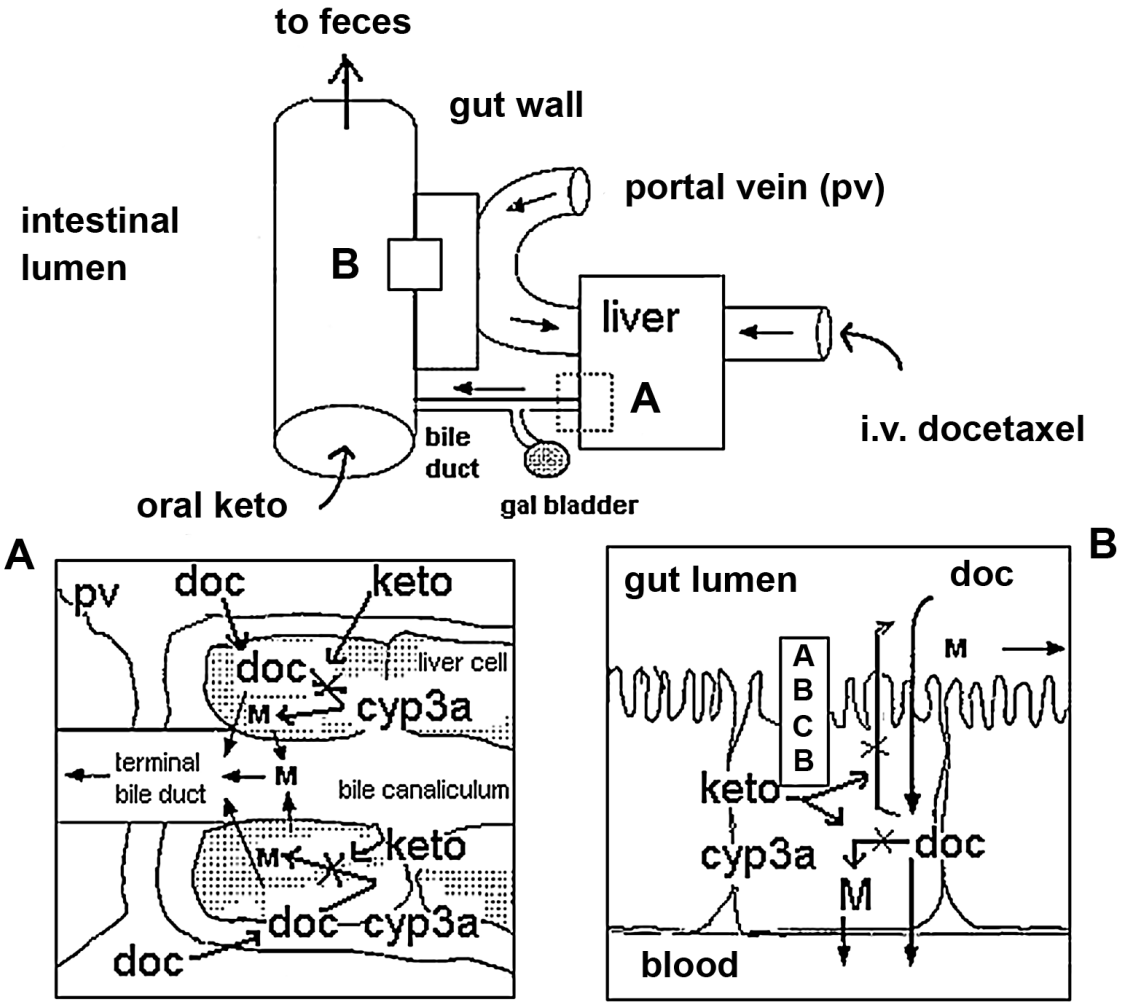

Figure 3. *Schematic representation of the role of hepatic CYP3A and intestinal ABCB1 in docetaxel metabolism and elimination in humans.*

A. Model of the hepatic-cellular plate showing the bile collecting system with (i) inhibition by ketoconazole of cytochrome P450 3A isozyme-mediated hepatic metabolism of docetaxel from the systemic circulation or the portal vein after re-absorption following biliary secretion and (ii) its subsequent secretion in the terminal bile duct.

B. Model of the intestinal epithelium showing (i) re-uptake of docetaxel into the intestinal lumen, (ii) ABCB1-mediated efflux of docetaxel and its inhibition by ketoconazole and (iii) intestinal metabolism by the cytochrome P450 3A enzyme system and its inhibition by ketoconazole. Abbreviations: ABCB, ABCB1; doc, docetaxel; keto, ketoconazole; M, docetaxel metabolite M4; CYP3A, enzymes of the cytochrome P450 family, isoform 3A.

A second consequence of temporarily inhibiting CYP3A-mediated metabolism is a reduction in the fraction of formed metabolites. The difference between faecal recovered radioactivity (i.e. parent drug *and* all excreted metabolites) and faecal parent drug excretion reflects the faecal excretion of metabolites. We observed a lower fraction of excreted metabolites in the presence of ketoconazole (21.0 *vs* 37.1 %), although the difference was not statistically significant ($P = 0.14$). The lack of significance is possibly also a consequence of concomitant ABCB1-inhibition or because alternative routes of metabolism then prevail or due to our limited sample size (N = 5).

The cumulative fraction of faecal recovered radioactivity was not significantly lower in the presence of ketoconazole ($P = 0.23$). This is in line with the concept of mass conservation, stating that (tritium-labelled) mass is neither created nor destroyed during a chemical reaction, i.e. metabolism. Thus, despite CYP3A-inhibition, the total fraction of excreted parent drug and all metabolites (i.e. recovered radioactivity) should not differ between the two courses.

Urinary excretion plays a minor role in the elimination of docetaxel. We determined urinary parent drug excretion and urinary excretion of metabolites each to be approximately 2 %. Interestingly, substantially increasing the ketoconazole dose significantly reduced parent drug excretion during the first 24 hours post-infusion relative to total parent drug excretion (ratio fe_u 0 – 24 h : fe_u 0 – 72 h). This effect is most likely attributable to ketoconazole-mediated ABCB1-inhibition as, with the exception of one, all patients were deficient in renal CYP3A(5) activity. Ketoconazole-mediated inhibition of ABCB1 transport function is concentration dependant [42], therefore it is likely that the higher ketoconazole dose effectively inhibits ABCB1 localized on the apical surface of renal tubular epithelial cells [43] thereby limiting ABCB1-mediated renal parent drug secretion. Again, (the effect of) renal ABCB1-inhibition seems most pronounced shortly after ketoconazole administration. However, ketoconazole-mediated ABCB1-inhibition was not marked enough to affect the cumulative urinary elimination of docetaxel.

The mass balance for cumulative parent drug excretion was increased upon concomitant ketoconazole administration, yet not as marked as one may have expected, due to the discussed influence of concomitant intestinal ABCB1-inhibition. The mass balance for the fraction of recovered radioactivity, although lower in the presence of ketoconazole, did not differ significantly between the two courses, due to above-mentioned reasons.

To our knowledge, the faecal and urinary excretion of docetaxel has not previously been evaluated as function of genotype. As our patient group was limited in size we only tentatively correlated parent drug excretion (in the absence of ketoconazole) to genotype. The mean cumulative faecal excretion for patients carrying the ABCB1 T/T genotype was more than 3-fold that observed for the (single) patient with the C/C genotype which is consistent with data suggesting higher expression of intestinal ABCB1 for the T/T genotype [44]. Interestingly, the observed higher faecal excretion for homozygous variant allele carriers

(T/T) compared to the homozygous wild-type (C/C) patient is inversed for urinary excretion and could be a reflection of different ABCB1 3435C>T expression among tissues [44,45].

We are aware of several limitations in our study; (1) the number of patients was limited, especially the sub-group administered [^{3}H]-radiolabelled docetaxel, yet more than in previous excretion studies using radiolabelled docetaxel [15]; (2) specimens were collected up to 72 hours post-infusion, the period of hospital admission; ideally specimens should be collected up to 7 days post-infusion yet this poses practical and logistical difficulties; (3) although patients consented to collection of all faecal and urinary specimens, it is possible that collection is not complete; (4) we did not analyse the faecal and urinary excretion of the major metabolites due to unavailability of reference compounds and (5) it is likely that by assuming that 1 gram unhomogenized faeces is equivalent to 1 mL, we have underestimated the cumulative faecal excretion. However, despite these limitations, this study has further elucidated the elimination pathways of docetaxel. Moreover, to the best of our knowledge, for the first time faecal and urinary excretion of docetaxel was evaluated after temporarily inhibiting the drug's major route of inactivation.

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Chapter 9

Influence of Medicinal Cannabis on the Pharmacokinetics of Irinotecan and Docetaxel

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Submitted

Abstract

Background: To date, data regarding the potential of cannabinoids to modulate cytochrome P450 (CYP) isozyme 3A activity are contradictory. Recently, a standardized medicinal cannabis product (variety Bedrocan®), was introduced in The Netherlands. We anticipated an increased use of medicinal cannabis concurrent with anticancer drugs, and undertook a drug-interaction study to evaluate the effect of concomitant medicinal cannabis on the pharmacokinetics of irinotecan and docetaxel, both subject to CYP3A-mediated biotransformation.

Methods: Twenty-four cancer patients were treated intravenously with irinotecan (600 mg, N = 12) or docetaxel (180 mg, N = 12), followed three weeks later by the same drugs concomitant with medicinal cannabis (200 mL herbal tea, 1 g/L) for 15 consecutive days, starting 12 days before the second treatment. Blood samples were obtained up to 55 hours after dosing and analysed for irinotecan and its metabolites (SN-38, SN-38G) and docetaxel. Pharmacokinetic analyses were performed during both treatments. Results are reported as the mean ratio (95 % confidence interval, CI) of the observed pharmacokinetic parameters with and without concomitant medicinal cannabis.

