New Treatment Modalities and Pharmacologic Refinements for Metastatic Breast Cancer

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New Treatment Modalities and Pharmacologic Refinements for Metastatic Breast Cancer

Nieuwe cytotoxische behandelingen en farmacokinetiek van paclitaxel bij het gemetastaseerd mammacarcinoom

PROEFSCHRIFT

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de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
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Hoe zal ik dit uitleggen, dit waarom
wat wij vinden niet is
wat wij zoeken?

Laten we de tijd laten gaan
waarheen hij wil,

en zie dan hoe weiden hun vee vinden,
wouden hun wild, luchten hun vogels,
uitzichten onze ogen

en ach, hoe eenvoud zijn raadsel vindt.

Uit “enkele andere overwegingen”
Rutger Kopland

In herinnering aan René Mathijs

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CHAPTER 1

Introduction to the Thesis

INTRODUCTION TO THE THESIS

Breast cancer is the most frequently diagnosed malignancy in women in western countries. In the Netherlands, breast cancer is found in 10,000 women every year, and presents the most common cancer-related cause of death in women aged 55 years or older [1]. As its incidence rises with advancing age, in the near future the number of breast cancer patients is expected to increase substantially due to the aging population. Unfortunately, few trials have investigated the feasibility of well known anticancer agents in elderly cancer patients and most clinical cancer studies exclude patients aged 65 years or older [2].

Despite advances in prevention, early detection and adjuvant therapy, nearly one third of breast cancer patients without lymph node metastases and more than half of those with lymph node metastases at initial diagnosis will sooner or later develop recurrent disease. Breast cancer once metastasized, has to be considered as a systemic disease, and unfortunately is currently still incurable. Even with intensive multi-modality treatment only a few metastatic patients become long-term survivors [3]. While local treatment, such as radiotherapy or hyperthermia, and systemic endocrine and cytotoxic treatment often result in objective tumor responses associated with relevant relieve of symptoms, the gain in survival they yield is much less impressive and can only be suggested by using retrospective comparisons of data on the natural behaviour of the disease [4,5,6]. Chemotherapy for metastatic breast cancer should therefore aim at a maximum of palliation and prolongation of life, at the cost of a minimal of toxicity [7]. It is indicated in patients who are hormone resistant or who have rapidly progressive disease, especially at visceral sites. At present, anthracyclines and taxanes (paclitaxel and docetaxel) are among the most effective agents in metastatic breast cancer. A combination chemotherapy schedule containing an anthracycline seems to result in higher response rates as compared to monotherapy or a polychemotherapy schedule without an anthracycline [8]. Its efficacy is unfortunately counterbalanced by its considerable toxicity such as total alopecia and severe myelosuppression. Other cytotoxic agents, such as the antimetabolites 5-fluouracil (5-FU) and methotrexate, have also shown to be valuable in metastatic breast cancer. In search of improving chemotherapeutic options, novel agents, new combinations of agents, new schedules of administration as well as high-dose chemotherapy are currently been explored.

This thesis on new chemotherapeutic modalities in metastatic breast cancer comprises studies ultimately aiming at the balance between safety and efficacy on the one hand, and patient convenience as well as physician convenience on the other hand. The thesis includes two phase II studies in breast cancer patients with two new cytotoxic agents, miltefosine and gemcitabine, respectively. The use of a new oral 5-FU analogue, capecitabine, as monotherapy or in a combination schedule in breast cancer is being discussed. A combination of anti-tumor agents does not always improve their respective anti-tumor activities. Drug interaction may result in synergism, not only of efficacy but also of toxicity.

Combination therapy may even result in antagonism of anti-tumor activity. The combination of methotrexate and docetaxel, both effective single agents in breast cancer, has been investigated in a dose-finding study. Subsequently the combinations of an antimetabolite and a taxane in solid oncology are critically reviewed. Finally two pharmacokinetic studies on paclitaxel are presented. Although paclitaxel is frequently used in oncology, data on the exposure to the unbound drug fraction are limited. Pharmacokinetic data of paclitaxel have been investigated in metastatic breast cancer patients aged 70 years or older, while another study has examined the impact of the common way of dose calculation using the body-surface area of the patient, on the variability of exposure to paclitaxel.

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CHAPTER 2

Phase II Study of Miltefosine 6% Solution as Topical Treatment of Skin Metastases in Breast Cancer

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SUMMARY

Topical treatment of skin metastases with a cytotoxic agent is attractive for its easy self-administration and absence of major systemic interference. Miltefosine exerts its cytotoxicity by acting on cell membrane phospholipids, and can be administered topically.
Twenty breast cancer patients with progression of skin metastases were treated with a 6% solution of miltefosine, which was topically administered once daily during the first week and twice daily thereafter. Sixteen out of 20 patients also had metastatic disease at other sites. Concomitant systemic treatment when ongoing for at least 2 months prior to study entry was permitted, and consisted of chemotherapy and hormonal therapy in seven and nine patients, respectively. Prior palliative cytotoxic and hormonal therapy had been administered to 11 and 19 patients, respectively.

No grade 3 and 4 toxicity occurred. Miltefosine therapy was discontinued in two patients due to nausea and in 1 patient due to skin toxicity. Grade 1 and 2 adverse skin reactions, and nausea and vomiting were seen in 11 and two patients respectively.

In 18 patients evaluable for response, four partial responses were noted (response rate 22%), while 7 patients had stable disease. Three partial responses were observed in patients in whom the skin lesions were smaller than 1.5 cm^2. Median duration of response was 2.5 months and median time to progression for all patients was 1.9 months.

In this study topically applied miltefosine for metastatic skin lesions of breast cancer showed modest activity in a relatively heavily pretreated patient population, without serious systemic toxicity.

INTRODUCTION

Skin metastases of breast cancer are often located at the chest wall and may present as the single metastatic site or may occur concomitantly with metastatic disease at other sites. In patients with isolated skin metastases or skin metastases together with stable disease at other sites, topical application of a cytotoxic drug is attractive as an additive local treatment option, while avoiding further systemic toxicity [1]. Moreover, topical treatment may be of value in skin metastases at the chest wall, in which penetration of systemic chemotherapy is hampered by vascular damage due to previous surgery and/or radiotherapy.

Miltefosine (hexadecylphosphocholine, He-PC) is an alkylphosphocholine. The drug exerts its cytotoxic action by interfering with the metabolism of the cell membrane phospholipids [2]. The cytotoxicity of the agent was shown both after incubation of human leukemic cell lines *in vitro* and after oral administration in chemically induced breast carcinomas in rats *in vivo* [3]. These results stimulated performance of clinical phase I-II

studies [4-6]. For the purpose of topical administration He-PC was dissolved in a fixed mixture of alkylglycerols and water, increasing its penetration into the skin. A phase I study in heavily pretreated breast cancer patients with progressive skin metastases showed excellent tolerability of the topical treatment with miltefosine solution at a concentration of 20 mg/ml to 80 mg/ml (2% to 8%), without evident systemic toxicity [5]. Concomitant systemic therapy in that study was not allowed. As the 8% miltefosine solution showed marked erythema of previously untreated skin, a concentration of 6% was recommended for phase II studies.
In this article we report on the activity and tolerability of a topically applied 6% solution of miltefosine in pretreated patients with progression of skin metastases of breast cancer without progression at other metastatic sites.

PATIENTS AND METHODS

Patients with histologically confirmed breast cancer and progressive skin metastases without progressive disease at other metastatic sites were eligible for this non-randomized single-center study. Concomitant cytotoxic or hormonal therapy was permitted provided it had been started at least 2 months prior to study entry and was continued at an unchanged dose. Further eligibility criteria required a WHO performance status ≤ 2, a life expectancy > 3 months, white blood cell count (WBC) $\geq 2.5 \times 10^{9}$/l, platelet count $\geq 100 \times 10^{9}$/l, serum creatinine ≤ 177 umol/l, alkaline phosphatase ≤ 2 x the upper limit of normal (UNL) (except in the case of bone metastases), bilirubin ≤ 2 x UNL, transaminases ≤ 4 x UNL. Exclusion criteria were: patients requiring radiotherapy of indicator lesions, progressive disease requiring immediate initiation or change of systemic therapy, brain metastases or severe and insufficiently controlled other disease. Informed consent was obtained. The study protocol was approved by the institutional ethics board.

Pretreatment evaluation included: medical history, physical examination, description, measurement and photography of the indicator skin lesions, assessment of other metastatic sites, hematology and biochemical laboratory analysis including blood cell count, sodium, potassium, Ca, creatinin, AP, ASAT, ALAT, LDH and total protein. During therapy, physical examinations and toxicity assessments were performed at week 1, 2, 4 and every 4 weeks thereafter. Hematology and chemical laboratory analysis were done at week 2 and 4 and every 4 weeks thereafter, and objective tumor assessment was performed every 4 weeks. Responses and toxicity were evaluated using standard WHO criteria.

A 6% solution of miltefosine (Asta Medica AG, Frankfurt, Germany) was applied topically to the skin metastases once daily during the first week, and in the absence of toxicity twice daily thereafter. The solution was smoothly rubbed over the affected areas, using one drop of solution per 10 cm^2 of treated skin area. If tolerated, treatment was continued for at least 8 weeks. After reaching complete response, treatment was to be

continued for 4 more weeks. In case of partial response or stable disease it was continued until progression.

RESULTS

Table 1. Patient characteristics

Characteristic	
Total number of patients entered	20
Number of patients evaluable	
for response	18
for toxicity	20
Median age (years)	61
Range	43-79
Performance status	
0	8
1	11
unknown	1
Dominant site of disease	
Skin only	4
Visceral	6
Bone	6
Soft tissue	4
Prior anticancer therapy	
Surgery	19
Radiotherapy	19
adjuvant	15
palliative	10
Palliative hormonal therapy	19
Chemotherapy	11
adjuvant	2
palliative	11
Concomitant anticancer therapy	
None	4
Hormonal therapy	9
Chemotherapy	7

Twenty patients were entered into the study. Patient characteristics are depicted in Table 1. One patient was not eligible, because systemic cytotoxic treatment had been discontinued only 18 days before entry in the study. Another patient was formally not evaluable, because miltefosine was stopped after 5 weeks of administration due to early

systemic progression and poor tolerability. However, there was no progression of the treated skin lesions. Sixteen patients (80%) had metastatic disease at other sites and received concomitant systemic antitumor therapy. Only 3 patients had received prior palliative radiotherapy for skin metastases.

Median duration of topical treatment was 10.5 weeks (range 3-46 weeks). Seventeen out of 20 patients could be treated according to the scheduled twice daily application without necessity for dose reduction or delay. Toxicity led to discontinuation of therapy in 3 patients after 29, 36 and 51 days: two patients with a large treated skin area of 60 and 100 cm^2, respectively, experienced continuous possible miltefosine-related nausea grade 2 and 1 respectively, another patient stopped treatment due to skin toxicity grade 2. All patients were evaluable for toxicity (Table 2). Grade 3 and 4 toxicity did not occur. Side effects did not require hospitalisation. Eleven patients reported adverse skin reactions, mainly grade 1 (9 patients). In patients not receiving concomitant chemotherapy no hematologic or biochemical toxicity was observed. One patient, who did not receive concomitant systemic antitumor therapy, developed anemia and thrombocytopenia grade 3 and elevated serum bilirubin grade 4 due to progressive disease, and died after 46 weeks. One patient on palliative hormonal therapy developed elevated serum transaminases grade 1, another patient with bone metastases showed an elevated serum alkaline phosphatase grade 2.

Table 2. Toxicity in 20 evaluable patients

Toxicity	Grade 1 (%)	Grade 2 (%)
Local toxicity		
Skin atrophy	4 (20)	-
Skin exfoliation	3 (15)	2 (10)
Erythematous rash	2 (10)	2 (10)
Pruritis	2 (10)	-
Pain	3 (15)	-
Dry skin	2 (10)	-
Teleangiectasis	1 (5)	-
Any skin toxicity	9 (45)	2 (10)
Systemic toxicity		
Nausea and vomiting	1 (5)	1 (5)
Anorexia	1 (5)	-
Fatigue	1 (5)	-

A partial response was observed in 4 patients (22%), with a median duration of response of 2.5 months (range 1.0-9.1). Stable disease was noted in 7 patients , lasting 1.8-5.9 months with a median of 3.7 months. For the group of evaluable patients, the median time to progression was 1.9 months (range 0.7-9.1 months). A partial response was achieved in 3 out of 6 patients (50%) with a maximum size of treated skin lesions < 1.5 cm^2 , compared to only 1 out of 6 patients (17%) with a maximum size of treated skin lesions > 3.5 cm^2 . In the 6 patients with stable metastatic disease at other sites, a partial response of the skin metastases was seen in 3 patients and stable disease in the other 3 patients. Four out of 9 patients having progressive systemic disease at other sites also had progression of skin lesions. In the other patient with systemic disease the response of the other sites was not evaluable.

Table 3. Results of topical administration of miltefosine 6% in skin metastases of breast cancer

Author	Number of patients evaluable	Patients with prior palliative chemotherapy	Concomitant anticancer therapy	CR	PR	RR%	Reference
Unger [1]	24	20	-	4	3	29	5
Gaafar	17	17	-	0	7	41	10
Terwogt	30	25	-	7	6	43	11
Clavel	23	23	+	0	8	35	12
Khayat	20	20	20	2	6	40	13
Clive	14	+	7	0	7	50	14
Clive	25	19	15	1	2	12	15
Smorenburg	18	11	16	0	4	22	this study

[1] This study used miltefosine at a concentration of 2% -8%

DISCUSSION

Most patients with skin metastases of breast cancer will also present with distant metastatic disease or sooner or later develop such distant metastases [7]. As metastatic breast cancer is still an incurable disease, its treatment should focus on optimal palliation at the expense of minimal toxicity.Although maximal and sometimes aggressive treatment for loco-regional cutaneous disease may be warranted to prevent untreatable and invalidating symptoms of ulceration, bleeding, infection and pain, topical treatment of skin metastases is

very attractive because of the easyness of self-administration in an outpatient setting and its lack of major systemic toxicity. Topical administered miltefosine has shown activity and a good tolerability in breast cancer. However, while oral adminstered miltefosine appeared feasible and effective in the treatment of visceral leishmaniasis, it lacked activity as an antineoplastic drug [8,9].