Results: Medicinal cannabis administration did not significantly influence exposure to, and clearance of irinotecan (1.04; CI = 0.96 – 1.11 and 0.97; CI = 0.90 – 1.05, respectively) or docetaxel (1.11; CI = 0.94 – 1.28 and 0.95; CI = 0.82 – 1.08, respectively).

Conclusion: Coadministration of medicinal cannabis, as herbal tea, in cancer patients treated with irinotecan or docetaxel does not significantly influence the plasma pharmacokinetics of these drugs. The evaluated variety of medicinal cannabis can be administered concomitantly with both anticancer agents without dose adjustments.

Introduction

For the past 4,000 years [1], patients and doctors of each era have resorted to cannabis when conventional treatments were ineffective or lacking [2,3]. Indeed, in oncology beneficial effects have been reported for cancer-associated anorexia, (delayed) chemotherapy-induced nausea and vomiting, and palliation [4-8]. However, largely due to the lack of well designed clinical trials, much controversy remains regarding the claimed benefits [9].

The only FDA-approved medicinal cannabis products are an oral formulation containing dronabinol (Marinol®; Solvay Pharmaceuticals Inc, Marietta, GA, USA), the synthetic version of delta9-tetrahydrocannabinol (THC), the main pharmacologically active cannabinoid [10] and capsules containing nabilone, an analogue of dronabinol (Cesamet®, Valeant Pharmceuticals Int., Costa Mesa, CA, USA). In Canada, where seriously ill patients can apply for medicinal cannabis under the Canadian Marihuana Medical Access Regulations, the government licensed the prescription sale of an oromucosal spray called

Sativex® (GW Pharm Ltd, Salisbury, United Kingdom) containing both THC and cannabidiol (CBD) in April 2005. However, many patients claim (subjectively), that a whole or partially purified extract of *Cannabis sativa L*. offers advantages over a single isolated ingredient [10-12]. In The Netherlands, the unavailability of a legal product forced patients to frequent 'coffeeshops', which, although not prosecuted according to the Dutch soft-drugs policy, remain illegal. In September 2003, in order to stimulate the conduct of representative clinical trials evaluating the safety and efficacy of medicinal cannabis, while simultaneously offering patients access to a prescription product meeting pharmaceutical quality standards (standardized content; free of microbiological impurities) [13], a legal medicinal cannabis product was introduced in The Netherlands [14]. However, as it is not an officially registered drug, pharmacokinetic drug-interactions have not been evaluated as recommended for new drug applications [15]. Yet it has previously been shown that pharmacokinetic drug-interactions, also with herbal products (increasingly used by cancer patients) [16,17], can result in under- or overdosing [18-20].

Cannabinoids appear able to modulate the catalytic activity of several hepatic cytochrome P450 (CYP) isozymes, including isozyme 3A, responsible, in part, for the metabolism of 37 % of all currently FDA-approved anticancer drugs [21]. The majority of *in vitro* and animal data suggest an inhibitory effect on CYP3A-mediated metabolism [22-25], yet induction of CYP3A has been observed after repeated administration [26,27]. In vivo data are also contradictory; both CYP3A inhibition [28] and induction [29] have been reported. Moreover, clinical drug-interaction studies adequately assessing the effect of medicinal cannabis on the pharmacokinetics of concomitantly administered (anticancer) drugs are absent [30,31].

We anticipated that the introduction of a legal cannabis product in The Netherlands would result in increased use of medicinal cannabis concomitant with cytotoxic drugs, many of which are highly toxic and characterized by narrow therapeutic windows. The postulated, albeit contradictory, effects of cannabinoids on CYP3A function and the absence of clinical drug-interaction studies, led us to initiate a drug-interaction study to assess the influence of medicinal cannabis on the pharmacokinetics of the anticancer drugs irinotecan and docetaxel, both CYP3A-substrates [32,33]. We here report on the plasma pharmacokinetics of irinotecan and docetaxel after intravenous infusion to cancer patients, with and without concomitant oral medicinal cannabis administration.

Patients and methods

Patients and treatment

Patients were eligible if they had a histologically or cytologically confirmed diagnosis of (metastatic) cancer for which irinotecan or docetaxel was considered an adequate option, which was refractory to conventional treatment or for which there was no standard regimen. Eligibility criteria were identical to those documented elsewhere [20,34]. In addition, patients

with a history of, or current cannabis use were not eligible. The protocol was approved by the Institutional Review Board of the Erasmus MC and written informed consent was obtained from all patients prior to study entry.

The primary study endpoint was a measurable effect of medicinal cannabis on the plasma pharmacokinetics of irinotecan and its metabolites SN-38 and SN-38-glucuronide (SN-38G) or on docetaxel plasma pharmacokinetics. Based on the assumption that the within-patient standard deviation of the response variable (i.e. irinotecan or docetaxel pharmacokinetic parameters) for two measurements is 0.2 (20 %), a power (1-β) of 0.9 (90 %), a clinically relevant difference of 30 %, and a two-sided significance level of 0.05 (5 %), a sample size of (at least) twelve patients per treatment-arm (i.e. irinotecan or docetaxel) was required in a paired two-sided analysis [35]. It was assumed that the interval between the two treatments was an adequate washout period, with no carryover effects.

Patients meeting eligibility criteria received their first treatment of either irinotecan, as a 90-minute intravenous (i.v.) infusion or docetaxel, as a 1-hour i.v. infusion, at a fixed dose of 600 mg or 180 mg, respectively, followed three weeks later by a second treatment of the same drug in combination with medicinal cannabis. The decision to administer a fixed dose instead of a body surface area (BSA)-based dose, was based on analyses demonstrating that BSA-based dosing does not substantially decrease interindividual variability in drug clearance for these two drugs [36-39]. For the second treatment, the first three patients were dosed irinotecan and docetaxel at 75 % (450 mg and 135 mg, respectively), after which a protocol-scheduled safety interim-analysis, including a pharmacokinetic analysis, was performed to determine if subsequent dose adjustments were necessary. If no clinically relevant [40,41] pharmacokinetic interaction or increased haematological toxicity was observed, the following nine patients were to be administered the same dose as in the first treatment. Dose reductions for the second treatment were allowed and based on the worst toxicity observed during the previous treatment.

Irinotecan (Campto®, Pfizer, Capelle aan den IJssel, The Netherlands) and docetaxel (Taxotere®, Sanofi-Aventis, Gouda, The Netherlands) were diluted in 250 mL 0.9 % (wt/vol) sodium chloride prior to drug administration. Patients received oral and written instructions to prepare the medicinal cannabis (*Cannabis Sativa L. Flos*, variety Bedrocan®, Office for Medicinal Cannabis, The Hague, The Netherlands) containing 18 % THC and 0.8 % CBD, as 200 mL herbal tea (1 g/L), and to administer it once daily in the evening [26] at home, for a total of 15 consecutive days as recommended [15], starting on day 10 of the first treatment. During both treatments, patients administered irinotecan received granisetron (1 mg i.v.) and dexamethasone (10 mg i.v.) 30 minutes prior to chemotherapy. Atropine (0.25 mg) was administered subcutaneously as treatment or prophylaxis for irinotecan-induced acute cholinergic syndrome. To prevent allergic reactions and oedema, for patients treated with docetaxel, premedication consisted of dexamethasone (8 mg, orally) given twice daily for three consecutive days, starting on the evening before docetaxel infusion.

During both treatments, physical examination, toxicity assessment [42], a complete blood count with differential and serum chemistry tests, including creatinine, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total bilirubin, and albumin were performed weekly.

Pharmacokinetic analyses

Irinotecan, its metabolites (SN-38, SN-38G) and docetaxel pharmacokinetic analyses were performed during both treatments. For irinotecan and docetaxel pharmacokinetics blood samples (approximately 7 mL in lithium-heparinized tubes) were collected up to 54 and 47 hours after end of infusion, respectively, according to previously published sampling strategies [18,43]. All samples were processed to plasma by centrifugation for 10 minutes at 3,000 *g* (4 °C), and stored at –80 °C until analysis. Irinotecan and its metabolite concentrations were determined by validated assays based on reversed-phase high-performance liquid chromatography (HPLC) with fluorescence detection [44,45]. Docetaxel plasma concentrations were determined using HPLC with tandem mass-spectrometric detection [46].

Based on a previously developed population model [47], and the observed individual plasma concentrations, individual pharmacokinetic parameter estimates for irinotecan and its metabolites were obtained by Bayesian (POSTHOC) analysis using non linear mixed-effect modelling implemented in the NONMEM software program (double precision, version V; level 1.1) [48]. The area under the plasma concentration-time curve (AUC) was simulated for irinotecan and its metabolites from time 0 to 100 hours, to 500 hours, and to infinity after start of infusion for both treatments. (Metabolic) Clearance was defined as dose divided by AUC. Metabolic ratios, that is the relative extent of conversion (REC; $AUC_{0\text{-}100}$ ratio of SN-38 to irinotecan) and the relative extent of glucuronidation (REG; $AUC_{0\text{-}100}$ ratio of SN-38G to SN-38) were calculated based on individual Bayesian predicted AUC values.

For docetaxel, individual pharmacokinetic parameters were estimated using model dependent methods implemented in WinNonLin 4.0 (Pharsight, CA, USA). Concentration-time data were fit with a three-compartment model with reciprocal squared prediction weighting. Model adequacy was guided by inspection of the coefficient of variation of the fitted pharmacokinetic parameters, and by the Akaike information criteria [49]. Maximum plasma concentrations were obtained from the model-estimated plasma concentration at the end of infusion. Calculated secondary parameters included systemic exposure (AUC), total systemic clearance, half-life during the terminal phase of the disposition curve, and (apparent) volume of distribution.

Cannabis screening

A urine sample was collected just before start of the second treatment and stored at –80 ºC until analysis. Samples were screened semi-quantitatively (i.e. results are reported as

'positive' that is above, or 'negative' that is below the threshold level of 50 μg/L) for presence of the primary urinary metabolite of orally ingested THC (11-nor-THC-9-carboxylic acid) using a validated cannabinoids assay (TDx/FLx® Cannabinoids assay, Abbott® Laboratories, IL, USA). The presence of cannabinoids and/or metabolite(s) in urine indicates previous cannabis exposure [50].

Statistics

All parameter estimates are reported as mean values with 95 % confidence intervals (CIs) in parenthesis unless stated otherwise. The difference in irinotecan and docetaxel pharmacokinetic parameters between the first and second treatment was evaluated by calculating 95 % CIs for the geometric mean ratios of the observed pharmacokinetic parameters in the presence and absence of medicinal cannabis (e.g. 95 % CI for ratio $CL_{treatment2}$: $CL_{treatment1}$) [51]. CIs for the geometric mean ratio provide an estimate of the distribution of the observed systemic exposure measure ratio of substrate and interacting drug *vs* substrate alone and convey a probability of the magnitude of the interaction. The difference in haematological toxicity for the two treatments was evaluated statistically using non-parametric two-tailed, Wilcoxon signed rank tests for paired observations, and the significance level was set at $P < 0.05$. Statistical calculations were performed with SPSS, version 11.5 (Chicago, IL, USA).

Results

Patient accrual

For both the irinotecan- and docetaxel treatment-arm, twelve patients completed two treatments, did not use comedication and/or dietary supplements known to modulate CYP3A-function, took their medicinal cannabis as prescribed (based on cannabis screening, patient oral declaration and patient treatment-diaries) and were evaluable for irinotecan and docetaxel pharmacokinetic analyses, respectively. Table 1 lists a summary of the baseline characteristics of the twelve patients in both treatment groups.

Irinotecan treatment and pharmacokinetics

All patients were administered 600 mg irinotecan during the first treatment. Two patients enrolled after the interim analysis, which did not demonstrate a substantial change in irinotecan pharmacokinetics or increased haematological toxicity, also received a reduced second irinotecan-dose due to toxicity, i.e. grade 3 diarrhoea (450 mg) and grade 3 liver function abnormalities (300 mg), respectively. All other patients (N = 7) were administered 600 mg during the second treatment.

Table 1. Baseline patient characteristics (N = 12 per treatment arm)

Characteristic	Irinotecan	Docetaxel
Age (years)	58 (27 – 66)	55 (40 – 67)
Sex		
Male	7	7
Female	5	5
Body-surface area (m^2)	1.90 (1.56 – 2.20)	1.78 (1.50 – 2.16)
WHO performance status	1 (0 – 1)	1 (0 – 1)
Tumour type		
Pancreas	5	1
Breast	–	4
Melanoma	–	3
Head & Neck	–	2
ACUP	2	–
Lung	1	1
Gastric	1	1
Other	3	–
Haematology		
WBC (x 10^9/L)	7.4 (4.4 – 13.5)	6.5 (4.3 – 15.6)
ANC (x 10^9/L)	4.9 (2.1 – 11.2)	4.2 (2.8 – 14.5)
Platelets (x 10^9/L)	233 (116 – 447)	293 (144 – 620)
Haemoglobin (mmol/L)	8.2 (5.8 – 9.3)	8.2 (6.6 – 10.5)
Clinical Chemistry		
ASAT (U/L)	16 (31 – 104)	30 (14 – 64)
ALAT (U/L)	10 (35 – 133)	21 (12 – 65)
Alk Phos (U/L)	66 (109 – 323)	96 (61 – 401)
Total Bilirubin (μmol/L)	8 (4 – 21)	7 (3 – 25)
Total Protein (g/L)	75 (66 – 88)	64 (48 – 80)
Serum Albumin (g/L)	42 (29 – 45)	39 (32 – 48)
Serum Creatinine (μmol/L)	63 (51 – 88)	64 (48 – 80)
Serum AAG (g/L)	1.41 (0.74 – 2.84)	0.71 (0.47 – 2.16)

Abbreviations: WHO, World Health Organization; ACUP, adeno carcinoma of unknown primary; WBC, white blood cell count; ANC, absolute neutrophil count; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; Alk Phos, alkaline phosphatase; AAG, alpha-1 acid-glycoprotein. Values are given as median with range in parentheses (except for sex and tumour type).

Upon concurrent medicinal cannabis use, irinotecan clearance and dose-normalized AUC were not significantly affected, as reflected by the geometric mean ratios and the corresponding 95 % CIs for both parameters being 0.97 (0.90 – 1.05) and 1.04 (0.96 – 1.11), respectively. Similarly, metabolic clearance and dose-normalized AUC of SN-38 and SN-38G were not significantly changed. Table 2 summarizes the pharmacokinetic parameters for irinotecan with and without concomitant medicinal cannabis administration. The mean (N =

12) irinotecan, SN-38 and SN-38G plasma concentration-time curves for both treatments furthermore illustrate the similarity between the two treatments (Figure 1).

Table 2. Irinotecan pharmacokinetic parameters (N = 12) in the absence (–) and presence (+) of medicinal cannabis

Parameter[1]	Cannabis –	Cannabis +	Ratio[2]
Absolute dose (mg)	600	525 (461 – 589)[3]	NA
Irinotecan			
CL (L/h)	29.3 (23.8 – 34.7)	28.4 (22.7 – 34.0)	0.97 (0.90 – 1.05)
$AUC_{0\text{-}inf}$[4] (ng·h/mL)	22,825 (17,141 –28,509)	23,644 (17,703 –29,932)	1.04 (0.96 – 1.11)
SN-38			
CL (L/h)	400 (330 – 469)	341 (290 – 392)	0.90 (0.74 – 1.05)
$AUC_{0\text{-}100}$[4] (ng·h/mL)	422 (325 – 519)	448 (364 – 532)	1.11 (0.98 – 1.23)
SN-38G			
CL (L/h)	53.7 (36.6 – 70.9)	45.8 (30.4 – 61.2)	0.93 (0.74 – 1.12)
$AUC_{0\text{-}100}$[4] (ng·h/mL)	3,837 (2,217 – 5,457)	4,101 (2,385 – 5,818)	1.10 (0.94 – 1.26)
Relative AUCs			
REC (%)	1.95 (1.48 – 2.41)	2.04 (1.58 – 2.49)	1.07 (0.94 – 1.20)
REG	7.39 (5.30 – 10.93)	6.90 (5.40 – 10.28)	0.98 (0.87 – 1.09)

Abbreviations: Cannabis –, medicinal cannabis absent; Cannabis +, medicinal cannabis present; CL, clearance; AUC, area under the plasma concentration-time curve; $_{0\text{-}inf}$ AUC extrapolated to infinity; $_{0\text{-}100}$, AUC up to 100 hours; NA, not applicable; REC, relative extent of conversion ($AUC_{0\text{-}100}$ SN-38 over $AUC_{0\text{-}100}$ irinotecan); REG, relative extent of glucuronidation ($AUC_{0\text{-}100}$ SN-38G over $AUC_{0\text{-}100}$ SN-38). [1]Values are reported as mean with 95 % confidence intervals in parentheses; [2]Geometric mean ratios of the observed pharmacokinetic parameters with medicinal cannabis and without medicinal cannabis, a significant difference exists when the value 1.00 is not included within the 95 % confidence interval; [3]Four patients received a reduced dose of 450 mg (75 %), and one of 300 mg (50 %); [4]Dose-normalized to 600 mg.

Docetaxel treatment and pharmacokinetics

In the absence of medicinal cannabis, all patients were administered 180 mg docetaxel. In the presence of medicinal cannabis, three patients, enrolled after the interim-analysis, which did not demonstrate a substantial change in docetaxel pharmacokinetics or increased haematological toxicity, also received a reduced dose (135 mg) due to treatment-related haematological toxicity (leukopenia and neutropenia grade 4). Table 3 summarizes the pharmacokinetic parameters for docetaxel with and without concomitant medicinal cannabis administration.

Table 3 Docetaxel pharmacokinetic parameters (N = 12) in the absence (–) and presence (+) of medicinal cannabis

Parameter[1]	Cannabis –	Cannabis +	Ratio[2]
Absolute dose (mg)	180	158 (143 – 172)[3]	NA
CL (L/h)	40.4 (35.4 – 45.5)	37.9 (31.7 – 44.2)	0.95 (0.82 – 1.08)
AUC[4] ($ng \cdot h \cdot mL^{-1} \cdot mg^{-1}$)	25.7 (22.2 – 29.2)	28.3 (22.9 – 33.7)	1.11 (0.94 – 1.28)
C_{max}[4] ($ng/mL \cdot mg^{-1}$)	17.8 (15.7 – 20.0)	19.5 (15.8 – 23.2)	1.10 (0.94 – 1.27)
V_{ss} (L)	304 (250 – 358)	359 (264 – 454)	1.18 (0.94 – 1.43)
$T_{1/2, \gamma}$ (h)	22.0 (17.9 – 26.1)	26.7 (21.3 – 32.2)	1.24 (1.00 – 1.48)

Abbreviations: Cannabis –, medicinal cannabis absent; Cannabis +, medicinal cannabis present; CL, clearance; AUC, area under the plasma concentration-time curve; C_{max}, peak plasma concentration; V_{ss}, apparent volume of distribution; $T_{1/2, \gamma}$, terminal elimination half-life; NA, not applicable. [1]Values are reported as mean with 95 % confidence intervals in parentheses; [2]Geometric mean ratios of the observed pharmacokinetic parameters with medicinal cannabis and without medicinal cannabis, a significant difference exists when the value 1.00 is not included within the 95 % confidence interval; [3]Six patients were administered a reduced dose of 135 mg (75 %); [4]Dose-normalized, i.e. divided by dose.