Treatment results of 7 phase I-II studies using topically applied miltefosine for skin metastases in patients with breast cancer are summarized in Table 3. The median response rate in these studies is 38% (range 12%-50%). In our study, a 6% solution of miltefosine showed modest activity, with 4 partial responses and additionally 7 times stable disease. The response rate of 22% contrasts unfavourably to those reported in other studies [5,10-15]. This may be due to more advanced disease in our study population, since 16 out of 20 patients also had distant metastatic disease (of whom 6 had visceral disease). It may also be due to more extensive prior systemic anticancer therapy which consisted of 2 or more lines of palliative hormonal or cytotoxic therapy in 12 and 6 of our patients, respectively. A higher response rate was indeed suggested in patients in whom the skin lesions were smaller than 1.5 cm^2 and in patients without systemic progressive disease. In an overview analysis of topical miltefosine in metastatic breast cancer, Sinderman et al. identified size of tumor lesions and depth of infiltration as significant prognostic factors for tumor response [16].The present study confirmed the good tolerability as seen in other clinical studies, with mild skin reactions and gastrointestinal toxicity in 12 and 2 patients, respectively.

In conclusion, topically administered miltefosine may effectively control skin metastases (especially small lesions) in patients with metastatic breast cancer, without inconvenient side effects or hospitalized care.

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CHAPTER 3

Phase II Study of Weekly Gemcitabine in Patients with Metastatic Breast Cancer Relapsing or Failing Both an Anthracycline and a Taxane

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SUMMARY

A phase II study was performed to investigate the efficacy and tolerability of gemcitabine as third-line chemotherapy for patients with metastatic breast cancer, previously treated with both an anthracycline- and taxane-containing regimen. Twenty-three patients were treated with gemcitabine 1200 mg/m^2 in a 30-min infusion on day 1, 8 and 15 of a 28 day cycle. Seventy-four percent of the patients had visceral metastases.

No complete or partial responses were observed. Six patients (26%) had stable disease with a median duration of 4.0 months. The median time to progression was 1.9 months and the median survival time was 7.8 months. Neutropenia grade 3 and 4 was observed in four patients (18%). Non-hematological toxicity grade 3 included nausea and vomiting in 14%, skin toxicity in 9% and elevation of transaminases in 23% of the patients. Gemcitabine is ineffective as third-line single agent therapy in patients failing anthracycline and taxane treatment for metastatic breast cancer.

INTRODUCTION

Metastatic breast cancer is an incurable disease, with a median survival of 2-3 years, although patients with a hormone-responsive tumor may live substantially longer. Once the malignant cells have become hormone-resistent and chemotherapy has to be applied, only very few patients become long-term survivors [1]. Chemotherapy for metastatic disease should therefore aim at maximum palliation and prolongation of life, at the cost of minimal toxicity.

Anthracyclines, together with taxanes, are at present the most active agents in the treatment of metastatic breast cancer. Traditional combination chemotherapy using an anthracycline, such as FAC or FEC (5-fluorouracil, doxorubicin or epirubicin and cyclophosphamide), results in response rates varying from 50-60%, which are usually better than those observed with CMF (cyclophosphamide, methotrexate and 5-fluorouracil) or single-agent chemotherapy for which the response rates are about 40% or less [2,3]. Taxanes have demonstrated activity as single agent in first- and second-line and are investigated in combination schedules [4,5]. However, after failure on both anthracyclines and taxanes, only capecitabine has shown relevant efficacy, but further active agents are still lacking [6].

Gemcitabine (2'2'-difluorodeoxycytidine dFdC) is a new cytotoxic agent that has shown single-agent activity in phase I-II studies in patients with a variety of solid tumors [7]. The drug is phosphorylated intracellularly to active diphosphate and triphosphate metabolites, along a pathway of enzymes including deoxycytidine kinase [8]. Gemcitabine triphosphate competes with deoxycytidinetriphosphate (dCTP) for incorporation into DNA, inhibiting further DNA synthesis. The diphosphate form inhibits ribonucleotide reductase, which results in

lower pools of cellular deoxynucleotide. The subsequent decrease in dCTP potentiates the incorporation of gemcitabine triphosphate into DNA, and enhances phosphorylation of gemcitabine, since dCTP is an inhibitor of deoxycytidine kinase. These self-potentiating mechanisms prolong retention of active gemcitabine metabolites in tumor cells, rendering the drug attractive for the treatment of a relatively slowly proliferating tumor such as breast cancer with few cells in the active phases of the cell cycle.

Early clinical studies with gemcitabine as first-or second-line treatment in metastatic breast cancer reported response rates ranging from 0-46% [9]. The schedule used most frequently was gemcitabine at a dose of 1000-1200 mg/m^2 administered on day 1, 8 and 15 of a 4-weekly cycle. In this schedule gemcitabine appeares to be well tolerated, with mild myelotoxicity, little nausea and vomiting and no hair loss.

Based on these data we initiated a phase II study of gemcitabine in patients with metastatic breast cancer, previously treated with both an anthracycline- and a taxane-containing regimen.

PATIENTS AND METHODS

Study design

Eligibility criteria included measurable or evaluable metastatic breast cancer, progressive after previous chemotherapy for metastatic disease, that consisted either of an anthracycline-based regimen followed by second-line single agent paclitaxel or docetaxel, or an anthracycline-docetaxel combination, age less than 70 years, WHO performance score ≤ 2, life expectancy of at least 3 months, normal bone marrow functions with white blood cell count (WBC) > 3.0 x 10 ^{9}/l and a platelet count > 100 x 10 ^{9}/l, acceptable liver functions with a normal serum bilirubin, ASAT and ALAT ≤ 2.5 x the upper normal limit (UNL) and alkaline phosphatase ≤ 5 x UNL. Patients with leptomeningeal or brain metastases, a second malignancy other than basal cell carcinoma of the skin or carcinoma *in situ* of the cervix, extensive radiotherapy within the previous 4 weeks, uncontrolled cardiac disease, active infections and/or peptic ulcer were ineligible. Every patient gave written informed consent before entering the study.

Pretreatment evaluation included medical history, physical examination, tumor measurement, electrocardiogram, complete blood cell counts and biochemistry (including sodium, potassium, creatinine, bilirubin, alkaline phosphatase, serum transaminases), bone scan, bone and chest X rays and ultrasound of the liver (CT scan in case of liver metastases). During therapy blood cell counts and toxicity assessment were performed weekly, physical examination and biochemistry every 4 weeks, and objective tumor assessment every 8 weeks. Responses and toxicity were assessed using standard Common Toxicity Criteria.

Table 1. Summary of Patient Characteristics

Characteristic	No
Total number of patients entered	23
Number of eligible patients	22
Number of patients evaluable	
for response	20
for toxicity	22
Median age (years)	53
Range	31-70
Performance status (WHO)	
0	6
1	16
2	1
Estrogen receptor (ER)	
ER-	9
ER+	11
ER?	13
Number of organ systems involved	
1	2
2	8
≥ 3	13
Prior anticancer therapy	
Hormonal therapy for metastatic disease	21
Chemotherapy	
Adjuvant	11
For metastatic disease	23
Prior chemotherapy for metastatic disease	
1 line	3
2 lines	14
3 lines	5
4 lines	1

Gemcitabine was administered at the outpatient clinic as a 30-min intravenous infusion at a dose of 1200 mg/m^2 on day 1, 8 and 15 of a 28 day cycle. Prophylactic use of anti-emetic drugs was not scheduled, but permitted if indicated. Dose modifications were based on blood cell counts before each infusion and on liver enzymes on day 1 of every cycle. If on day 1 WBC was < 3.0 x 10 ^{9}/l and/or platelets were < 100 x 10 ^{9}/l, and/or ASAT/ALAT were >

5 x UNL, treatment was postponed for a week. A 50% dose reduction was required if WBC was 2.0-3.0 x 10^9/l and/or platelets were between 50-100 x 10^9/l at day 8 or 15, and if ASAT, ALAT or alkaline phosphatase were between 2.5-5 x UNL on day 1. Gemcitabine was omitted in case of grade 3-4 hematologic toxicity on day 8 or 15.

RESULTS

Twenty-three patients entered the study. Patient characteristics are presented in Table 1. One patient was considered ineligible because of an elevated serum bilirubin level due to liver metastases. Two patients were not evaluable for response as they showed early disease progression within 4 weeks after entry into the study. However, in an intent to treat analysis these patients were included in the denominator for response rate. All eligible patients were evaluable for toxicity. All patients but one had measurable disease. Most common sites of metastatic disease were bone (57%), liver (52%) and lungs (35%). No patient had bone metastases only. The majority of patients (74%) had visceral disease, while 13 out of 23 patients (57%) had 3 or more organ systems involved. Ninety-one percent of the patients had received two or more lines of chemotherapy as adjuvant and palliative treatment, and 57% were already pretreated with three or more lines of chemotherapy. Five patients had progressive disease during any prior chemotherapy regimen for metastatic disease.

A total of 53 treatment courses was given to the 22 eligible patients, and 142 (93%) out of 153 scheduled doses were administered. Eleven doses were omitted due to toxicity. Thirty-four of the planned doses were reduced because of leukopenia (n=21) or elevated ASAT/ALAT (n=13). The mean number of cycles given was 2.4 (median 2) with a range of 1-8. The mean dose per infusion was 985 mg/m^2 (range 600-1200 mg/m^2).

All eligible patients were evaluable for toxicity (Table 2). Side effects were generally mild and did not require hospitalisation. Two patients discontinued therapy because of side effects. One patient stopped after 4 cycles due to myalgia grade 2 and one patient went off study during the second cycle because of skin toxicity grade 3 with a painful erythema and induration of both legs. Neutropenia grade 3 or 4 was observed in 4 patients, but neutropenic fever did not occur. Non-hematologic toxicity was generally mild. Drug-induced fever, flu-like symptoms and transient reversible increase in liver transaminases were reported. Hair loss did not occur in our patients.

No complete or partial remission was noted, six out of 23 patients in an intent to treat analysis (26%) had stable disease with a median duration of 4.0 months. Both patients with only one site of metastatic disease achieved stable disease and two other patients with stable disease had two metastatic sites. Progressive disease occured in 13 patients within the first two cycles of gemcitabine. The median time to progression was 1.9 months (range

1.0-4.4), the median survival time 8.1 months for the patients with stable disease and 7.8 months for all patients.

Table 2. Worst toxicity in 22 patients

	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4(%)
Neutropenia	2 (9)	4 (18)	3 (14)	1 (5)
Thrombocytopenia	-	-	1 (5)	-
Nausea/Vomiting	10 (45)	3 (14)	3 (14)	-
Skin	3 (14)	4 (18)	2 (9)	-
Myalgia	5 (23)	2 (9)	-	-
Fatigue	7 (32)	4 (18)	1 (5)	-
Fever	2 (9)	7 (32)	-	-
Stomatitis	2 (9)	-	-	-
Flu-like symptoms	7 (32)	2 (10)	-	-
ASAT elevation	5 (23)	5 (23)	3 (14)	-
ALAT elevation	3 (14)	4 (18)	5 (23)	-

DISCUSSION

In spite of the responses that can be achieved with anthracycline- and taxane-based chemotherapy in metastatic breast cancer, recurrence is inevitable. Although it is known that effectivity declines with each subsequent regimen, patients and physicians have difficulty with accepting only symptomatic treatment without the possibility of systemic anticancer effects [10]. Therefore there is a need for effective drugs as third-line therapy after anthracycline and taxane failure. Gemcitabine is a new drug that has shown efficacy with excellent tolerability in breast cancer in early phase II studies [9]. Treatment results of seven studies (including our study) using weekly gemcitabine as a single agent in metastatic breast cancer are shown in Table 3.

Gemcitabine at a dose of 800 mg/m^2 given as a 30-min infusion in pretreated patients yielded response percentages of 0% and 25% respectively [11,12]. In view of the low toxicity profile, in subsequent studies the dose was increased to 1000-1200 mg/m^2, yielding response rates of 14% and 37% respectively in first-line chemotherapy in metastatic breast cancer patients, and 28% in second-line chemotherapy in patients failing anthracycline-based regimens [13-15]. No prior treatment with a taxane was reported in any of these studies. Prolonged infusion of gemcitabine in a similar schedule resulted in a similar response rate, at a far lower but maximum tolerable dose of 250 mg/m^2 [16,17].

Table 3. Weekly gemcitabine monotherapy in metastatic breast cancer

Author	Number of patients	Dose in mg/m ^{2} and duration of administration	Previous lines of palliative chemotherapy	Response rate	Reference
Possinger	18	800 in 30 min	1-2	0	11
Carmichael	44	800 in 30 min	0-1	25	12
Blackstein	39	1200 in 30 min	0	37	13
Possinger	42	1000 in 30 min	0	14	14
Spielmann	47	1200 in 30 min	1	28	15
Schmid	20	250 in 6 h	0-4	25	17
Smorenburg	23	1200 in 30 min	2-3	0	this study

In all studies, gemcitabine was administered on day 1, 8 and 15 of a 28 day cycle.

We investigated the activity of gemcitabine single agent therapy in patients with metastatic breast cancer, failing both an anthracycline and a taxane. The scheduled dose of 1200 mg/m^2 gemcitabine is similar to the dose used in other studies [13-15]. The average dose of 985 mg/m^2 delivered in our study is even somewhat higher than the mean dose of 942 mg/m^2 delivered in the study of Possinger et al [14]. However, despite this satisfactory dose intensity, no objective responses were observed in our patients. The extent of metastatic disease (a majority of patients had predominant visceral disease (74%) or 3 or more organ systems involved (57 %)), together with heavy pretreatment, may in part account for the short median time to progression and median survival time in our study.

Few other studies have adressed the therapy in patients with metastatic breast cancer, previously treated with both anthracyclines and taxanes. Dose-intensive vinorelbine with G-CSF support or continuous infusion of vinorelbine resulted in response rates of 25% and 16% respectively, but at the cost of considerable stomatitis, neutropenia and infections [18-19]. In a small study, 2-weekly vinorelbine showed efficacy with less toxicity [20]. Blum et al conducted a multicenter phase II study with capecitabine, a new oral fluoropyrimidine carbamate, in 163 patients previously treated with paclitaxel (100%) and an anthracycline (91%) [6]. A total of 110 patients (68%) had predominant visceral disease. The oral treatment schedule appeared tolerable, and resulted in a response rate of 20% and a median survival of 55 weeks. Based upon this study capecitabine is currently registered for third-line use in the USA. Weekly administration of taxanes, enabling a higher total dose per time period, is being explored in several phase I-II studies and preliminary data suggest promising results in patients progressive after anthracyclines and standard 3-weekly taxane schedules [21-24].

Since several studies have reported modest activity of gemcitabine in non-pretreated patients with metastatic breast cancer and because of non-overlapping modes of action and toxicity, the value of adding gemcitabine to anthracycline or taxane containing regimens is being investigated [25,26].

From our study we conclude that the use of gemcitabine as single agent for metastatic breast cancer after prior anthracyline and taxane treatment is not effective.

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CHAPTER 4

Capecitabine in Breast Cancer. Current Status

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ABSTRACT

Anthracyclines, together with taxanes, are at present the most active agents in metastatic breast cancer, while single agent bolus of 5-fluorouracil (5-FU) is not very active in this setting. In view of encouraging results and tolerable toxicity of continuous infusion of 5-FU in gastrointestinal cancer, innovative oral 5-FU agents such as capecitabine have been developed. Capecitabine is a prodrug that is converted into the active compound 5-FU preferentially at the tumor site. An intermittent dosing schedule of capecitabine twice daily at a dose of 2,510 mg/m^2 day on day 1-14 in a 3-weekly cycle appeared to be feasible and resulted in a high dose-intensity.

A large phase II study investigating capecitabine in 135 advanced breast cancer patients, pretreated with anthracyclines and taxanes, observed three complete and 24 partial responses (response rate, 20%), with a mean duration of 8.0 months. Preliminary results of a study comparing capecitabine with paclitaxel in 42 breast cancer patients failing anthracyclines indicate that the efficacy of capecitabine is comparable to that of paclitaxel with response rates of 36% and 21%, respectively. Another study reported a response rate of 25% for capecitabine as first-line therapy for advanced breast cancer in women aged ≥ 55 years, which tended to be better than combination chemotherapy with cyclophosphamide, methotrexate and 5-FU (CMF). In all studies, capecitabine side effects were mainly mild, and treatment related grade 3-4 toxicity consisted of diarrea (8-11%), nausea (4-11%), hand-foot syndrome (10-18%), neutropenia (3-20%) and bilirubin elevation (6%).

Capecitabine is clearly an active agent for the treatment of breast cancer. It is currently registered in various countries for use in third-line treatment of metastatic disease. Its further role will have to be precised from data of randomized phase III studies.

INTRODUCTION

Although metastatic breast cancer still remains an incurable disease, its treatment with chemotherapy offers relevant relief of symptoms and a modest gain in survival in many patients. At present, anthracyclines and taxanes are the most active classes of anticancer drugs in the treatment of breast cancer. When given as first- or second-line treatment, single-agent anthracyclines achieved response rates ranging from 25-62% [1]. A recent meta-analysis of randomized trials in metastatic breast cancer indicated improved response rates and survival for combination chemotherapy including anthracyclines, usually combined with cyclophosphamide and 5-FU, compared with anthracycline monotherapy [2]. Single agent paclitaxel or docetaxel yield response rates in first-line treatment of 25-46% and 38-68%, respectively [3]. The efficacy of both anthracyclines and taxanes as palliative treatment in breast cancer is coincided by its relevant toxicity, with myelosuppression, fatigue and

alopecia, together with neurotoxicity for taxanes and cardiotoxicity and nausea for anthracyclines.

In an attempt to improve the chemotherapeutic options, high dose chemotherapy, new combinations of cytotoxic agents, new schedules of administration, and new agents are currently being explored.

5-FU IN BREAST CANCER

While 5-FU is a commonly used and appreciated agent in the treatment of several solid tumors, especially colorectal and head-and-neck cancer, single agent bolus 5-FU has never shown impressive results in patients with breast carcinoma. Clinical studies adding leucovorin to bolus infusion of 5-FU in metastatic breast cancer reported slightly higher response rates of 17-48% [4-9]. Lokich et al have reintroduced treatment with 5-FU administered as a continuous infusion in patients with advanced gastrointestinal cancer [10]. This was based on the concept of increasing the duration of exposure of the small fraction of cancer cells that are in the active phases of the cell cycle and, hence, are susceptible for chemotherapy to the cell-cycle specific cytotoxic drug 5-FU with its short serum half-life of only 11 minutes. A review on the use of continuous infusion of 5-FU in heavily pretreated breast cancer patients reported an overall response rate of 29%, with encouraging responses even in patients pretreated with bolus 5-FU [11]. In contrast to bolus infusion, which has a dose limiting toxicity of myelosuppression, continuous delivery of 5-FU is mainly coincided by mucositis and the hand-foot syndrome as major side effects.

In view of encouraging results and tolerable toxicity of continuous infusion of 5-FU and to circumvent the necessity and inconvenience of an intravenous access device, new oral 5-FU agents have been developed. For breast cancer, of these new oral agents, capecitabine has been studied most extensively. This review will discuss the mechanism of action of capecitabine and early clinical trial results, as well as its use in metastatic breast cancer.

ACTIVATION AND PHARMACOLOGY OF CAPECITABINE

Capecitabine (Xeloda ®) is an innovative fluoropyrimidine carbamate, developed as an orally administered precursor of 5'-deoxy-5-fluorouridine (5'DFUR) [12]. Along a pathway with 3 enzymatic steps, capecitabine is finally converted into the active compound 5-FU, preferentially at the site of tumor tissue (figure 1). After transformation of capecitabine in the liver into 5'-deoxy-5-fluorocytidine (5'-DFCR) by hepatic carboxylesterase, cytidine deaminase converts 5'-DFCR in the liver and tumor tissues into 5'-DFUR. The final step is conversion to 5-FU by the tumor-associated angiogenetic factor thymidine phosphorylase (TP), which is overexpressed in tumor cells. The enzyme TP appears to be essential for the antitumor activity of capecitabine. A study in human cancer xenograft models confirmed a

correlation between TP levels and tumor susceptibility for capecitabine and reported tumor growth inhibition in many more tumor xenografts for capecitabine than with 5-FU [13]. A study in patients with metastatic colorectal cancer who had been treated with capecitabine confirmed its tumor selectivity by measuring 3.2 and 1.4 times greater concentrations of 5-FU in the primary tumor and the liver metastases, respectively, compared to concentrations in surrounding healthy tissue [14].

Extensive pharmacokinetic studies have shown that after oral administration of capecitabine, the intact molecules are rapidly absorbed by the intestinal mucosa [15,16]. Plasma concentrations of 5-FU and 5'-DFUR reach a peak at 2 h, followed by a swift decline with a plasma half life of around 1 h [15]. Finally, more than 70% of capecitabine and its metabolites are excreted in the urine [16]. Despite extensive hepatic transformation of capecitabine, mild to moderate hepatic dysfunction only slightly, but not significantly, increases serum C_{max} and AUC of capecitabine, 5'-DFUR and 5-FU [17].

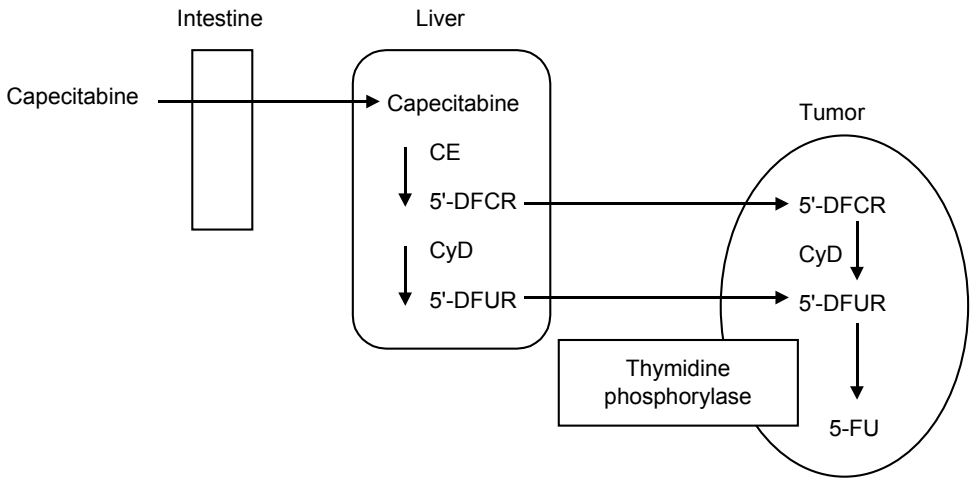

Figure 1. Conversion of capecitabine to active 5-fluouracil
Abbreviations: 5'-DFCR= 5'-deoxy-5-fluorocytidine; 5'-DFUR=5'-deoxy-5-fluorouridine; 5-FU= 5-fluouracil; CE= carboxylesterase; CyD=cytidine deaminase

Studies in human colon cancer xenografts in nude mice reported induction of TP by paclitaxel, docetaxel, mitomycin C and cyclophosphamide, while combination therapy of paclitaxel or docetaxel with capecitabine resulted in synergistic antitumor activity [18]. Oral

administration of cyclophosphamide in a human breast cancer xenograft model in nude mice also elevated TP tumor levels [19]. Likewise, the efficacy of the combination of cyclophophamide and capecitabine was found to be more than additive. These *in vivo* data support studies on the clinical use of capecitabine in combination chemotherapy regimens.

DOSE AND TOXICITY IN PHASE I STUDIES IN CANCER PATIENTS

In a phase I study on continuous treatment with capecitabine twice daily in patients with a solid tumor, a maximum tolerable dose (MTD) of 1657 mg/m^2 day was noted [20]. Another trial examining an intermittent schedule of 2 treatment weeks of twice daily dosing followed by 1 week of rest showed an MTD of 3000 mg/m^2 day [15]. Recommended doses for further studies were 1331 and 2510 mg/m^2 day for the continuous and intermittent schedule, respectively. The combination of continuous treatment with capecitabine and leucovorin did not appeared to be tolerable at the starting dose of capecitabine of 1004 mg/m^2 day, due to toxicity. The addition of leucovorin at a fixed dose of 60 mg/day to the intermittent schedule of capecitabine turned out to be feasible, with an MTD of capecitabine of 2000 mg/m^{2}day [21]. Dose limiting toxicities (DLT's) in all phase I studies were diarrhea and hand-foot syndrome.

Recently, a randomized phase II study in patients with advanced colorectal cancer compared all three described regimens of capecitabine [22]. The combination of leucovorin and capecitabine in an intermittent schedule improved neither tumor responses nor the time to progression. The intermittent schedule of single-agent capecitabine on day 1-14 in a 3-weekly cycle yielded a higher dose-intensity as well as a longer median duration of treatment and has been selected for further phase II-III studies.

Table 1. Single-agent capecitabine in metastatic breast cancer

Author	Daily dose (mg/m^2)	Prior chemo-therapy [1]	No.of patients evaluable	CR	PR	RR%	Median time to progres-sion (days)	Ref.
Blum	2510	A+T	135	3	24	20	93	23
Blum	2500	A+T	74	?	?	24	111	24
Wong	2500	A+T	22	0	6	27	?	25
O'Reilly	2510	A	22	3	5	36	92	26
Cervantes	2500	T	32	2	11	41	?	27
O'Shaughnessy	2510	no	61	0	15	25	132	28

In all studies capecitabine was administered twice daily on day 1-14 every 3 weeks

[1] A = anthracycline, T = taxane

SINGLE AGENT CAPECITABINE IN METASTATIC BREAST CANCER

Treatment schedule and results of clinical trials exploring capecitabine in advanced breast cancer are summarized in Table 1.

Blum et al. conducted a single-arm phase II study on capecitabine in patients with advanced breast cancer in 25 centers in North America [23]. The treatment was given for a minimum of 43 days and discontinued when progressive disease appeared. All 162 patients who entered the study were refractory on prior paclitaxel therapy. No less than 91% and 82% of the patients had also been pretreated with anthracyclines and 5-FU, respectively. Visceral disease was predominant in 110 patients. One patient was ineligible due to early progressive disease, another eight patients had incomplete data for response evaluation. No data were reported on the incidence of dose reduction or treatment delay. Of 135 patients with measurable disease, 27 (20%) had a complete (n=3) or a partial response (n=24), with a mean duration of 8.0 months. Responses were achieved irrespective of the site of metastatic disease. In 27 patients with assessable but not measurable disease five responses (19%) were observed. Importantly, all responses had been subject to review. For all 162 patients the median time to progression was 93 days and the median survival time 384 days. Despite extensive pretreatment, capecitabine seemed tolerable. Grade 3/4 toxicity consisted mostly of hand-foot syndrome (14%), diarrhea (11%), fatigue (7%), nausea and vomiting (4%), stomatitis (3%), neutropenia (3%) and a rise in serum bilirubine unrelated to liver metastases (6%). An important, confirmatory, phase II trial studied 74 breast cancer patients who were refractory to prior treatment with an anthracycline and a taxane [24]. Both in paclitaxel and in docetaxel pretreated patients a response rate of 24 % was observed, with a median duration of time to progression of 111 days.

Early data of a small separate non-randomized study confirmed activity of capecitabine in third-line use [25]. A majority of 63% of 22 evaluable patients had been pretreated with both anthracyclines and taxanes. In six out of 22 evaluable patients, a partial response (27%) was observed after 2 cycles of capecitabine.

Capecitabine vs paclitaxel

A randomized, multicenter, phase II study compared capecitabine with paclitaxel as second-line treatment in patients failing anthracyclines [26]. Forty-four patients were treated with either capecitabine (n=22) or with paclitaxel (n=22) at a dose of 175 mg/m^2 on day 1 in a 3-weekly cycle. Data on other patient characteristics and treatment duration are lacking. The study was prematurely closed due to poor accrual, as potential eligible patients often refused randomization to any treatment arm. Treatment with capecitabine resulted in three complete and five partial responses (RR 36%), while a response rate of 21% with no complete and four partial responses was noted in paclitaxel treated patients. Median time to progression was similar for both treatment arms.

In a non-randomized trial of 32 patients failing on prior taxanes, a response rate of 41% was obtained [27]. No information on time to progression or median survival time was given.