Upon concurrent medicinal cannabis use docetaxel clearance and dose-adjusted AUC were not significantly affected, as reflected by the geometric mean ratios and the corresponding 95 % CIs for both parameters being 0.95 (0.82 – 1.08) and 1.11 (0.94 – 1.28), respectively. Furthermore, for both parameters, interpatient variability, expressed as coefficient of variation, was only marginally increased in the presence of medicinal cannabis (from 20 % to 26 % and from 21 % to 30 %, respectively), yet within previously reported ranges [52,53]. The mean (N = 12) docetaxel plasma concentration-time curves for both treatments illustrate the similarity between the two treatments (Figure 2).

Cannabis screening

All urine samples tested 'positive' for cannabinoids and/or metabolites. Although this is no definite confirmation of patient adherence, we have no reason to believe that patients did not take their medicinal cannabis as prescribed, which could explain the lack of a pharmacokinetic drug-interaction.

Toxicity

For both drug treatments, haematological toxicity was the predominant side-effect. Upon concurrent medicinal cannabis use, the relative haematological toxicity (expressed as percentage decrease in white blood cell count (WBC) and percentage decrease in absolute neutrophil count (ANC), at nadir compared to baseline) in those patients who received full dose docetaxel (180 mg) during both treatments (N = 6) was not significantly affected,

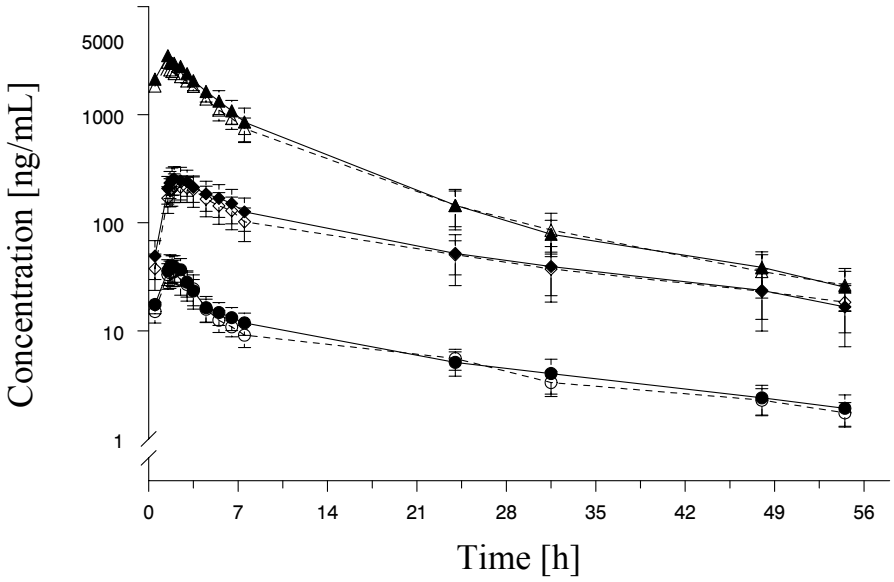

Figure 1. *Mean (95 % confidence interval, N = 12) plasma concentrations of irinotecan (dose 600 mg) in the absence (solid line, closed symbols and error bars) and presence (dose-normalized to 600 mg, dashed line, open symbols and error bars) of medicinal cannabis. Triangles, diamonds and circles represent concentrations of irinotecan, SN-38G, and SN-38, respectively.*

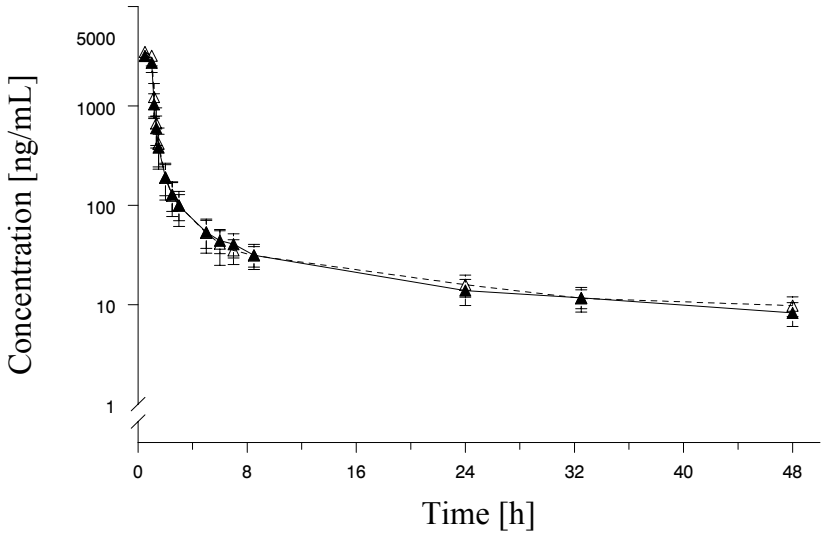

Figure 2. *Mean (95 % confidence interval, N = 12) plasma concentrations of docetaxel (dose 180 mg) in the absence (solid line, closed symbols and error bars) and presence (dose-normalized to 180 mg, dashed line, open symbols and error bars) of medicinal cannabis.*

mean values (95 % confidence interval) for the first and second treatment being 82.6 % (75.2 – 90.1 %) *vs* 80.6 % (73.2 – 88.0 %) and 91.3 % (85.7 – 96.8 %) *vs* 92.0 % (87.4 – 96.5 %), respectively (P = 0.75, Table 4). Patients treated with full-dosed irinotecan (600 mg) during both treatments (N = 7) showed a smaller percentage decrease in WBC, 38.8 % (20.2 – 57.4 %) *vs* 23.5 % (11.1 – 35.8 %) and ANC, 44.4 % (22.0 – 66.7 %) *vs* 25.4 % (10.9 – 40.0 %) during the second treatment ($P < 0.04$, Table 4). However, the nadir values for WBC and ANC were identical for both treatments being 4.8 x 10^9/L (3.1 – 6.4 x 10^9/L) *vs* 4.6 x 10^9/L (3.6 – 6.0 x 10^9/L) and 3.0 x 10^9/L (1.91 – 4.0 x 10^9/L) *vs* 2.9 x 10^9/L (1.91 – 3.9 x 10^9/L), respectively, $P > 0.60$).

For each treatment-arm the incidence and severity of non-haematological toxicities (irinotecan: fatigue, nausea, vomiting and diarrhoea; docetaxel: fatigue, increased hepatic transaminases and bilirubin) appeared similar between the first and second treatment, although the small number of patients and low incidence precluded statistical evaluation. Patients tolerated the medicinal cannabis tea well, the majority of patients indicated to sleep better and only a minority complained of minor headaches, mood disturbances or weird dreams.

Discussion

Our study shows that medicinal cannabis (variety Bedrocan®), ingested as herbal tea during 15 consecutive days, starting 12 days before intravenous administration of irinotecan or docetaxel, two anticancer drugs for which CYP3A is a major route of metabolism, does not influence the systemic pharmacokinetics, and does not negatively affect the haematological toxicity of these drugs. Furthermore, besides being inactivated by CYP3A, irinotecan is subject to carboxylesterase-mediated activation resulting in SN-38. SN-38 is subsequently detoxified in the liver to its glucuronide SN-38G by UDP glucuronosyltransferase 1A isoforms, in particular UGT1A1 [54]. Since both exposure to, and clearance of SN-38 and SN-38G, as well as the metabolic ratios for these two irinotecan metabolites were equal for the first and second treatment, it seems unlikely that the evaluated variety of medicinal cannabis affects these enzyme systems. We have no indications that patients were non-adherent, which could have explained the lack of a drug-interaction.

Several aspects regarding the observed lack of a (statistically) significant and clinically relevant effect of medicinal cannabis on the pharmacokinetics of irinotecan and docetaxel require attention. Firstly, our conclusions apply specifically to the investigated medicinal cannabis variety. In The Netherlands, currently medicinal cannabis is available in two varieties (Bedrocan® and Bedrobinol®), both containing a standardized content of THC (18 % and 13 %, respectively) and CBD (0.8 % and 0.2 %, respectively). At present, there are plans to introduce a third variety with a significantly higher content of CBD, claimed to be beneficial for syndromes associated with spasticity.

Table 4. Summary of irinotecan (N = 7) and docetaxel (N = 6) haematological pharmacodynamics in the absence (–) and presence (+) of medicinal cannabis for patients who received two full-dosed treatments

Parameter[1]	Cannabis – Irinotecan	Cannabis + Irinotecan	*P*-value[2]	Cannabis – Docetaxel	Cannabis + Docetaxel	*P*-value[2]
Leukocytes % decrease WBC	38.8 (20.2 – 57.4)	23.5 (11.1 – 35.8)	0.04	82.6 (75.2 – 90.1)	80.6 (73.2 – 88.0)	0.75
Nadir (x 10^9 /L)	4.8 (3.1 – 6.4)	4.6 (3.2 – 6.0)	0.69	1.14 (0.61 – 1.67)	1.47 (0.95 – 1.98)	0.17
Neutrophils % decrease ANC	44.4 (22.0 – 66.7)	25.4 (10.9 – 40.0)	0.03	91.3 (85.7 – 96.8)	92.0 (87.4 – 96.5)	0.75
Nadir (x 10^9 /L)	3.0 (1.91 – 4.0)	2.9 (1.91 – 3.9)	0.60	0.41 (0.13 – 0. 69)	0.49 (0.15 – 0.84)	0.75

Abbreviations: Cannabis –, medicinal cannabis absent; Cannabis +, medicinal cannabis present; WBC, white blood cell count; ANC, absolute neutrophil count; % decrease WBC and ANC defined as [(pretreatment value - nadir value) / (pretreatment value)]*100; Nadir, absolute lowest point during follow-up. [1]Values are reported as mean with 95 % confidence interval in parentheses. [2]Non-parametric paired analysis for those patients for which the dose for the first and second treatment was identical (i.e. 600 mg irinotecan or 180 mg docetaxel).