Capecitabine vs CMF

First-line use of capecitabine was investigated in a randomized phase II study in women aged 55 years or older [28]. A standard dose of capecitabine in 61 patients was compared with CMF combination chemotherapy administered intravenously on day 1 of a 3-4-weekly schedule in 32 patients. Again, data on patient characteristics, treatment duration and achieved dose-intensity are yet missing. While 15 responses, confirmed by review, were obtained in the capecitabine group (response rate 25%), only five patients responded in the CMF group (response rate 16%). Capecitabine resulted in a longer median time to progression of 132 days, compared to 94 days in the CMF group. Although recent studies have already suggested that the true response rate to CMF, when measured according to modern standards, is likely less than 30%, the even lower response rate to CMF in this study raises some doubts of the obtained dose-intensity. A clear dose-response relation has surely been established for CMF combination chemotherapy in advanced breast cancer [29].

In the above summarized studies in metastatic breast cancer, the efficacy of single agent capecitabine in an intermittent schedule appears comparable to paclitaxel and intravenous CMF, at the cost of mild side efffects, with treatment related grade 3-4 toxicity consisting of diarrhea (8-11%), nausea (4-11%), hand-foot syndrome (10-18%), neutropenia (3-20%) and bilirubin elevation (6%).

A retrospective analysis of the impact of dose reduction of capecitabine in 131 patients with metastatic breast cancer showed no detrimental effects on response, response duration, time to treatment failure or survival [30]. Another retrospective study noticed improved tolerability and similar efficacy at lower doses of capecitabine [31].

COMBINING CAPECITABINE WITH OTHER AGENTS

Based on preclinical *in vivo* studies showing synergism for certain cytotoxic drugs and capecitabine, several clinical dose finding studies investigated capecitabine in a polychemotherapy regimen. A total of 17 patients with solid tumors received continuous daily capecitabine at a dose of 1004-1657 mg/m^2 and paclitaxel at a dose of 135 or 175 mg/m^2 on day 1 in a 3-weekly schedule [32]. While no DLT's occured at the lower dose level of paclitaxel, both patients treated with 1657 mg/m^2 day capecitabine and the higher dose level of paclitaxel experienced dose-limiting neutropenic fever.

All other studies examining capecitabine in combination therapy for various solid cancers administered it in the intermittent schedule, together with docetaxel, oxaliplatin, interferon-alpha, gemcitabine or irinotecan [33,39]. A phase I study investigated the combination of capecitabine (1650-2500 mg/m^2) and docetaxel (75-100 mg/m^2) in 33 patients

with a solid tumor [33]. As asthenia was considered to be a DLT, feasible maximal doses were capecitabine 1650 mg/m^2 combined with docetaxel 100 mg/m^2, or capecitabine 2,500 mg/m^2 with docetaxel 75 mg/m^2.

Table 2. Capecitabine in combination with other chemotherapy agents in metastatic breast cancer

Author	Capecitabine daily dose (mg/m^2)	Other agent (mg/m^2, day 1)	Prior chemotherapy [1]	No. of patients evaluable	RR%	Ref.
O'Shaughnessy	2500	docetaxel 75	A	255	42	40
Villalona	1650-2000	paclitaxel 175	A	13	46	41
Batista	2000	paclitaxel 175	A	64	63	42
Nole	1000-2250	vinorelbine 12,5-20 [2]	yes	22	36	43
Venturini	1530-2120	epirubicin 75 docetaxel 75	no	23	74	44
Bangemann	2250	trastuzamab 2 [3]	A+T	13	47	45

In all studies capecitabine was administered twice daily on day 1-14 every 3 weeks
[1] A = anthracycline, T=taxane; [2] Day 1 and 3; [3] Day 1 weekly

Capecitabine plus docetaxel

Table 2 shows results of capecitabine in combination chemotherapy in metastatic breast cancer. O'Shaughnessy et al have just reported a large randomized phase III study of docetaxel with or without capecitabine in anthracycline-pretreated patients [40]. A total of 511 patients was treated with either the standard dose of capecitabine (2500 mg/m^2) and docetaxel (75 mg/m^2) (n=255) or docetaxel monotherapy (100 mg/m^2, n=256) in a 3-weekly cycle. An independent review committee confirmed responses. The combination schedule resulted in a response rate of 42% versus a response rate of 30% with docetaxel monotherapy. Progression free survival and median survival with the combination were 6.1 and 13.7 months, respectively, versus 4.2 and 11.1 months with docetaxel. While more gastrointestinal toxicity and hand-foot sydrome were noticed in the combination schedule, docetaxel resulted in more neutropenic fever.

Capecitabine plus paclitaxel

In a phase I study sixteen anthracycline-pretreated patients with metastatic breast cancer received paclitaxel at a dose of 175 mg/m^2 on day 1 in a 21-day cycle together with capecitabine at a daily dose of 1650-2000 mg/m^2 [41]. The hand-foot syndrome and febrile

neutropenia occured as DLT's at the highest dose level of capecitabine, while no DLT was noticed at a dose of 1650 mg/m^2. Six out of 13 evaluable patients responded. A phase II study evaluated a similar combination and schedule of capecitabine and paclitaxel at a dose of 2000 mg/m^2 and 175 mg/m^2, respectively [42]. A response rate of 63% (14 complete and 26 partail responses) was noted in 64 patients refractory to previous anthacyclines.

Other combinations

In a dose finding study capecitabine at a dose of 1000-2250 mg/m^2 day was combined with 3-weekly vinorelbine on day 1 and 3 at a dose of 12,5-20 mg/m^2 [43]. Only 1 out of 26 pretreated patients with advanced breast cancer experienced neutropenic fever as a DLT. Eight out of 22 evaluable patients had a partial respons. A study in 23 previously untreated patients investigated epirubicin (75 mg/m^2) and docetaxel (75 mg/m^2) on day 1 in a 3-weekly schedule in combination with daily capecitabine at a dose of 1530-2120 mg/m^2 [44]. Febrile neutropenia was noticed at all dose levels, while at the highest dose level three out of three patients had a DLT of mucositis, sepsis and neutropenia, respectively. Seventeen patients showed a response. In another study, treatment of 18 her2/neu-overexpressing patients with capecitabine and trastuzamab, after prior therapy including an anthracyclines and a taxane, resulted in a response rate of 47% [45].

Given the preclinical data on synergism, the feasibility of combining capecitabine with some other cytotoxic agents, and promising results on activity, further studies in breast cancer on combination chemotherapy schedules including capecitabine are either planned or ongoing. A phase III study is under way, compairing capecitabine with CMF or AC chemotherapy in postmenopausal patients with early breast cancer.

CONCLUSION

Based on a large reviewed trial of Blum et al, capecitabine has been approved by the FDA in the United States for metastatic breast cancer failing an anthracyline- and taxane-containing regimen [23]. Capecitabine clearly has shown promising efficacy in patients with metastatic breast cancer patients, at the cost of tolerable toxicity and with a benefit of oral administration. Its further role as monotherapy or in combination schedules will have to be precised from data of randomized phase III studies.

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CHAPTER 5

Altered Clearance of Unbound Paclitaxel in Elderly Patients with Metastatic Breast Cancer

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SUMMARY

Background:

The pharmacokinetic behaviour of anticancer drugs may be altered with aging due to (for example) differences in body composition and decreased hepatic and renal function.

Patients and methods:

To address this issue for paclitaxel, we studied the pharmacokinetics of the drug in 8 elderly women with metastatic breast cancer [median age (range), 77 years (70-84)] and a control group of 15 patients aged <70 years [median age (range), 54 years (22-69)]. Paclitaxel was administered as a 1-h i.v. infusion at a dose of 80 (elderly) or 100 mg/m^2 (<70 years), and serial blood samples were obtained at baseline, and up to 24 h after the end of infusion. Paclitaxel concentration-time profiles were fitted to a linear 3-compartment model without any demonstration of saturable behavior.

Results:

The apparent clearance of unbound paclitaxel was 124 $\pm$ 35.0 (elderly) vs 244 $\pm$ 58.8 L/h/m^2 (<70 years) (P = 0.002), and was inversely related to patient's age (r^2 = 0.857; P < 0.00001). Total plasma clearance of the formulation vehicle Cremophor EL was 150 $\pm$ 60.7 vs 115 $\pm$ 39.2 mL/h/m^2 (P = 0.04).

Conclusions:

These data indicate a 50% change in total body clearance of unbound paclitaxel and a concomitant significant increase in systemic exposure with age, most likely as a result of altered Cremophor EL disposition. The clinical relevance of these observations with respect to toxicity profiles and antitumor efficacy requires further evaluation.

INTRODUCTION

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

The cytotoxic agent paclitaxel (Taxol) is registered for the treatment of advanced breast cancer, for which it is usually administered in second line therapy as a single agent every 3 weeks at a dose of 175-225 mg/m^2. Frequently encountered side effects are neutropenia, neuropathy, asthenia and alopecia. Weekly administration of paclitaxel has demonstrated sustained efficacy together with a more favorable toxicity profile lacking severe myelotoxicity [5]. While the related agent docetaxel, despite a dose reduction of 75% of the standard dose of 100 mg/m^2 every 3 weeks, appeared to be too toxic in non-pretreated patients aged >70 years with metastatic breast cancer, a weekly schedule at a dose of 36 mg/m^2 in heavily pretreated elderly patients indeed appeared effective and well tolerated [6, 7]. Yet unpublished data suggest similar efficacy for weekly paclitaxel. This way of administering paclitaxel therefore seems an attractive chemotherapeutic alternative for elderly women with metastatic breast cancer, although no pharmacologic data are yet available. Here, we studied the pharmacokinetics of paclitaxel and its formulation vehicle CrEL in patients with breast cancer aged ≥70 years treated in a weekly schedule, and compared the results with a control group of patients aged <70 years treated in a similar way.

PATIENTS AND METHODS

Eligibility criteria

Two groups of patients were studied based on age; patients aged ≥ 70 years were eligible if they had histologic or cytologic confirmed breast cancer, unresponsive to hormonal therapy, while patients aged between 18 and 70 years were eligible if they had any histologic or cytologic confirmed metastatic solid tumor for which treatment with paclitaxel was a viable option. Prior to recruiting male patients in the control group, it was confirmed that there are no sex-related differences in unbound paclitaxel clearance. This was investigated in unpublished data from a historic patient population treated at the Rotterdam Cancer Institute (Rotterdam, the Netherlands) with single agent paclitaxel given as a 1-h i.v. infusion at dose levels ranging between 70 and 200 mg/m^2. The group consisted of 10 males (median age, 58 years; range, 46 – 70 years) and 30 females (median age, 57 years; range, 29 – 71 years). The mean (± SD) values for clearance of unbound paclitaxel in male and female patients were 200 ± 35.6 and 195 ± 48.3 L/h/m^2, respectively, which is a not statistically significant difference [P = 0.75; mean difference (± SE), 5.26 ± 16.3 L/h/m^2; 95% confidence limits for the mean difference, -27.8 and 38.3; unpaired two-tailed Student's t-test].

Other criteria for patient enrollment were (*i*) acceptable performance status according to the World Health Organization criteria (0-2), (*ii*) an adequate bone marrow function (defined by pretherapy values of hemoglobin ≥ 6.0 mM, absolute neutrophil count > 1.5 × 10^9/l, and platelet count > 160 × 10^9/l), (*iii*) adequate renal function (creatinine levels <175 μM) and (*iv*) adequate hepatic function (bilirubin levels < 25 μM). Patients with other malignancies during

the past 5 years, neuropathy graded ≥ 2, symptomatic cardiac disease, and/or signs of central nervous system involvement were excluded. All patients gave written informed consent, and the study protocol was reviewed and approved by the Rotterdam Cancer Institute review board.

Treatment Schedule and Patient Evaluation

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

Pharmacokinetics and Pharmacodynamics

Blood samples for pharmacokinetic analysis were collected from all patients on day 1 of the first administration from a vein in the arm opposite to the one used for drug infusion. Blood samples of 5 ml were obtained at the following time points: before infusion, at 0.5 and 1 h (end of infusion) during infusion, and at 5, 15, 30 minutes and 1, 2, 4, 8, 12 and 24 h after end of infusion. Samples were collected in tubes containing lithium heparin as anticoagulant and were subsequently centrifuged at 3000 × *g* for 10 minutes at 4ºC to separate plasma and cells. Plasma samples were stored frozen at -80ºC until analysis.

In view of the profound nonlinear disposition of paclitaxel in patients [8], the pharmacological consequences of the treatment in patients with increasing age can not be predicted based on total plasma levels alone when different dose groups are compared. Since the area under the plasma concentration-time curve (AUC) of unbound paclitaxel is a linear function of the dose administered [9, 10], we focused here on comparing the fraction unbound paclitaxel between the 2 groups. Concentrations of total paclitaxel in plasma samples were determined by a validated reversed-phase high-performance liquid chromatography with ultraviolet detection as described earlier [11]. The free drug fraction of paclitaxel was measured by using a reproducible equilibrium dialysis method using a tritiated-paclitaxel tracer [9]. Coinciding levels of Cremophor EL (CrEL) were measured by a colorimetric dye-binding microassay, as published [12]. The kinetics of paclitaxel and CrEL were evaluated for each patient separately by a linear 3-compartment model and by model-independent methods, respectively, using the Siphar v4.0 software package (InnaPhase, Philadelphia, PA). This program determines the slopes and intercepts of the logarithmically plotted curves of multiexponential functions using non-linear least-squares, iterative steps.