To what extent a higher exposure to CBD, recently shown to inhibit the transporter protein ABCB1 (P-glycoprotein) *in vitro* [55] influences the pharmacokinetics of concomitantly prescribed drugs, remains to be investigated. Although it was anticipated that the availability of medicinal cannabis in Dutch pharmacies would decrease the need to resort to 'coffeeshops' more than 80 % of the patients still frequent the illegal circuit [56]. The high price in pharmacies, complaints of decreased effectiveness, and the hesitation of physicians to prescribe medicinal cannabis seem to be the major reasons underlying this finding. Since our conclusions do not apply to 'illegal products', oncologists should recommend patients who wish to use cannabis for medicinal purposes, to resort to prescription-based, legally produced cannabis instead of cannabis of unknown origin and quality.

Secondly, the evaluated dose is the initial recommended dose, which may be increased according to an individual's need. Again, it is possible that a higher cannabinoid exposure might yet result in an undesirable drug-interaction. Thirdly, we have evaluated orally administered medicinal cannabis. An alternative recommended route of administration is inhalation [15]. Due to extensive first-pass metabolism and high lipid solubility only 10 % to 20 % of orally administered THC reaches the systemic circulation unchanged [57]. In contrast, up to 50 % of THC can be absorbed from the lungs, resulting in higher systemic exposure. From our data, we cannot draw justified conclusions regarding the potential effects of inhaled medicinal cannabis on the pharmacokinetics of concomitantly administered irinotecan and docetaxel or other (anticancer) drugs.

The lower percentage decrease in WBC and ANC in patients administered irinotecan concomitant with medicinal cannabis, observed in our exploratory evaluation, is not on forehand attributable to a pharmacodynamic interaction given the fact that nadir values of WBC and ANC were almost identical for both treatments, yet is most likely to be of multi-factorial origin or related to the limited sample size. Indeed, the study was not designed to detect statistically significant differences in pharmacodynamic parameters. Furthermore, the observed differences do not translate in different grades of neutropenia [42].

Despite the low prescription rate of legal medicinal cannabis, there remains a need for clinical trials to evaluate the efficacy and safety of medicinal cannabis for specific indications and in combination with other drugs with a narrow therapeutic index, as well as research into adequate dosage forms. If, in the mean time, cancer patients wish to use medicinal cannabis (variety Bedrocan®, orally administered as recommended) concomitantly with irinotecan or docetaxel or other drugs primarily detoxified by CYP3A, we do not recommend any dose adjustments *a priori*.

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Chapter 10

Summary, Conclusions and Future Perspectives

Summary, Conclusions and Future Perspectives

Summary and Conclusions

Since clinical evaluations started in the early nineties, the anticancer drug docetaxel has obtained a prominent place in the treatment of various human malignancies. Marketing approval currently includes the treatment of patients with breast cancer, non-small-cell lung cancer, androgen-independent prostate cancer (AIPC) and patients with advanced gastric cancer. Following initial introduction much research has focused on docetaxel's clinical pharmacological properties in an attempt to improve the risk-benefit ratio for docetaxel treatment. In particular, reducing the substantial degree of interindividual variability in docetaxel pharmacokinetics, which is associated with the observed variability in toxicity, is an aspect, which has received much attention.

Chapter 2 provides an overview of research, which has focused on various pharmacological approaches, including reducing interindividual pharmacokinetic and pharmacodynamic variability, optimizing schedule and route of administration, reversing drug resistance and the development of structurally related second-generation taxanes, in an attempt to overcome current limitations associated with docetaxel treatment and to improve treatment outcome.

Chapter 3 compares the efficacy and toxicity of the currently recommended treatment schedule based on intravenous docetaxel infusion once every 3 weeks (3-weekly) to the regimen involving weekly docetaxel administrations. Efficacy appears similar for the two schedules, while weekly docetaxel is significantly less myelotoxic. However, this benefit comes at the cost of more hyperlacrimation, cumulative skin- and nail toxicity and negatively affects quality of life. Currently, 3-weekly docetaxel remains the standard schedule whereas the weekly schedule offers a possibility of treatment individualization for those patients where the risk of severe myelosuppression is considered unacceptable.

Chapter 4 documents research focused on the development of less toxic, solvent-free formulations for the delivery of docetaxel, while continuing to maximize the drug's antitumour efficacy through preferential uptake of the drug at the tumour. This pharmaceutical strategy has lead to several alternative, solvent-free drug formulations including fibrinogen-coated olive oil droplets loaded with docetaxel, PEGylated docetaxel liposomes, docetaxel immunoliposomes, and docetaxel-encapsulated nanoparticle-aptamer bioconjugates, which are all currently in different preclinical stages of development, and with different advantages and disadvantages. Whether the observed advantages (improved pharmacokinetics, selective tumour uptake, increased survival) over the current docetaxel

formulation translate to clinical benefits remains to be seen. Clinical evaluations with a submicronic polysorbate-80 free dispersion formulation of docetaxel are limited to two phase I studies. However, based on toxicity considerations the development of this formulation was discontinued.

In **Chapter 5** the effect of the potent cytochrome P450 (CYP) 3A inhibitor ketoconazole on the pharmacokinetics of docetaxel was investigated. This drug combination is currently being evaluated for the treatment of patients with AIPC. Concomitant administration of a drug that inhibits the activity of CYP3A, responsible for the metabolism of docetaxel, has the potential to result in undesirable clinical consequences. Indeed, a 50 % decrease in docetaxel clearance has been shown to increase the odds of developing grade 4 neutropenia and febrile neutropenia 4.3-fold and 3-fold, respectively. Concomitant ketoconazole reduced docetaxel clearance by 49 % (P = 0.018, N = 7). Mean (± SD) clearance values were 35.0 L/h (± 11.8 L/h) and 18.2 L/h (± 9.68 L/h) in the absence and presence of ketoconazole, respectively. The range for the degree to which docetaxel clearance was reduced was 32 – 74 % indicating substantial interindividual variability. Furthermore, the extent to which docetaxel clearance was reduced was weakly related to systemic ketoconazole exposure. Overall, this report shows that substantial dose reductions are required when docetaxel is administered with potent CYP3A inhibitors.

Variable response (efficacy and toxicity) to docetaxel-based treatment is presumed to be largely related to interindividual variation in docetaxel pharmacokinetics, notably in the pharmacokinetic parameters systemic drug exposure and clearance. Moreover, phenotypic expression of CYP3A activity has been identified as a strong predictor of docetaxel clearance and observed variation in (intrinsic) CYP3A activity has been suggested to account to a large extent for the significant interindividual variability in docetaxel pharmacokinetics. The aim of the study presented in **Chapter 6** was to reduce the interindividual variability in docetaxel systemic exposure and clearance in an attempt to improve the risk-benefit ratio for docetaxel therapy. It was hypothesized that by temporarily eliminating the cause of variability, i.e. variable CYP3A catalytic activity, using the potent CYP3A inhibitor ketoconazole, interindividual variability in docetaxel systemic exposure and clearance could be reduced. The results reported in **Chapter 5** showed that there was wide variation in the extent to which docetaxel clearance was reduced, and that the degree to which clearance was decreased, was related to systemic ketoconazole exposure. Furthermore, interindividual variability in clearance (expressed as coefficient of variation) was increased upon concomitant ketoconazole administration compared to single agent treatment (34 % *vs* 53 %). It was hypothesized that an increase in ketoconazole dose (compared to **Chapter 5**) would result in sufficiently high ketoconazole exposure in each patient and thus in maximum CYP3A-inhibition overall, thereby reducing the variability in the extent to which docetaxel clearance

is reduced. Docetaxel clearance (mean ± SD) was reduced by 50 % from 32.8 L/h ± 13.7 L/h to 16.5 L/h ± 8.15 L/h (P = 0.018, N = 7) upon ketoconazole coadministration, albeit with large interindividual variability (range, 34 – 69 % reduction). In the presence of ketoconazole, interindividual variability in docetaxel clearance and systemic exposure, expressed as coefficient of variation, was increased from 41.6 % to 49.5 % and from 28.0 % to 35.1 %, respectively, and not, as hypothesized, reduced. In addition, it was assessed if part of the resulting interindividual variability in decrease of docetaxel clearance after ketoconazole treatment could be dependent on functional CYP3A4 and/or CYP3A5 genetic variants. None of the patients carried the CYP3A5*1 allele indicating that for each evaluated patient total CYP3A activity is solely attributable to CYP3A4. No patient carried any of the currently known, potentially clinically relevant CYP3A4 single nucleotide polymorphisms (SNPs). Thus, it is highly unlikely that genetic differences contributed to the observed interindividual variability in the decrease of docetaxel clearance. It was concluded that the evaluated approach is unsuitable as method to achieve a uniform docetaxel pharmacokinetic profile.

In **Chapter 7** the development and analytical method validation of a rapid and simple combustion method to quantify [^{3}H]-docetaxel excreted in human faeces and urine is described. An important reason to administer a non-toxic tracer amount of radiolabelled drug to a patient is to quantify the drug in biological samples (e.g. faeces, urine, ascites) at concentrations below the lower limit of quantification for routinely used analytical techniques, often with the purpose of assessing a mass balance. Radiochemicals used in clinical research include [^{3}H]- or [^{14}C]-radiolabelled compounds and liquid scintillation counting is the most efficient method to quantify these low energy beta-emitters. However, most radiolabelled biological samples require extensive sample preparation to reduce quenching interference before quantification of radioactivity is possible. Combustion of tritiated faeces and urine samples is a simple, rapid, sensitive, precise and reproducible method with high recovery. It can be applied to quantify [^{3}H]-docetaxel excretion after intravenous administration.

In **Chapter 8** the influence of ketoconazole on the faecal and urinary disposition of docetaxel parent drug and [^{3}H]-docetaxel was explored. Besides being a potent CYP3A inhibitor, ketoconazole is also a weak to modest inhibitor of the transmembrane ATP-binding cassette efflux transporter protein ABCB1, for which docetaxel is also a substrate. The influence of ABCB1 on the plasma pharmacokinetics of docetaxel is minimal to absent however, ABCB1 plays a prominent role in the faecal elimination of docetaxel. Concomitant ketoconazole increased faecal parent drug excretion (mean ± SD of the administered absolute docetaxel dose) 2-fold from 2.6 % ± 2.8 % to 5.2 % ± 5.4 % (P = 0.03, N = 14) but did not

affect urinary parent drug excretion, which was 2.6 % ± 1.4 % and 2.6 % ± 1.1 % in the absence and presence of ketoconazole, respectively (P = 0.69, N = 15). The sum of faecal and urinary parent drug excretion was 5.3 ± 3.0% for docetaxel alone and 7.8 % ± 5.6 % in the presence of ketoconazole (P = 0.04, N = 14). Total recovered radioactivity values in the absence and presence of ketoconazole were 45.8 % ± 19.1 % and 32.4 % ± 19.7 % of the administered [^{3}H]-docetaxel dose, respectively (P = 0.23, N = 5) which is as expected given the 72 hour interval post-infusion during which faecal and urinary samples were collected. It was hypothesized that a more pronounced increase in faecal parent drug excretion upon concomitant ketoconazole administration was not achieved most likely due to concomitant intestinal ABCB1-inhibition.

The fact that docetaxel is primarily metabolized by CYP3A makes the agent subject to a host of enzyme-mediated drug-interactions. In September 2003 a standardized medicinal cannabis product (variety Bedrocan®) was introduced in The Netherlands. Beneficial effects attributed to medicinal cannabis have been reported for cancer-associated anorexia, (delayed) chemotherapy-induced nausea and vomiting, and palliation. As *in vitro*- and *in vivo* data regarding the potential of cannabinoids to modulate CYP3A activity are contradictory, a drug-interaction study was conducted in cancer patients (N = 24) to evaluate the effect of concomitant medicinal cannabis on the pharmacokinetics of irinotecan and docetaxel, both dependent on CYP3A-mediated metabolism (**Chapter 9**). Coadministration of medicinal cannabis, as herbal tea, did not significantly influence the plasma pharmacokinetics of docetaxel or irinotecan (P > 0.31). Docetaxel systemic exposure and clearance, reported as the mean ratio in the presence and absence of medicinal cannabis (95 % confidence interval) was 1.11 (0.94 – 1.28) and 0.95 (0.82 – 1.08), respectively indicating the lack of a clinically relevant drug-interaction. The evaluated variety of medicinal cannabis can be administered concomitantly with both anticancer agents without dose adjustments.

Future Perspectives

The work presented in this thesis aimed to optimize docetaxel-based treatment through a reduction of the interpatient pharmacokinetic variability and to further elucidate the role of CYP3A- and ABCB1-mediated elimination pathways for docetaxel. Both aims were based on purposeful modulation of the drug's pharmacokinetic profile using ketoconazole, a potent CYP3A inhibitor and, to a lesser degree, also inhibitor of ABCB1. Reducing interindividual variability in docetaxel pharmacokinetics by temporarily inhibiting the source of the observed variability (i.e. variable CYP3A catalytic activity) has however not proved feasible. Indeed, upon concomitant ketoconazole administration, a substantial degree of interpatient pharmacokinetic variation was still present, and could not be accounted for by

variable systemic exposure to ketoconazole or genetic diversity in the genes encoding the CYP3A4 and CYP3A5 proteins.

These disappointing findings led us to initiate several currently ongoing studies, which evaluate alternative approaches to optimize docetaxel treatment. Monitoring plasma levels and pharmacokinetic-guided individualized dose adjustments is most often referred to as 'therapeutic drug monitoring' (TDM). Although widely practised in other areas of medicine routine TDM is limited in oncology. Application of limited sampling strategies (typically requiring 2 to 4 samples to be collected on an outpatient basis) in combination with a population pharmacokinetic model and Bayesian analysis allows individual pharmacokinetic parameters to be accurately estimated, and makes TDM a potential approach for anticancer drugs. Indeed, the performance and feasibility of pharmacokinetic-guided dosing of paclitaxel was recently successfully evaluated. In our centre we are currently in the final stages of conducting a study (N = 30) which applies a limited sampling strategy and a previously developed population pharmacokinetic model, incorporating albumin, alpha-1 acid-glycoprotein, age, body surface area and elevated hepatic function, to evaluate the feasibility and performance of pharmacokinetic-guided individualized docetaxel dosing. In addition, the same limited sampling strategy and population pharmacokinetic model are being applied to evaluate the relationship between docetaxel pharmacokinetics and potentially clinically relevant CYP3A4, CYP3A5 and ABCB1 single nucleotide polymorphisms (SNPs) in a large population study (current inclusion N > 100). A similar population study is also being conducted to evaluate the relationship between paclitaxel pharmacokinetics and relevant polymorphisms in the drug's metabolizing enzymes (current inclusion N > 100). At present pharmacokinetic-pharmacogenetic studies aimed at determining the clinical relevance of different CYP3A4, CYP3A5 and ABCB1 variant alleles in relation to docetaxel pharmacokinetic variability or the pharmacokinetic variability of other CYP3A or ABCB1 substrates and/or probes, have yielded controversial results, in part due to underpowered studies given the allele frequency of the potentially clinically relevant SNPs.

In clinical oncology it is generally accepted that the efficacy of anticancer drugs depends on the maintenance of adequate unbound (i.e. pharmacologically active) drug concentrations at the target tissue, the tumour site. However, very little is known about intratumoural pharmacokinetics due to the fact that up until now unbound drug concentrations could rarely be (easily) measured at the site of action. For this reason in clinical pharmacokinetics one typically measures total parent drug concentrations (and/or metabolite concentrations) in a more accessible, surrogate site of action, plasma (blood). Total plasma drug concentrations are then correlated to clinical parameters such as drug efficacy and/or toxicity. Yet, the pharmacokinetic behaviour of drugs in tumour tissue may be completely different from the kinetics in plasma due to tissue- or tumour specific metabolizing enzymes. Thus, interpatient variability in drug response may be explained for by (wide) interpatient pharmacokinetic variability at the target site. Microdialysis is a

relatively novel and minimally invasive sampling technique based on the diffusion of (endo- and exogenous) analytes from the interstitial compartment through a semi-permeable membrane. We are currently evaluating the feasibility of microdialysis sampling to gain insight in the tissue- and tumour pharmacokinetics of docetaxel, and in docetaxel-induced intratumoural pharmacodynamic changes in cancer patients intravenously administered docetaxel once every 3 weeks.

Samenvatting, Conclusies en Toekomstige Ontwikkelingen

Samenvatting, Conclusies en Toekomstige Ontwikkelingen

Samenvatting en Conclusies

Sinds klinische evaluaties begin jaren 90 begonnen, heeft het antikanker middel docetaxel een prominente plaats veroverd bij de behandeling van diverse vormen van kanker. Op dit moment is docetaxel geregistreerd voor behandeling van patiënten met borstkanker, niet-kleincellig longkanker, hormoon-ongevoelig prostaatkanker en maagkanker. In aansluiting op de initiële introductie, heeft veel klinisch onderzoek zich gericht op de klinisch farmacologische eigenschappen van docetaxel met als doel de balans tussen effectiviteit en toxiciteit te verbeteren. In het bijzonder vormt het reduceren van de aanzienlijke interindividuele variatie in docetaxel farmacokinetiek, welke gerelateerd is aan de interpatient variabiliteit in bijwerkingen, een belangrijk aandachtspunt.

Hoofdstuk 2 levert een overzicht van het onderzoek dat zich gericht heeft op verschillende farmacologische invalshoeken, waaronder het reduceren van de interindividuele variatie in farmacokinetiek en farmacodynamiek, optimalisatie van toedieningsschema en route, terugdringen van geneesmiddelresistentie en de ontwikkeling van structuur gerelateerde tweede-generatie taxanen, met als doel de huidige beperkingen gerelateerd aan de behandeling met docetaxel te overwinnen en de therapie-uitkomst te verbeteren.

Hoofdstuk 3 vergelijkt de effectiviteit en de toxiciteit van het aanbevolen toedieningsschema gebaseerd op intraveneuze infusie van docetaxel eens per 3 weken (3-wekelijks) met het schema van wekelijkse docetaxel infusies. Effectiviteit lijkt vergelijkbaar te zijn voor de twee schema's, terwijl wekelijks docetaxel aanzienlijk minder myelotoxisch is. Echter, dit voordeel gaat ten koste van een toename in overmatige traanafscheiding, cumulatieve huid- en nagel toxiciteit en heeft een negatief effect op de kwaliteit van leven. Op dit moment blijft 3-wekelijks docetaxel derhalve het standaard toedieningsschema, terwijl het wekelijks schema een mogelijkheid biedt voor individualisatie van de behandeling bij die patiënten waarbij de kans op ernstige beenmergsuppressie onacceptabel wordt geacht.