Initial parameter estimates were determined by an automated curve-stripping procedure. The mathematical equations describing the drug concentration $C_{(t)}$ at any time t during (eq. 1) and after i.v. administration (eq. 2) are given by:

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

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

In these equations, λ_i is the component of the *i*-th exponential term, C_i is the initial concentration of the *i*-th component of the curve, and T_{inf} is the infusion duration. In all cases, paclitaxel-concentration-time curves were best described with a tri-exponential model, which gave the lowest Akaike information criterion, without any demonstration of saturable behavior ($r^2 = 0.996 \pm 0.002$, root mean square error = $13.5 \pm 3.53\%$). The curve fitting procedure with this model yields the parameters C_1, C_2, C_3, λ_1, λ_2, and λ_3. Individual AUC values were determined on the basis of the best fitted curve as the exact integral of the concentration-time plots from time zero with extrapolation to infinity using the terminal disposition rate constant. The apparent clearance was defined as dose (expressed in mg/m^2) divided by AUC. The apparent volume of distribution at steady-state was calculated as the product of clearance and the mean residence time, estimated from the AUC and the area under the (first) moment-time curve. Peak plasma concentrations were put on par with observed (experimental) drug levels immediately following the end of infusion. The fraction unbound paclitaxel was defined as the ratio of unbound paclitaxel AUC and total paclitaxel AUC. Pharmacodynamics was assessed by calculation of the relative hematologic toxicity of white blood cell count (WBC) and absolute neutrophil count (ANC), defined as:

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

Statistical Evaluation

ll pharmacologic parameters are expressed as mean values $\pm$ SD. Differences in any of the studied pharmacokinetic and pharmacodynamic parameters between the 2 age groups or within the control group between male and female patients were evaluated statistically using an unpaired two-tailed Student's t-test after testing for normality. The relationship between clearance of unbound paclitaxel and age was evaluated using least-squares linear regression analysis and adjusted R^2 values to compensate for the expected chance prediction when the null hypothesis is true. The level of significance was set at $P<0.05$. All statistical calculations were performed using Number Cruncher Statistical System v5.X (Jerry Hintze, East Kaysville, UT; 1992).

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

Characteristic	patients ≥70 years	patients <70 years
No. studied	8	15
Age (years)	77 (70-84)	54 (22-69)
BSA (m^2)	1.75 (1.45-1.91)	1.76 (1.31-2.37)
Weight (kg)	71.6 (54.0-84.3)	68.1 (36.6-116)
Height (cm)	160 (150-167)	165 (157-185)
Sex (M/F)	0/8	7/8
Serum albumin (g/dl)	4.2 (3.8-4.7)	3.8 (2.4-4.7)
Total serum protein (g/dl)	7.4 (6.9-8.0)	6.9 (4.9-7.9)
Hematocrit (l/l)	0.35 (0.27-0.40)	0.35 (0.29-0.44)

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

RESULTS

Patient Characteristics

A total of 8 elderly patients and 15 patients aged <70 years was studied (Table 1), and all were evaluable for paclitaxel pharmacokinetics and toxicity. The median age in the groups was 77 years (range, 70-84) and 54 years (range, 22-69), respectively. Other patient characteristics and baseline clinical chemistry values were similar between the 2 groups (Table 1). In the elderly patient group, 7 of 8 patients had received prior hormonal therapy for metastatic disease, and a total of 76 paclitaxel infusions was administered, with a mean number of 4 cycles (range, 1-6) per patient. Only 4 administrations (5%) were delayed, of which 1 was due to erysipelas and 3 were due to non-therapy related morbidity. Dose reductions were not required in any patient from both groups.

Pharmacokinetics

Unbound paclitaxel concentration-time curves for both groups are shown in Fig. 1. Overall, the interpatient variability in unbound paclitaxel clearance was moderate (coefficient of variation, 30.8%). A summary of pharmacokinetic data of unbound paclitaxel, total paclitaxel and CrEL is shown in Table 2.

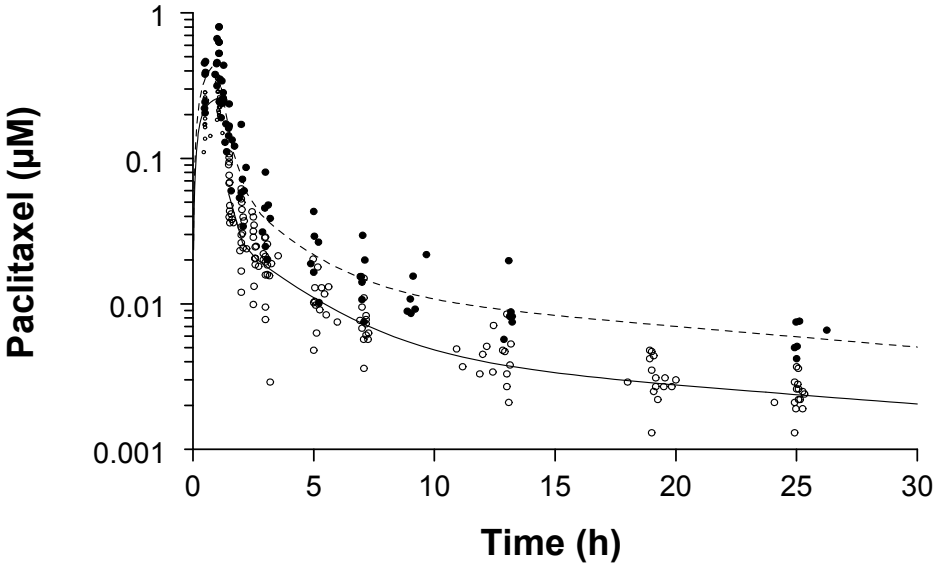

Figure 1. Plasma concentration-time profiles of unbound paclitaxel in elderly (≥ 70 years) patients (n = 8; closed symbols and dotted line) and patients < 70 years (n = 15; open symbols and solid line) receiving a 1-h i.v. infusion of paclitaxel at a dose levels of 80 and 100 mg/m^2, respectively. Data from the elderly group were normalized to a paclitaxel dose of 100 mg/m^2, by multiplying unbound paclitaxel concentrations (Cu) by the dose difference [Cu × (100/80)]. The mathematical equations describing the drug concentration ($C_{(t)}$) at any time (t) during (eq. 1) and after i.v. administration (eq. 2) are given by: $C_{(t)} = \Sigma \{C_i / (\lambda_i \times T_{inf}) \times (1 - e^{(-\lambda i \times t)})\}$ (eq. 1) and $_{(t)} = \Sigma \{C_i / (\lambda_i \times T_{inf}) \times (e^{(-\lambda i \times [t - Tinf])} - e^{(-\lambda i \times t)})\}$ (eq. 2). The model parameters were C_1 = 1.19 µM, C_2 = 0.076 µM, C_3 = 0.013 µM, λ_1 = 2.96 h^{-1}, λ_2 = 0.444 h^{-1}, and λ_3 = 0.033 h^{-1} for elderly patients, and C_1 = 0.976 µM, C_2 = 0.033 µM, C_3 = 0.005 µM, λ_1 = 4.26 h^{-1}, λ_2 = 0.350 h^{-1}, and λ_3 = 0.029 h^{-1} for younger patients.

In the control group, there were no significant sex-related differences in unbound paclitaxel clearance (males vs females, 251 ± 74.3 vs 237 ± 43.0 L/h/m^2; P = 0.67), total paclitaxel clearance (18.4 ± 5.63 vs 16.6 ± 2.69 L/h/m^2; P = 0.43), the fraction unbound paclitaxel (0.084 ± 0.007 vs 0.085 ± 0.005; P = 0.76), and the clearance of CrEL (115 ± 41.7 L/h/m^2 vs 114 ± 39.2 L/h/m^2; P = 0.94). Therefore, pharmacokinetic data were directly compared between the groups in spite of gender being unequally represented in the elderly and younger patients.

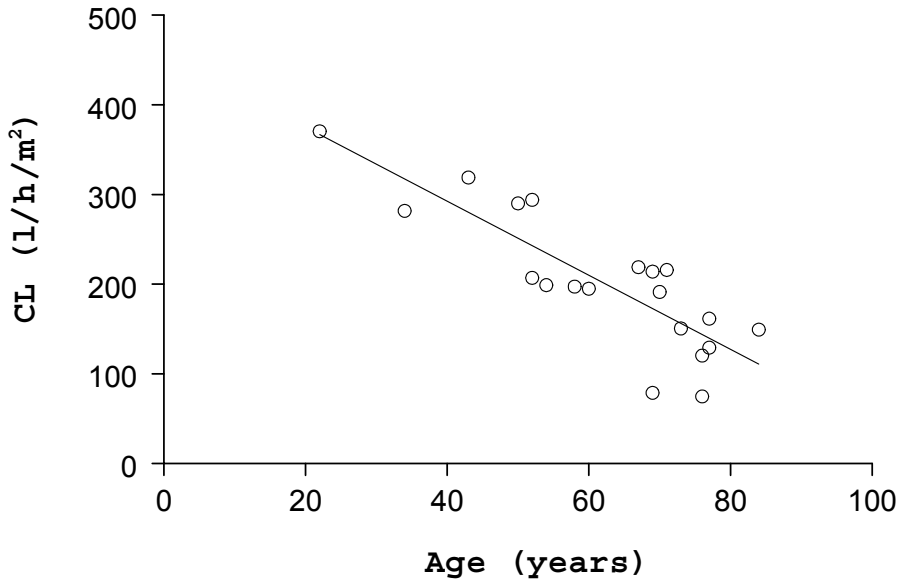

Figure 2. Relationship between patient age and unbound paclitaxel clearance (CL). The solid line indicates the fit of a least-squares linear regression analysis [CL=(-4.127×age)+457.5; adjusted r^2=0.847; P<0.00001].

The apparent clearances of unbound paclitaxel and total paclitaxel were significantly different between the 2 age groups, with mean values (elderly vs younger) of 124 ± 35.0 vs 244 ± 58.8 l/h/m^2 (P = 0.002) and 13.9 ± 2.31 vs 17.4 ± 4.52 l/h/m^2 (P = 0.04), respectively (Table 2). The difference in unbound paclitaxel clearance remained significant when the 8 females in the elderly group were compared to the 8 females in the control group (124 ± 35.0

vs 237 ± 43.0 L/h/m^2; P = 0.002). In the entire patient population, a significant negative correlation was observed between age and unbound paclitaxel clearance [Fig. 2; clearance (in l/h/m^2) = (-4.127 × age) + 457.5; adjusted R^2 = 0.847; P < 0.00001]. The unbound paclitaxel volume of distribution at steady state was also significantly smaller in the elderly patients (1105 ± 300 vs 2546 ± 754 L/m^2; P = 0.04), whereas the terminal disposition half-life was similar (18.0 ± 7.40 vs 21.7 ± 4.33 h; P = 0.14). The clearance of CrEL was significantly faster in elderly patients as compared to the control group (150 ± 60.7 vs 115 ± 39.2 ml/h/m^2; P = 0.04).

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

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

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

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

Toxicity profiles

In the elderly group, one patient experienced a grade 3 toxicity (neutropenia and skin toxicity with generalized erythroderma), while no grade 3-4 toxicities were noted in any of the other patients. In spite of the difference in paclitaxel dose administered, no significant difference was observed in hematologic pharmacodynamics between the 2 groups as defined by the percent decrease in WBC (40.7 ± 7.96 vs 45.9 ± 15.5 %; P = 0.39) and the percent decrease in ANC (50.8 ± 14.6 vs 56.3 ± 14.8 %; P = 0.40).

DISCUSSION

In the present study we have described for the first time the pharmacokinetics of unbound paclitaxel in cancer patients as a function of age. Overall, our data indicate that the clearance of unbound paclitaxel, following weekly administration as a 1-h i.v. infusion, is approximately 50% reduced in elderly patients (≥ 70 years) as compared to younger patients, and that age is a significant predictor of paclitaxel disposition in the population studied. These data complement previous knowledge on the clinical pharmacology of paclitaxel, and may have important practical implications for its optimal use. Indeed, while some studies examined the efficacy and feasibility of chemotherapy in elderly patients with metastatic breast cancer, little is known about the pharmacokinetic behavior of the anticancer agents involved, with the notable exception of some anthracyclines and *Vinca* alkaloids [4]. For doxorubicin a trend for delayed clearance in elderly cancer patients has been documented, while the AUC of daunorubicinol, an active metabolite of daunorubicin, was significantly increased in 13 elderly patients with acute leukemia [13,14]. In patients aged ≥ 70 years the clearance of vinorelbine was reduced by 30-40%, as compared to adult patients [15]. To adjust for decreasing renal function with age, a study investigating combination chemotherapy with cyclophosphamide, methotrexate and 5-fluorouracil in women aged ≥ 65 years used creatinine clearance for calculation of appropriate doses of cyclophosphamide and methotrexate [16]. While indeed less toxicity resulted, unfortunately no pharmacokinetic analysis was performed.

For paclitaxel, only scarce data are available on the effect of aging on the agent's pharmacokinetic behavior. Nakamura et al. performed a retrospective analysis investigating total paclitaxel pharmacokinetics in 120 lung cancer patients, of whom 28 were elderly, treated at a dose of 210 mg/m^2 given over 3 h in a 3-weekly regimen [17]. These authors could not detect any differences in AUC, peak concentration, terminal disposition half-life, and time above the threshold of 0.1 μM between patients aged < 70 years and those > 70 years [17]. Likewise, Fidias et al. recently reported that the clearance of total paclitaxel in a group of 8 patients with non-small cell lung cancer (age, ≥ 70 years) treated with a dose of 90 mg/m^3 as a 1-h i.v. infusion was comparable to values that have been reported for studies

involving younger patients [18]. However, these apparent inconsistencies with our current findings need to be interpreted with great caution, as in the study performed by Fidias et al. no control group involving younger patients was studied, and a host of confounding factors might influence their overall conclusions, including differences in paclitaxel dose administered between the comparative trials, variability in analytical methods employed, and parameter calculation procedures used. In contrast to conclusions drawn in the above studies [17, 18], Lichtman et al. recently reported in abstract form a significant difference in AUC and clearance of total paclitaxel with advancing age in 113 patients treated with paclitaxel at a dose of 175 mg/m^2 administered as a 3-h infusion [19]. The total paclitaxel clearances in patients aged 55-64 years and in 28 patients > 75 years were 10.9 and 8.21 l/h/m^2, respectively, which was significant at P = 0.012. Unfortunately, these investigators used a limited-sampling strategy for AUC calculation using only few timed samples early after dosing (up to 7 h), which may have caused a serious flaw in that any alteration in drug elimination as a result of aging (e.g., metabolic and excretory routes) may remain undetected by such methodology. Moreover, as it cannot be excluded that any alteration in paclitaxel disposition is (partially) associated with changes in CrEL pharmacokinetics as a function of age (see below), the use of total plasma concentrations and subsequent calculation of total plasma clearance, as done in the mentioned studies [17-19], may be essentially less meaningful. The results of the various investigations performed to date further emphasize the need to simultaneously study paclitaxel pharmacokinetics in a control group of younger patients when evaluating the role of patient age in drug disposition.