Hoofdstuk 4 levert een overzicht van het onderzoek dat gericht is op het ontwikkelen van minder toxische, oplosmiddel-vrije formuleringen voor de toediening van docetaxel, waarbij tegelijkertijd getracht wordt de antitumor effectiviteit te verbeteren door specifieke opname van docetaxel ter plekke van de tumor. Deze farmaceutische aanpak heeft geleid tot verschillende alternatieve, oplosmiddel-vrije formuleringen van docetaxel waaronder, olijfolie druppels geladen met docetaxel en gecoat met fibrinogeen, gePEGyleerde docetaxel liposomen, docetaxel immunoliposomen, en aptameer bioconjugaat nanopartikels geladen met docetaxel, allen op dit moment in verschillende stadia van preklinische ontwikkeling en

met verschillende voor- en nadelen. Of de waargenomen voordelen (verbeterd farmacokinetisch profiel, selectieve tumor opname, toegenomen overleving) ten opzichte van de huidige docetaxel formulering zich ook vertalen in klinische voordelen moet nog worden afgewacht. Klinische evaluaties met een polysorbate-80 vrije formulering van docetaxel zijn beperkt tot twee fase I studies. Echter, de ontwikkeling van deze formulering is op basis van toxiciteitsoverwegingen gestaakt.

In **Hoofdstuk 5** is het effect van de sterk werkzame cytochroom P450 (CYP) 3A remmer ketoconazol op de farmacokinetiek van docetaxel onderzocht. Deze geneesmiddelcombinatie wordt thans onderzocht voor de behandeling van hormoon-ongevoelig prostaatkanker. Gelijktijdige toediening van een (genees)middel dat de activiteit van CYP3A, verantwoordelijk voor het metabolisme van docetaxel, remt, kan leiden tot ongewenste klinische consequenties. Zo is aangetoond dat een 50 % afname in docetaxel klaring de kans op het ontwikkelen van graad 4 neutropenie of neutropene koorts met een factor 4.3 respectievelijk 3 doet toenemen. Bij gelijktijdige toediening van ketoconazol werd de klaring van docetaxel met 49 % gereduceerd (P = 0.018, N = 7). De gemiddelde (± SD) docetaxel klaring was 35.0 L/h (± 11.8 L/h) en 18.2 L/h (± 9.68 L/h) in de af- respectievelijk aanwezigheid van ketoconazol. De spreiding in de mate waarin de klaring van docetaxel werd gereduceerd liep van 32 – 74 %, hetgeen wijst op aanzienlijke interindividuele variabiliteit. Bovendien was de mate waarin de klaring van docetaxel afnam gerelateerd aan de systemische blootstelling aan ketoconazol. Dit onderzoek laat zien dat een aanzienlijke dosis reductie vereist is als docetaxel gelijktijdig wordt toegediend met sterk werkzame CYP3A remmers.

Er wordt verondersteld dat de variabele respons (effectiviteit en toxiciteit) op behandeling met docetaxel grotendeels gerelateerd is aan de interindividuele variatie in docetaxel farmacokinetiek, met name in de farmacokinetische parameters blootstelling en klaring. Bovendien is de fenotypische activiteit van CYP3A geïdentificeerd als een sterke voorspeller van docetaxel klaring en heeft men gesuggereerd dat de geobserveerde (intrinsieke) variatie in CYP3A activiteit voor een groot deel de aanzienlijke interindividuele variatie in docetaxel farmacokinetiek kan verklaren. Het doel van het onderzoek gepresenteerd in **Hoofdstuk 6** was het reduceren van de interindividuele variabiliteit in docetaxel blootstelling en klaring in een poging de balans tussen effectiviteit en toxiciteit voor de behandeling te verbeteren. Als werk hypothese gold dat door tijdelijk de oorzaak van de variabiliteit, d.w.z. variabele CYP3A activiteit, te onderdrukken, middels de sterk werkzame CYP3A remmer ketoconazol, de interindividuele variabiliteit in docetaxel blootstelling en klaring zou kunnen worden gereduceerd. De resultaten gepresenteerd in **Hoofdstuk 5** lieten zien dat er een aanzienlijke variatie was in de mate waarin docetaxel klaring werd gereduceerd en dat deze mate van reductie gerelateerd was aan de systemische

ketoconazol blootstelling. Bovendien was de interindividuele variabiliteit in klaring (uitgedrukt als variatie coëfficiënt) toegenomen als gevolg van ketoconazol toediening vergeleken met docetaxel monotherapie (34 % *vs* 53 %). Verondersteld werd dat een toename in ketoconazol dosis (vergeleken met **Hoofdstuk 5**) zou leiden tot voldoende hoge blootstelling aan ketoconazol bij elke patiënt en dus in maximale CYP3A-remming, met als gevolg een afname van de variabiliteit in de mate waarin de klaring van docetaxel wordt gereduceerd. De klaring van docetaxel (gemiddelde ± SD) werd met 50 % gereduceerd van 32.8 L/h ± 13.7 L/h naar 16.5 L/h ± 8.15 L/h (P = 0.018, N = 7) als gevolg van gelijktijdige ketoconazol toediening, echter met grote interindividuele variabiliteit (uitersten, 34 – 69 % afname). In aanwezigheid van ketoconazol, nam de interindividuele variabiliteit in docetaxel klaring en systemische blootstelling (uitgedrukt als variatie coëfficiënt) zelfs toe van 41.6 % naar 49.5 % en van 28.0 % naar 35.1 %, respectievelijk, en niet, zoals verondersteld, af. Aansluitend is geëvalueerd of een deel van de interindividuele variabiliteit in de afname van docetaxel klaring onder ketoconazol behandeling afhankelijk was van functionele CYP3A4 en/of CYP3A5 genetische varianten. Geen van de patiënten was drager van het CYP3A5*1 allel, hetgeen aangeeft dat voor ieder geëvalueerde patiënt totale CYP3A activiteit uitsluitend toegeschreven kan worden aan CYP3A4. Geen enkele patiënt was drager van één van de, op dit moment bekende, potentieel klinisch relevante polymorfismen in het CYP3A4 gen. Het is derhalve zeer onwaarschijnlijk dat genetische verschillen bijgedragen hebben aan de waargenomen interindividuele variabiliteit in de afname van docetaxel klaring. Geconcludeerd is dat de geëvalueerde aanpak ongeschikt is als methode om een uniform farmacokinetisch profiel voor docetaxel te bereiken.

In **Hoofdstuk 7** wordt de ontwikkeling en analytische validatie van een snelle en eenvoudige verbrandingsmethode om [^{3}H]-docetaxel, uitgescheiden in humaan feces en urine te kwantificeren, beschreven. Een belangrijke reden om een minimale en onschadelijke hoeveelheid van een radioactief-gelabelde verbinding aan een patiënt toe te dienen is om deze verbinding in een biologische matrix (bijv. feces, urine, ascites) te kwantificeren bij concentraties die onder de detectie limiet van de gebruikelijke analyse technieken liggen, veelal met als doel het bepalen van een massabalans. In klinisch onderzoek worden onder meer [^{3}H]- of [^{14}C]-radioactief-gelabelde verbindingen gebruikt; hierbij is vloeistof scintillatie telling de meest efficiënte methode om deze laag energetische beta-stralers te kwantificeren. Echter, de meeste radioactief-gelabelde biologische matrices vereisen een uitgebreide en bewerkelijke monstervoorbewerking procedure om verstoring door 'quenching' (uitdoving) te reduceren, alvorens tot kwantificering van de radioactiviteit kan worden overgegaan. Verbranding van getritieerde feces en urine monsters is een eenvoudige, snelle, gevoelige, precieze en reproduceerbare methode met hoge opbrengst. Deze methode kan worden gebruikt om de uitscheiding van [^{3}H]-docetaxel, na intraveneuze toediening, te kwantificeren.

In **Hoofdstuk 8** is de invloed van ketoconazol op de uitscheiding van docetaxel en [^{3}H]-docetaxel in feces en urine onderzocht. Ketoconazol is naast een sterk werkzame CYP3A remmer, ook een zwakke tot matige remmer van de transmembraan gelokaliseerde 'ATP-binding cassette' transporter-eiwit ABCB1, waar docetaxel ook substraat van is. De invloed van ABCB1 op de plasma farmacokinetiek van docetaxel is minimaal tot afwezig echter ABCB1 speelt een prominente rol in de fecale uitscheiding van docetaxel. Onder invloed van ketoconazol nam de fecale uitscheiding van docetaxel (gemiddelde ± SD van de toegediende absolute docetaxel dosis) met een factor 2 toe van 2.6 % ± 2.8 % tot 5.2 ± 5.4 % (P = 0.03, N = 14) maar had geen effect op de urine excretie welke 2.6 % ± 1.4 % en 2.6 % ± 1.1 % bedroeg in de af- respectievelijk aanwezigheid van ketoconazol (P = 0.69, N = 15). De som van de uitscheiding in feces en urine bedroeg 5.3 % ± 3.0 % voor docetaxel monotherapie en 7.8 % ± 5.6 % in aanwezigheid van ketoconazol (P = 0.04, N = 14). De totale hoeveelheid gemeten radioactiviteit in af- respectievelijk aanwezigheid van ketoconazol bedroeg 45.8 % ± 19.1 % en 32.4 % ± 19.7 % van de toegediende dosis [^{3}H]-docetaxel (P = 0.23, N = 5) hetgeen overeenstemt met de verwachting gezien het 72-uurs interval na-infusie gedurende welk feces en urine monsters zijn verzameld. Verondersteld wordt dat een meer uitgesproken toename in de fecale excretie van docetaxel onder invloed van ketoconazol niet heeft plaatsgevonden als gevolg van gelijktijdige remming van intestinaal ABCB1.

Het feit dat docetaxel grotendeels door CYP3A wordt gemetaboliseerd stelt deze verbinding bloot aan een scala van enzym-gemedieerde geneesmiddel interacties. In September 2003 is in Nederland een gestandaardiseerd medicinaal cannabis product (variëteit Bedrocan®) geïntroduceerd. Gunstige effecten toegeschreven aan medicinaal cannabis zijn gemeld voor kanker-geïnduceerde anorexie, (vertraagd)-chemotherapie geïnduceerde misselijkheid en braken en voor palliatie. Daar *in vitro*- en *in vivo* resultaten betreffende de mogelijkheid dat cannabinoïden de activiteit van CYP3A kunnen beïnvloeden tegenstrijdig zijn, is een geneesmiddelinteractie studie uitgevoerd bij kanker patiënten (N = 24) om het effect van gelijktijdige toediening van medicinaal cannabis op de farmacokinetiek van docetaxel en irinotecan, beiden afhankelijk van CYP3A-gemedieerd metabolisme, te onderzoeken (**Hoofdstuk 9**). Gelijktijdige toediening van medicinaal cannabis, als kruidenthee, had geen invloed op de plasma farmacokinetiek van docetaxel of irinotecan (P > 0.31). Docetaxel systemische blootstelling en klaring, gerapporteerd als de gemiddelde ratio in de aan- en afwezigheid van medicinaal cannabis (95 % betrouwbaarheidsinterval) bedroeg 1.11 (0.94 – 1.28) en 0.95 (0.82 – 1.08), respectievelijk, hetgeen niet wijst op een klinisch relevante geneesmiddelinteractie. De geëvalueerde variëteit van medicinaal cannabis kan zonder dosis aanpassing worden toegediend in combinatie met beide antikanker middelen.

Toekomstige ontwikkelingen

Het beschreven werk in dit proefschrift had tot doel de behandeling met docetaxel te verbeteren door een reductie in de interpatient farmacokinetische variabiliteit en meer inzicht te verkrijgen in de rol van CYP3A- en ABCB1-gemedieerde eliminatie routes voor docetaxel. Beide doelen waren gestoeld op bewuste beïnvloeding van het farmacokinetisch profiel van docetaxel door gebruik te maken van ketoconazol, een sterk werkzame remmer van CYP3A en, in mindere mate, ook een remmer van ABCB1. Echter, reductie van de interindividuele variabiliteit in docetaxel farmacokinetiek door tijdelijk de bron van de geobserveerde variabiliteit (d.w.z. variabele CYP3A katalytische activiteit) te remmen, is niet haalbaar gebleken. Bij gelijktijdige toediening van ketoconazol was er nog steeds sprake van aanzienlijke interpatient farmacokinetische variatie welke niet kon worden verklaard door variabele systemische blootstelling aan ketoconazol of door genetische diversiteit in de genen die de CYP3A4 en CYP3A5 eiwitten coderen.

Als reactie op deze tegenvallende resultaten hebben wij een aantal thans lopende nieuwe studies opgezet, die alternatieve uitgangspunten onderzoeken om de behandeling met docetaxel te optimaliseren. Het volgen van plasma concentraties in de tijd en farmacokinetisch-gestuurde individuele dosis aanpassingen wordt meestal gedefinieerd als ‘therapeutic drug monitoring’ (TDM). Hoewel TDM in andere medische deelgebieden regelmatig wordt toegepast is het gebruik van TDM in de klinische oncologie beperkt. Toepassing van een ‘limited sampling’ aanpak (waarbij men slechts 2 tot 4 bloedmonsters, die poliklinisch kunnen worden afgenomen, nodig heeft) in combinatie met een populatie farmacokinetisch model en Bayesiaanse analyse maakt het mogelijk om individuele farmacokinetische parameters nauwkeurig te schatten en maakt TDM derhalve een potentiële aanpak voor antikanker geneesmiddelen. Reeds eerder is de uitvoerbaarheid en toepasbaarheid van farmacokinetisch gestuurd doseren van paclitaxel succesvol geëvalueerd. In onze kliniek loopt thans een onderzoek (N = 30) ten einde waarbij de ‘limited sampling’ aanpak en een reeds eerder ontwikkeld populatie farmacokinetisch model, welke albumine, alfa-1 zure glycoproteïne, leeftijd, lichaamsoppervlak en verhoogde leverfuncties incorporeert, wordt toegepast om de uitvoerbaarheid en toepasbaarheid van farmacokinetisch-gestuurd geïndividualiseerd docetaxel doseren te evalueren. Daarnaast wordt dezelfde ‘limited sampling’ aanpak en het populatie farmacokinetisch model toegepast om de relatie tussen docetaxel farmacokinetiek en potentieel klinisch relevante CYP3A4, CYP3A5 en ABCB1 polymorfismen in een groot populatie onderzoek te evalueren (huidige inclusie N > 100). Eveneens wordt een vergelijkbare populatie studie uitgevoerd om de relatie tussen paclitaxel farmacokinetiek en relevante polymorphismen in de metaboliserende enzymen te evalueren (huidige inclusie N > 100). Tot op heden hebben farmacokinetisch-farmacogenetisch georiënteerde studies, gericht op het vaststellen van de klinische relevantie van de verschillende CYP3A4, CYP3A5 en ABCB1 variant allelen in relatie tot docetaxel farmacokinetische variabiliteit, of de farmacokinetische variabiliteit van andere CYP3A en

ABCB1 substraten en/of ‘probes’, wisselende resultaten opgeleverd, deels als gevolg van te klein opgezette studies gezien de allel frequentie van de potentieel klinisch relevante polymorfismen.

In de klinische oncologie is het over het algemeen geaccepteerd dat de effectiviteit van antikanker middelen afhankelijk is van het onderhouden van adequate, vrije, niet eiwit-gebonden (d.w.z. farmacologisch actieve) geneesmiddelconcentraties bij het doelorgaan, de tumor. Echter, er is zeer weinig bekend over de intratumorale farmacokinetiek als gevolg van het feit dat tot op heden vrije geneesmiddel concentraties zelden (eenvoudig) bij de plaats van werking gemeten konden worden. Om deze reden is het gebruikelijk om bij klinisch farmacokinetische evaluaties de totale geneesmiddelconcentratie (en/of metabolietconcentraties) te meten in een beter toegankelijk, surrogaat doelorgaan, namelijk plasma (bloed). Totale plasma geneesmiddelconcentraties worden vervolgens gecorreleerd aan klinische parameters zoals effectiviteit en/of toxiciteit. Echter, het farmacokinetisch gedrag van geneesmiddelen in tumorweefsel is wellicht volledig anders dan het kinetisch gedrag in plasma als gevolg van weefsel- of tumor-specifieke metaboliserende enzymen. De interpatient variabiliteit in geneesmiddelrespons zou derhalve mogelijk verklaard kunnen worden door (grote) interpatient farmacokinetische variabiliteit bij het doelorgaan. Microdialyse is een relatief nieuwe en minimaal invasieve monster afnametechniek gebaseerd op diffusie van (endo- en exogene) verbindingen vanuit de extracellulaire ruimte door een semi-permeabel membraan. Wij evalueren thans bij kankerpatiënten die behandeld worden met 3-wekelijks intraveneus docetaxel de toepasbaarheid van microdialyse om inzicht te krijgen in de weefsel- en tumor farmacokinetiek van docetaxel en in docetaxel-geïnduceerde intratumorale farmacodynamische veranderingen.

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Curriculum Vitae

Frederike Engels werd op 1 mei 1970 geboren te Leiden. Na een Engelstalige basisopleiding behaalde zij in 1988 het VWO-gymnasiumdiploma aan het Rijnlands Lyceum te Wassenaar. Van september 1988 tot mei 1989 studeerde zij Frans aan de Ecole de Langue et de Civilisation Françaises van de Universiteit van Genève, en behaalde daar het Certificat d'Etudes Pratiques de Français. In 1989 is zij gestart met de studie Farmacie aan de Universiteit van Utrecht. In het vijfde jaar van de studie vervulde zij de functie van vice-quaestrix in het Bestuur van de Utrechtsche Vrouwelijke Studenten Vereeniging / Nieuwe Vereniging van Vrouwelijke Studenten te Utrecht. Ter afsluiting van de doctoraal fase van de studie heeft zij gedurende zes maanden onderzoek gedaan naar hormonale invloeden op de expressie van Bcl-2, bij het Laboratoire d'Oncologie Moléculaire van de Universiteit van Parijs, waarna in september 1995 het doctoraal examen werd gehaald. De post-doctorale fase van de studie heeft zij vervolgens begin 1998 afgerond met het behalen van het apothekersdiploma. Aan het eind van de studie liep zij stage bij de Apotheek van het Erasmus MC. Na het behalen het apothekersdiploma is zij daar blijven werken en in juli 1999 aldaar begonnen met de opleiding tot ziekenhuisapotheker (opleider Prof.dr. A.G. Vulto). In juni 2003 werd de opleiding voltooid. In de Apotheek van het Erasmus MC was zij vervolgens werkzaam als ziekenhuisapotheker met als aandachtsgebied de ondersteuning van klinische trials en had zij vanuit die hoedanigheid zitting in de Medisch Ethische Toetsings Commissie (METC) van het Erasmus MC (van 1 februari 2003 tot 1 juli 2004). In februari 2003 is zij gestart met de werkzaamheden voor dit proefschrift onder leiding van Prof.dr. J. Verweij in het Erasmus MC - locatie Daniel den Hoed kliniek. Medio 2004 kwam zij volledig in dienst van de afdeling Interne Oncologie, Erasmus MC - locatie Daniel den Hoed kliniek (Prof.dr. G. Stoter) om uitsluitend wetenschappelijk onderzoek te verrichten.

Zij woont samen met Gneomar van Nispen en zij zijn in blijde verwachting van hun eerste kindje, dat zich medio januari aankondigt.

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