Previous investigations have demonstrated the importance of unbound paclitaxel AUC as a pharmacokinetic parameter to delineate exposure-toxicity relationships, both with 1-h and 3-h infusion schedules [10, 20]. Although intuitively the unbound fraction of paclitaxel accounts for the (cyto)toxic actions of the treatment, its concentration has never been investigated in elderly patients. We have recently shown that CrEL, the vehicle used for i.v. paclitaxel administration, has a substantial impact on the fraction unbound paclitaxel [21, 22]. Although the exact mechanism underlying this interaction has not yet been fully elucidated, the presence of CrEL in the circulation as large polar micelles is thought to entrap paclitaxel, thereby reducing cellular accumulation of paclitaxel in blood cells (e.g., erythrocytes) and altering the fraction unbound paclitaxel in whole blood. Since CrEL clearance increases with prolonged duration of infusion from 1- to 3- and 24-h, the systemic exposure to unbound paclitaxel and CrEL significantly depends on the duration of drug infusion [23]. Our current data on unbound paclitaxel levels in elderly patients should therefore not be compared with studies using other infusion schedules. In any event, the demonstration that CrEL clearance is significantly increased by 30% in elderly patients, combined with the notion that CrEL micelles act as the principal carrier of paclitaxel in the systemic circulation [24], suggests that this phenomenon likely contributes substantially to the changes in unbound paclitaxel clearance.

The mechanisms underlying the age-dependent pharmacokinetics of CrEL are not clear. In fact, the faster clearance of CrEL in the group of elderly patients is rather unusual, because for most xenobiotics that exhibit age-dependent pharmacokinetics, clearance tends to decrease with advancing age [25]. It has been shown previously that elimination routes of polyoxyethylated surfactants like CrEL are associated with esterase-mediated metabolic breakdown within the systemic circulation [22]. One possibility to explain the age-dependent pharmacokinetics of CrEL would be that CrEL biotransformation takes place at an accelerated rate as a result of elevated enzyme levels in the systemic circulation in elderly patients. This would be consistent with the observation that the clearance of CrEL is significantly higher (approximately 3 to 4 fold) in adult patients with moderate to severe hepatic dysfunction as compared to patients with normal hepatic function [26]. This and several other possibilities are currently under investigation.

As paclitaxel elimination is almost entirely caused by metabolic breakdown through cytochrome P450 (CYP) isoforms 3A4 and 2C8 [27], an alternative explanation for the altered paclitaxel clearance is an impaired hepatic function with advancing age. Although eligibility criteria excluded patients with an elevated bilirubin and all patients entered had normal values of aspartate and alanine aminotransferases, these laboratory values do not represent the actual capacity of hepatic metabolism [28]. One possibility to investigate the role of altered liver function in relation to the current findings would be to determine pretreatment CYP3A4 and CYP2C8 activity in each patient using a functional surrogate such as the erythromycin breath test [29]. Additional clinical and pharmacological information is currently being collected by implementation of such assay in ongoing trials with paclitaxel as well as docetaxel to further explore the role of enzyme capacity in taxane disposition in elderly patients.

Collectively, our study demonstrates that CrEL and unbound paclitaxel clearance are subject to considerable changes depending on age. In our patient population, hematologic toxicity was relatively mild and not clinically relevant due to the low paclitaxel doses, precluding detection of statistically significant differences between both age groups. More insight will be provided by the ongoing CALGB 9762 study, evaluating paclitaxel pharmacology in relation to patient age with drug administration over 3-h in a 3-weekly schedule at higher doses [19]. As the unbound fraction of paclitaxel is responsible for its cellular actions and its clearance is remarkably reduced in the elderly, this observation warrants further studies on efficacy and feasibility of paclitaxel in aged patients using dose-dense regimens.

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CHAPTER 6

Phase I Pharmacokinetic and Sequence Finding Study of the Combination of Docetaxel and Methotrexate in Patients with Solid Tumors

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SUMMARY

This phase I study was performed to assess the feasibility and possible enhanced anti-tumor activity of the sequential administration of methotrexate (MTX) and docetaxel in patients with solid tumors. Pharmacokinetic analysis was performed to investigate the pharmacokinetic interaction of the two agents.

A total of 22 patients were enrolled, a total of six dose levels were investigated. MTX (day 1+15) 30, 40 and 50 mg m^2 + Docetaxel (day 2 or day 1) 75 and 85 mg m^2 with supportive care measures. Both haematological and non-haematological toxicities were significant, preventing dose escalation above MTX 40 mg/m^2 + Docetaxel 75 mg/m^2. Four partial responses were documented, three in patients with breast cancer, one in a patient with urothelial cell cancer. Pharmacokinetic data did not give an explanation for the significant toxicity as they revealed no interaction of docetaxel and methotrexate kinetics. Methotrexate and 17-OH methotrexate kinetics seemed to be independent of the administration of docetaxel and the moment of docetaxel administration appeared not to influence methotrexate kinetics.

The sequential administration of methotrexate and docetaxel results in significant toxicity without any evidence of a clinical benefit.

INTRODUCTION

Docetaxel (D), a microtubule polymerisation promotor, has proven to be effective as a single agent in the second-line treatment of patients with metastatic breast cancer previously treated with anthracycline-based chemotherapy (FEC or FAC), with response rates up to 55% [1, 2]. To date, docetaxel single agent given at 100 mg/m^2 every 3 weeks has become standard second-line chemotherapy for patients with metastatic breast cancer [3]. Methotrexate (MTX), a dihydrofolate reductase inhibitor, also has demonstrated single agent activity in first- as well as second-line treatment of metastatic breast cancer [4, 5].

As anthracycline-based chemotherapy is considered to be more effective than classical CMF, and the first is better tolerated with the availability of $5HT_3$ antagonists, the FEC or FAC combination regimens have become the most widely applied first-line treatment in metastatic breast cancer [6]. As a consequence, methotrexate is not frequently used in first-line, and with the established role of docetaxel as second-line therapy, methotrexate is no longer incorporated in second-line regimens either.

We conducted a phase I study of the combination of docetaxel and methotrexate as a second-line regimen in breast cancer patients. Since docetaxel and methotrexate can be considered effective agents in other solid tumors, patients with non-small cell lung cancer, head and neck cancer, urothelial cancer or gastric cancer were also eligible for this dose finding study. The study included a pharmacokinetic analysis.

PATIENTS AND METHODS

Eligibility

Breast cancer patients relapsing after or progressing during anthracycline based combination chemotherapy were eligible for the study. Patients with other solid tumors for whom treatment with docetaxel and/or methotrexate was considered of therapeutic intent were also eligible for study entry. Additional eligibility criteria were: histologically-confirmed solid tumors; age ≥ 18 years; WHO performance status 0-2; adequate haematopoietic (absolute neutrophil count ≥ 1.5 x 10^9/liter and platelet count ≥ 100 x 10^9/liter), hepatic (total serum bilirubin < 1x upper normal limit, transaminases < 1.5 x upper normal limit, alkaline phosphatase < 2.5 x upper normal limit (except in the presence of only bone metastases and in absence of any liver disorders) and renal function (serum creatinine < 100 µmol/liter or creatinine clearance ≥ 60 ml/min); no extensive radiotherapy for at least 4 weeks prior to study entry; indicator lesions should not have been irradiated, previous chemotherapy had to be stopped for at least 4 weeks before study entry; hormonal treatment had to be stopped before study entry; life expectancy of at least twelve weeks; no childbearing potential or using adequate contraception; no previous chemotherapy for systemic disease comprising methotrexate or a taxoid; no clinically relevant contra-indications for the use of corticosteroids; no somatic or psychic illness that could interfere with the planned treatment or follow-up; no concomitant use of other investigational drugs or anticancer treatment. The clinical protocol was approved by the institutional ethics committee and all patients provided written informed consent.

Pretreatment and Follow-up

Before the start of treatment a medical history was taken and physical examination, laboratory studies, electrocardiogram and imaging studies for tumor measurement were performed. Laboratory studies included a complete blood cell count analysis and measurement of white blood cell differential, sodium, potassium, creatinine, creatinine clearance if indicated, serum calcium, total protein, albumin, bilirubin, alkaline phosphatase, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH) and urinanalysis.

History, physical examination and toxicity scoring according to National Cancer Institute Common Toxicity Criteria (NCI-CTC) were performed every week. Blood counts were performed weekly. The other laboratory tests were repeated on days 1 and 15. Tumor measurements were performed at 6 weeks intervals until documentation of progressive disease (PD). In patients with evaluable and measurable disease, standard WHO response criteria were used.

Drug administration

Methotrexate was administered as an i.v. push on days 1 and 15. Docetaxel was administered as a 1-hour infusion on day 2. The sequential administration of methotrexate and docetaxel was based on preclinical data showing synergistic antitumor activity when docetaxel was administered at least 12 hours after methotrexate [7, 8]. Following dose limiting toxicity (DLT) observed in the first 3 patients at the first dose level and a recent report on a more favourable dose-toxicity profile by the simultaneous administration of both agents on day 1 [9], the protocol was amended to test this regimen in the second cohort before proceeding with our original study. In this cohort, less toxicity was observed. Since, apparently, the 24 hour interval augmented the clinical interaction and in order to obtain the recommended dose and to investigate potential evidence for enhanced antitumor efficacy with that schedule, we continued our study with the initial MTX on day 1 and docetaxel on day 2 schedule, complemented with maximum supportive measures to reduce myelosuppression and mucositis. These measures consisted of leucovorin 15 mg orally (p.o.) 4 times daily, starting 24 hours after each methotrexate administration for a total of 2 days, plus lenograstim 263 µg subcutaneously (s.c.) once daily, days 4-10. Treatment was repeated every 3 weeks. All patients received premedication with dexamethasone, p.o. 8 mg twice daily (b.i.d.), starting 1 day before each infusion of docetaxel, and given for 3 days.

In each cohort, three patients were treated until dose limiting toxicity (DLT) during the first cycle was observed in one patient. If two or more DLTs were observed, that dose was considered too high. In the case of one DLT, the accrual of three additional patients was required. If DLT was seen in no more than one patient on that dose level, the dose was to be escalated. From then onwards six patients were to be entered at each dose level and dose escalation was to continue if DLT was seen in no more than one patient. The dose level at which two or more patients experienced DLT was to be considered the maximum tolerated dose (MTD). DLT was defined as grade 4 neutropenia lasting ≥ 5 days, or neutropenia with fever causing hospitalisation for administration of intravenous antibiotics, or grade 4 thrombocytopenia, or grade 2 mucositis lasting ≥ 5 days, or any grade 3 or 4 non-haematological toxicity (except grade 3 nausea). The following dose levels and escalating steps were planned: MTX 30 mg/m^2 – Docetaxel (D) 75 mg/m^2 ; MTX 30 mg/m^2 – D 85 mg/m^2 ; MTX 30 mg/m^2 – D 100 mg/m^2 ; MTX 40 mg/m^2 – D 100 mg/m^2 ; and MTX 50 mg/m^2 – D 100 mg/m^2 . If a patient required dose reductions after experiencing DLT, the next cycles in this patient were evaluated for toxicity at the lower dose level.

Sampling Schedule and Drug Analysis

Blood specimens were obtained in all patients during the first and second courses of treatment. Blood volumes of 5 mL were drawn directly into Vacutainer glass tubes containing lyophilized lithium heparin (Becton Dickinson, Meylan, France) from a peripheral venous access device. Samples for MTX analysis were collected immediately before treatment and

at 0.08 (end of infusion), 0.5, 2, 4, 6, 24, and 48 h after dosing. All blood samples were centrifuged immediately for 5 min at 3000 *g* to yield plasma, which was frozen in polypropylene vials at -80°C until the time of analysis by reversed-phase high-performance liquid chromatography (HPLC) as described [10]. In brief, samples were purified by protein precipitation with acetone followed by solvent extraction with a mixture of *n*-butanol and diethyl ether. The water phase was subsequently further processed with methanol and dried under N_2. The compounds of interest, including the unchanged parent drug and its 7-hydroxyl metabolite (7-OH-MTX), were separated on an analytical column (150 × 4.6 mm, I.D.) packed with (5 µm P.S.) RP Inertsil ODS-80A material (delivered by Alltech, Breda, The Netherlands) and were eluted in a solvent system containing 5% (v/v) tetrahydrofuran in water (pH 2.0). The column effluent was monitored by ultraviolet (UV) absorption measurements using a Spectra Physics Model UV-2000 detector (San Jose, CA, USA) set at 313 nm. Detection and integration of chromatographic peaks was performed by the Fisons ChromCard data analysis system connected to an ICW workstation (Milan, Italy). Calibration curves were fitted by weighted ($1/x^2$) linear-regression analysis by using the peak area of methotrexate versus the concentrations of the nominal standard (x). The detector response was linear in a concentration range of 10 - 10000 ng/mL, with a lower limit of quantitation of MTX and 7-OH-MTX of 10 ng/mL using 1-mL sample aliquots. Values for accuracy and within-run and between-run precision were 95.5 - 111% and 3.69 - 11.0%, respectively.

Evaluation of docetaxel pharmacokinetics was performed using plasma samples obtained before drug infusion and at 0.5, 1, 2, 4, 6, and 24 h after the start of drug infusion. Samples were analyzed by a HPLC method described in detail elsewhere [11]. This method is based on extraction of docetaxel from the plasma matrix with a mixture of acetonitrile and *n*-butyl chloride (1:4, v/v), using paclitaxel as an internal standard. Chromatographic separations were performed on the same analytical column (see above) using a mobile phase composed of water-methanol-tetrahydrofuran (37.5:60.0:2.5, v/v/v) containing 0.1% (v/v) ammonium hydroxide, with the pH adjusted to 6.0 (formic acid). During analysis the column was maintained at 60°C using a Spark Model SpH99 column oven (Meppel, The Netherlands), and the eluent was monitored at a wavelength of 230 nm. The lower limit of quantitation was 10 ng/mL, with the accuracy and precision ranging from 95.5 - 106% and 0.33 - 3.34%, respectively.

Pharmacokinetic Data Analysis

Individual plasma concentration-time data were analyzed using the Siphar v4.0 software package (SIMED, Créteil, France), by determination of slopes and intercepts of the plotted curves with multi-exponential functions. The program determined initial parameter estimates and these were improved using an iterative numerical algorithm based on Powell's method [12]. Model discrimination was assessed by a variety of considerations, including

visual inspection of the predicted curves, dispersion of residuals, minimisation of the sum of weighted squares residuals, and the Akaike information criterion [13].

In all cases, concentration-time profiles of both MTX and docetaxel were best fitted to a bi-exponential equation after zero-order input with weighting according to y_{obs}^{-1}. Final values of the iterated parameters of the best-fit equation were used to calculate pharmacokinetic parameters using standard equations [14].

Statistical Considerations for Pharmacokinetics

Parameters of all compounds are reported as mean values ± standard deviation. The difference in pharmacokinetic parameters between the MTX administration days and between patient cohorts was evaluated statistically using a two-sided parametric matched-pairs Student's *t*-test (after testing for normality) *plus* the 95% confidence intervals and the Kruskal-Wallis statistic, respectively. The effect of docetaxel interval time on the pharmacokinetics of MTX was analysed by the Mann-Whitney test. Probability values (two-sided) of less than 0.05 were considered statistically significant. All calculations were done on the Number Cruncher Statistical Systems v5.× software package (J.L. Hintze, East Kaysville, UT, 1992).

Table 1. Patient characteristics

Patients treated			22
Number of cycles			79
Age median (range) yrs			49 (30-64)
WHO performance score	0		5
	1		14
	2		3
Sex	male		9
	female		13
Prior chemotherapy			
	No	Yes	Total
Primary tumor			
Breast cancer	-	9	9
Non-small cell lung cancer	-	3	3
Stomach cancer	2	-	2
Urothelial cell cancer	3	-	3
Head/neck cancer	3	-	3
Sarcoma	1	-	1
Adenomacarcinoma of the unknown primary	1	-	1

Table 2. Myelotoxicity (worst toxicity per cycle) at each dose level per cycle.

Mtx mg/m^2	Docetaxel mg/m^2	Pts. (n)	Cycles (n)	WBC Grade (CTC scale)				ANC Grade (CTC scale)				PLTS Grade (CTC scale)			
				1	2	3	4	1	2	3	4	1	2	3	4
30	75 d2	3	12	0	0	6	4	1	1	1	7 1*	1	0	0	0
30	75 d1	4	21	9	2	6	0	3	5	4	4 1*	4	0	0	0
30	75 d2 Supp. Care	3 (4)	15 (16)	1	2	10	1	0	2	3	7 1*	1	0	0	0
30	85 d2 Supp. Care	3	9	0	1	4	3	0	1	1	6	3	0	0	0
40	75 d2 Supp. Care	7 (9)	10 (19)	0	1	7	2	0	0	2	6 2*	0	0	0	0
50	75 d2 Supp. Care	2	2	0	0	0	1	0	0	0	1	0	0	0	0

* denotes dose limiting toxicity events
numbers in brackets ( ) represent total of patients / cycles evaluated for toxicity at a certain dose level, including patients treated / evaluable cycles at that dose level after dose reduction.

Table 3. Main toxicity's (worst per cycle) at each dose level, expressed in the number of cycles that they occurred

Mtx mg/m^2	Docetaxel mg/m^2	Pts. (n)	Cycles (n)	Mucositis Grade (CTC scale)				Fatigue Grade (CTC scale)				Myalgia Grade (CTC scale)			
				1	2	3	4	1	2	3	4	1	2	3	4
30	75 d2	3	12	6	1*	1*	0	2	5	1	0	1	2	0	0
30	75 d1	4	21	9	2	0	0	10	0	1	0	1	1	0	0
30	75 d2 Supp. Care	3 (4)	15 (16)	7	5	0	0	8	5	0	0	0	0	0	0
30	85 d2 Supp. Care	3	9	0	2 1*	1*	0	0	5	1*	0	1	1	0	0
40	75 d2 Supp. Care	7 (9)	10 (19)	5	3	0	0	4	7	0	0	2	5	0	0
50	75 d2 Supp. Care	2	2	0	0	0	0	0	1	1*	0	0	1	1*	0

* denotes dose limiting toxicity events
numbers in brackets ( ) represent total of patients / cycles evaluated for toxicity at a certain dose level, including patients treated / evaluable cycles at that dose level after dose reduction.

RESULTS

A total of 22 eligible patients entered this study, receiving a total of 79 cycles. Patient characteristics are shown in Table 1. All patients were evaluable for toxicity. Eighteen of the 22 patients were also evaluable for tumor response. Toxicity data are shown in Tables 2 and 3. Myelosuppression, mucositis and fatigue were the principal DLTs observed with this regimen. Neutropenia grade 4 prevented the administration of MTX day 15 in 33% of cycles at the first two dose levels. The following dose levels were explored:

1. MTX(d1+15) 30 mg/m^2 - Docetaxel(d2) 75 mg/m^2 3 pts;
2. MTX(d1+15) 30 mg/m^2 - Docetaxel(d1)75 mg/ m^2 4 pts;
3. MTX(d1+15) 30 mg/m^2 - Docetaxel(d2) 75 mg/m^2 + supportive care measures 3 (4) pts;
4. MTX(d1+15) 30 mg/m^2 - Docetaxel(d2) 85 mg/m^2 + supportive care measures 3 pts;
5. MTX(d1+15) 40 mg/m^2 - Docetaxel(d2) 75 mg/m^2 + supportive care measures 7 (9) pts
6. MTX(d1+15) 50 mg/m^2 - Docetaxel(d2) 75 mg/m^2 + supportive care measures 2 pts.

(Numbers of patients in brackets represent the total number of patients evaluated for toxicity at a dose level, including patients who were treated at this dose level after dose reduction).

At the first dose level (MTX30/D75 d2), all three patients obtained a DLT in the first course. Two patients had significant mucositis (grade 3 and 2 lasting more than 5 days). In the third patient, methotrexate day 15 was postponed because of neutropenia grade 4 lasting more than 5 days. According to the amended protocol, at the second dose level (MTX30/D75 d1) patients received methotrexate and docetaxel both on day 1 followed by methotrexate on day 15. One out of the four patients treated with this schedule experienced grade 4 neutropenia preventing the administration of methotrexate at day 15. There were no DLT events observed. Hence, the toxicity at this dose level compared favourably with that obtained with the sequential administration in dose level 1. At the next dose levels, again using the 24-hour interval between methotrexate and docetaxel the supportive care measures (s.c.m.) were introduced.

At dose level 3 (MTX30/D75 d2 + scm) febrile neutropenia was observed in one patient, which was considered DLT. No other significant toxicities were seen at this dose level. Since there were no additional cases of neutropenic fever amongst the six patients on dose level 1 and 3, escalation to dose level 4 was performed. All patients received methotrexate on day 15 in all courses. At dose level 4 (MTX30/D85 d2 + s.c.m.) one out of three patients experienced mucositis grade 2 concomitant with fatigue grade 3 which were considered dose-limiting and one patient obtained mucositis grade 3 in the second course that was also considered dose-limiting since this patient already experienced significant mucositis grade 2 in the first course. At dose level 5 (MTX40/D75 d2 + s.c.m.) febrile neutropenia was observed in one out of six patients treated. The first two patients who were treated at dose level 6

(MTX50/D75 d2 + s.c.m.) required dose reduction after obtaining DLT. One patient had grade 3 fatigue, the second patient had grade 3 myalgia. This dose level was considered MTD for the escalation of methotrexate. In the subsequent courses at reduced dose (level 5), no serious toxicities were reported. Dose level 5 (MTX40/D75 d2 + s.c.m.) was determined to be the recommended dose level. At dose levels with supportive care measures, neutropenia grade 4 was a common side effect (50 % of cycles), but this was generally brief and did not interfere with the planned treatment schedule as observed in dose level 1.

Overall, nausea and vomiting were mild (grade 1). Mucositis, fatigue and myalgia were significant non-haematological side effects.

Of the 10 (out of the total of 22) patients who obtained dose limiting toxicity, 7 had previously been treated with chemotherapy.

Table 4. Effect of docetaxel interval time on the pharmacokinetics of MTX[a]

Parameter	Interval time (h)		p value[a]
	0 (n=4)	24 (n=6)	
MTX			
C_{max} (ng/mL)[b]	4803±1828	9012±7339	0.286
AUC (µg.h/mL)	6.63±1.96	8.72±5.38	0.670
CL (L/h/m^2)	4.80±1.29	4.41±2.18	0.670
$T_{1/2}$ (h)	2.84±1.09	1.88±1.03	0.201
7-OH-MTX			
C_{max} (ng/mL)	110±30.0	160±42.7	0.088
T_{max} (h)	4.62±0.95	4.44±0.78	0.831
AUC (ng.h/mL)	2.06±0.69	2.97±1.34	0.166
$T_{1/2}$ (h)	7.06±0.31	8.56±2.90	0.831
REC	0.33±0.12	0.41±0.26	0.999

Data were obtained from patients treated with MTX at a dose level of 30 mg/m^2 with docetaxel (75 mg/m^2) given immediately following MTX or 24 h later. Abbreviations: C_{max}, peak plasma concentration; AUC, area under the plasma concentration-time curve; CL, total plasma clearance; $T_{1/2}$, apparent half-life of the terminal disposition phase; T_{max}, time to peak plasma concentration; REC, relative extent of conversion of MTX into 7-OH-MTX (i.e. $AUC_{7\text{-}OH\text{-}MTX}/AUC_{MTX}$). [a]Mann-Whitney test.

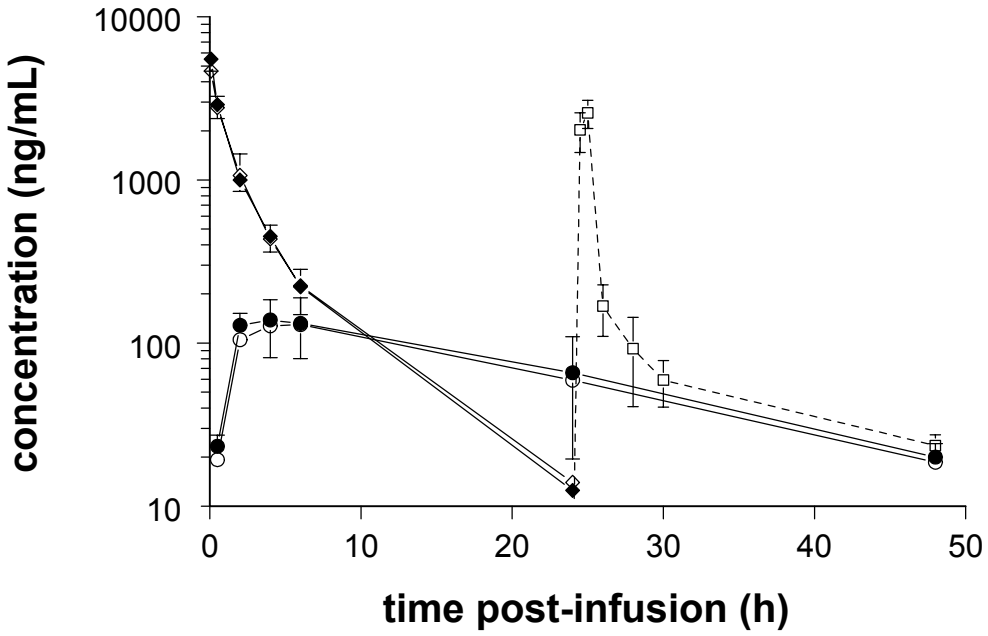

Figure 1. Plasma concentration-time profiles of MTX (diamonds) and 7-OH-MTX (circles) in patients treated with MTX alone at 40 mg/m^2 as a 5-min i.v. infusion (open symbols) or in combination with docetaxel (closed symbols) given on day 2 at 75 mg/m^2 as a 1-h i.v. infusion. The plasma concentration-time profile of docetaxel in indicated by squares and the dotted line (infusion from 24 to 25 h after MTX dosing). Data are presented as mean values of 6 pharmacokinetically evaluable patients (symbol) ± standard deviation (error bar).

Methotrexate and Docetaxel Pharmacokinetics

The possible effect of the drug schedule and interval time between drug administration on the pharmacokinetics of MTX was investigated in ten patients (four given docetaxel immediately following MTX and another six given docetaxel 24 hours later). Unpaired analysis indicated that changing the treatment interval to 24 hours had no significant influence on any of the studied parameters (P = N.S. Table 4). Plasma concentration-time profiles of MTX and its metabolite 7-OH-MTX in patients treated with MTX alone or in combination with docetaxel are displayed in Fig. 1. The time course of MTX concentrations in all patients was best described with a tri-exponential function. Concentrations of 7-OH-MTX increased slowly after MTX administration and peaked consistently at 4 hours after dosing. The metabolite data best fitted a two-compartmental model with a lag-phase of approximately 10 minutes preceding the appearance of 7-OH-MTX in plasma. A summary of

paired MTX pharmacokinetic parameters in the absence and presence of docetaxel obtained from 15 patients is given in Table 5. The total plasma clearance as well as the terminal disposition half-life of MTX was not significantly altered by docetaxel administration in any patient (P = N.S. Table 5). Similarly, the formation and subsequent disposition of the MTX metabolite was not substantially altered by docetaxel co-treatment (P>0.13), although it is possible that minor alterations were obscured by the substantial interindividual variability in the generated data. Over the various dose levels examined, docetaxel pharmacokinetics was not dependent on the MTX dose level, with an overall mean docetaxel clearance of 17.3±5.32 L/h/m2.

Table 5. Summary of paired MTX pharmacokinetics in the absence and presence of docetaxel

Parameter	MTX	MTX+doc	95% C.L.	p value[a]
MTX				
CL (L/h/m^2)	4.68±1.40	4.35±1.40	-0.78, 0.12	0.135
$T_{1/2}$ (h)	2.48±1.05	2.60±1.28	-0.45, 0.24	0.675
7-OH-MTX				
T_{max} (h)	5.16±1.04	4.61±1.20	-1.34, 0.24	0.155
CL_{app} (ng.h/mL)	16.9±22.7	17.2±16.2	-6.78, 7.45	0.921
$T_{1/2}$ (h)	8.63±3.90	9.23±3.40	-0.49, 1.68	0.260
REC	0.49±0.29	0.38±0.21	-0.26, 0.04	0.134

Data were obtained from 15 patients treated on the first day with MTX at a dose level of 30, 40, or 50 mg/m^2 and docetaxel given on day 2 at a dose level of 75 or 85 mg/m^2. Abbreviations: CL, total plasma clearance; $T_{1/2}$, apparent half-life of the terminal disposition phase; T_{max}, time to peak plasma concentration; CL_{app}, apparent total plasma clearance; REC, relative extent of conversion of MTX into 7-OH-MTX (i.e. $AUC_{7\text{-OH-MTX}}/AUC_{MTX}$); doc, docetaxel; 95% C.L., 95% confidence limits for the mean difference. [a]Two-sided paired Student's t-test.

Responses

Eighteen of the 22 patients were evaluable for response. Three patients with metastatic breast cancer treated at dose levels 1, 2, and 3 obtained a partial response, with a response

duration of 13, 9, and 6 months respectively. One additional patient with urothelial cancer treated at dose level 4 obtained a partial response that lasted 7 months. No complete responses were documented. Nine patients had stable disease for a median duration of 3 months (range 6 weeks - 6 months).

Table 6. Effect of MTX dose on the pharmacokinetics of docetaxel

D dose (mg/m^2)	75	75	75	85	
MTX dose (mg/m^2)	30	40	50	30	overall mean
n	6	7	2	4	19
C_{max} (ng/mL)	2413±672	2691±726	2694±478	2898±223	-
AUC (ng.h/mL)	3.92±1.34	4.85±0.99	4.97±1.04	6.06±1.62	-
CL ($L/h/m^2$)	21.1±7.33	15.9±2.66	15.4±3.23	14.9±4.34	17.3±5.32*
MRT (h)	4.51±3.23	10.4±6.33	5.87±3.33	5.56±4.73	7.04±5.26
$V_{d,ss}$ (L/m^2)	87.8±61.2	156±65.4	85.2±32.4	75.1±62.5	110±67.1
$T_{1/2}$ (h)	9.63±6.87	16.0±5.48	13.3±1.74	9.72±1.88	12.4±5.77

Abbreviations: C_{max}, peak plasma concentration; AUC, area under the plasma concentration-time curve; CL, total plasma clearance; MRT, mean residence time; $V_{d,ss}$, volume of distribution at steady-state; $T_{1/2}$, apparent half-life of the terminal disposition phase. * P=0.256, Kruskal-Wallis test.

DISCUSSION

In the present study, the feasibility and recommended dose of the sequential use of docetaxel and methotrexate was investigated. *In vitro* data had shown that the administration of edatrexate, a methotrexate analogue, 24 hours prior to docetaxel resulted in greater cytotoxicity than the simultaneous administration or administration of docetaxel prior to edatrexate [7]. This interaction was also reported *in vitro* with the combination of methotrexate and paclitaxel [8]. A phase I investigation of the sequential use of methotrexate given on day 1 followed by paclitaxel on day 2 showed the combination to be feasible, but pronounced myelotoxicity indicated enhanced toxicity [15].

In the current study, methotrexate was given as an i.v. push on days 1 and 15. Docetaxel was administered as a one-hour infusion on day 2. Treatment was repeated every 3 weeks. In the first cohort of sequential administration of the two agents, all 3 patients experienced dose limiting toxicity, consisting of mucositis and/or neutropenia grade 4. In a recent report by Guillot et al. [9], modest toxicity was reported when methotrexate and

docetaxel were both administered on day 1. In order to investigate this possible clinically significant schedule dependent difference in toxicity, by amending the protocol the next 4 patients received both agents on day 1 without changing the drug doses. Indeed, we found significantly less toxicity, suggesting a sequence-dependent pharmacodynamic interaction. Seeking suggestive evidence of enhanced cytotoxic interaction, we continued to study the original drug sequence, adding optimal drug support, consisting of lenograstim and leucovorin prophylaxis. Despite the administration of these supportive care measures, toxicity remained significant, prohibiting dose escalation of methotrexate above 40 mg/m^2 and docetaxel above 75 mg/m^2. The significant toxicity observed with this combination was not restricted to patients who had previously been treated with chemotherapy, as 3 out of the total of 10 DLT's were obtained in chemonaive patients. Moreover, the findings of no more than four partial responses in tumors potentially sensitive to docetaxel and/or methotrexate was disappointing and does not suggest enhanced anti-tumor effects.

We evaluated potential drug-drug interactions and the possible role of schedule of drug administration of methotrexate and docetaxel. Docetaxel undergoes significant hepatic metabolism, mainly by the cytochrome P-450 3A4 isozyme [16,17], and its use has been associated with kinetic interactions, for example with doxorubicin [18], epirubicin [19, 20], and topotecan [21]. In addition, a number of reports have shown that the docetaxel-formulation vehicle, polysorbate 80 (Tween 80), profoundly alters methotrexate pharmacokinetics by changing renal and biliary excretion profiles in mice [22]. The observed pharmacokinetic parameters of methotrexate and its aldehyde oxidase-mediated metabolite 7-OH-MTX demonstrated linear and dose-independent behaviour over the dose range studied, similar to single-agent data [23]. We also found that parameters of methotrexate and 17-OH-MTX pharmacokinetics were independent of concomitant administration of docetaxel, and comparable data were obtained in the schedule with a 24-hour interval between administration of both agents, indicating no apparent pharmacokinetic interaction. The lack of polysorbate 80 effect on methotrexate pharmacokinetics may be due to the fact that this surfactant is very extensively metabolised in humans within the systemic circulation into oleic acid and polyoxyethylene sorbitol [24]. In fact, the polysorbate 80 peak plasma levels observed in cancer patients receiving docetaxel (100 mg/m^2 over 1 hour) were only 0.16±0.05 µL/mL (mean ± SD), suggesting that it could not have interfered in methotrexate disposition in our patients [24]. The pharmacokinetic behaviour of docetaxel was also independent of methotrexate dose, and similar to single-agent data [25]. Thus, overall, our plasma pharmacokinetic data do not provide an explanation for the degree of toxicity observed with the combination of methotrexate and docetaxel. It is important to realise, however, that sequence and schedule-dependent differences in toxicity and pharmacokinetics can be obscured by large inter- and intraindividual variability in systemic exposure. Indeed, high (5- to 7-fold) variability has been reported in docetaxel and methotrexate AUC [25, 26]. However, because patients received methotrexate both in the

presence and absence of docetaxel, grossly abnormal plasma clearance values required to explain the toxicity of the combination should have been noted in the present study. It is more likely that alternative mechanisms, undetected by the current analytical methods, have contributed to the enhanced toxicity associated with the combination of methotrexate and docetaxel. One of these mechanisms might be an effect of docetaxel on the intracellular polyglutamation of methotrexate and 7-OH-MTX by the enzyme folylpolyglutamate synthetase (FPGS). This protein is an important pathway for the selective intracellular retention of naturally occurring folates, and is also an important determinant of methotrexate-induced cytotoxicity [27, 28]. Clearly, the pharmacological effects of the combination of antifolates and anti-microtubule anticancer agents seem to be rather complex and will need further (pre)clinical investigation to be fully understood. Hence, our pharmacokinetic data do not give an explanation for the amount of toxicity as they revealed no interaction of docetaxel and methotrexate kinetics. Methotrexate and 17-OH-MTX kinetics seemed to be independent of the administration of docetaxel and the moment of docetaxel administration appeared not to influence methotrexate kinetics.

We conclude that the sequential administration of methotrexate and docetaxel results in significant toxicity without evidence of an enhanced activity.

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CHAPTER 7

Combination Chemotherapy of Taxanes and Anti-Metabolites: Its Use and Limitations

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ABSTRACT

In an effort to improve response rates of chemotherapy, taxanes have been combined with other cytotoxic agents, such as antimetabolites. However, the use of some of these combinations in patients have been restricted by severe toxicity. The significance of sequence of drug administration in combining methotrexate (MTX) and taxanes was recognized in *in vitro* studies, showing synergistic effects for the sequence of MTX followed by paclitaxel, and antagonism for exposure in the reverse order. A possible explanation might be an MTX-induced synchronisation of cells in the S phase of the cell cycle, after which cells are more susceptible for the cytotoxic action of taxanes. Clinical studies using this sequence were hampered by severe neutropenia and mucositis at relatively low doses of both drugs. As no pharmacokinetic interactions were observed, the excess of toxicity may have been due to sequence-dependent synergistic actions on bone marrow and mucosa. In contrast, and confusingly, *in vitro* studies on 5-fluouracil (5FU) and taxanes indicate that 5FU preceeding or simultaneously given to paclitaxel impairs cytotoxicity as compared to paclitaxel monotherapy, while the reverse sequence results in additive or synergistic cytotoxicity. While almost all clinical studies have used the sequence of a taxane followed by 5FU, various schedules appeared feasible and effective. The combination of a 5FU analogue, capecitabine, and taxanes was supported by *in vitro* data. A large phase III trial confirmed the feasibility and superior efficacy of this combination in breast cancer patients relapsing after an anthracycline. Conflicting results exist on the benefit of combining gemcitabine and taxanes in tumor cell lines. Although the accumulation of gemcitabine triphosphate (dFdCTP) in mononuclear cells was significantly higher with an increasing dose of paclitaxel, no pharmacokinetic interactions for both agents were noticed. A pharmacokinetic analysis of the gemcitabine-docetaxel combination therapy has not been published in detail. Despite numerous trials, so far no optimimum schedule has been established. Regarding data on actually delivered dose intensities, a 2 or 3-weekly cycle seems favourable and feasible. However, possible severe pulmonary toxicity warrants cautious monitoring of patients treated with this combination. Different outcomes of preclinical and clinical studies reveal that combining two chemotherapeutc agents is not simply a matter of putting anti-tumor activities together. Drug interaction may result in synergism, not only of efficacy but also of toxic side effects. Adding two drugs may also implicate antagonism in drug efficacy, due to unwanted interference in cytotoxicity or pharmacokinetics. For agents acting at a specific phase of the cell cycle, the sequence of administration may determine the efficacy and toxicity of a combination therapy. Because of an observed discrepancy between *in vitro* data and clinical studies, we would like to emphasize the urge for adequate dose-finding clinical trials together with pharmacokinetic data analysis before examining any new combination chemotherapy in more detail in phase II studies.

INTRODUCTION

Curative cancer chemotherapy nearly always consists of a combination of cytotoxic agents. Increased efficacy of combination chemotherapy may result from the increase of total exposure to a cytotoxic effect due to the addition of other agents, especially if non-overlapping toxicities allow dose intensities for the combination to be similar to those of the single agents. Other rationales for combination chemotherapy are the possibility to overcome (multi)drug resistance and synergistic effects of certain antitumor drugs [1]. Concomitant administration of anticancer agents may affect the pharmacokinetic parameters such as absorption, distribution, metabolism or excretion of a drug, or may result in pharmacodynamic interactions at the level of cellular targets or the cell cycle, which can have both a positive or a negative impact on the cytotoxic effects of the drugs involved.

Since the late 1980s taxanes have proved to be effective agents in the treatment of a variety of solid tumors [2]. Paclitaxel is currently registered for the treatment of advanced breast cancer, ovarian cancer and non-small cell lung cancer and as a single agent it is usually dosed at 135-225 mg/m^2 as a 3 hour intravenous (iv) infusion every 3 weeks [3]. Docetaxel is registered for the treatment of metastatic breast cancer and non-small cell lung cancer and is most often given at a dose of 100 mg/m^2 as a 1 hour iv infusion in a 3-weekly schedule. Currently, weekly administration of taxanes, enabling a higher dose per time period and inducing less toxicity, is being explored in several phase I-II studies [4].

In view of the established efficacy of taxanes, numerous combinations of taxanes with other agents in various treatment schedules have been investigated in an effort to improve the response rates to palliative chemotherapy in solid tumors. Such combinations included those of taxanes with antimetabolites, but some of these yielded major problems in patients due to severe toxicity, which prevented maximum tolerable doses (MTD's) that were considered to be relevant for the single agents [5,6]. This contrasts with the feasibility of combining taxanes with other classes of cytotoxic agents almost at their respective recommended single doses. This review will summarize both preclinical and clinical studies on combinations of taxanes and the antimetabolites MTX, 5FU, capecitabine and gemcitabine, respectively, and consider possible mechanisms of interaction accounting for their efficacy and clinical feasibility. Combinations of other antimetabolites with taxanes have hardly been investigated and will therefore not be discussed.

PHARMACOLOGY OF TAXANES

Taxanes exert their cytotoxic effect by stabilising the assembly of intracellular microtubulus from tubulin dimers, thereby disrupting mitosis and other vital cellular functions.

In studies with hamster ovarian cell lines and human ovarian and leukemic cell lines paclitaxel appeared to be a phase-specific agent, being more cytotoxic to mitotic (M) cells than interphase (G_1, S, G_2) cells [7,8]. In studies with human leukemic cell lines paclitaxel induced a temporary accumulation of cells in G_2 and M phase [9,10]. As asynchronous human tumor cell cultures include paclitaxel-resistant interphase cells, the fraction of killed cells reaches a plateau despite an increasing concentration of paclitaxel [11]. In contrast, prolonging the exposure time of various human tumor cell lines to paclitaxel from 24 to 72 hour resulted in a marked increase in cytotoxicity [11]. Even in a synchronous cell culture of mainly mitotic cells, a period of exposure of at least 4-6 hour to paclitaxel at a concentration of 1.6 ug/ml was required to kill most cells, which emphasises the importance of duration rather than the drug concentration [8]. Besides inducing an arrest in the cell cycle in the G_2 and M phase, paclitaxel initiates apoptosis along various pathways, most of which are not yet resolved [12]. For docetaxel, a brief exposure (1 h) of a synchronous culture of HeLa cells in S phase was lethal without a subsequent block in the cell cycle [13]. After prolonging the exposure to 24 hours, both paclitaxel and docetaxel blocked the cell cycle of a human cancer cell line at the G_2-M phase [14].

The pharmacokinetics of paclitaxel and docetaxel show a large volume of distribution with extensive protein-binding, and a rapid elimination from the plasma with a short terminal half-life of 5 and 12 h, respectively, mainly due to hepatic metabolism, biliary excretion and tissue distribution [2,3]. Both paclitaxel and docetaxel are administered intravenously, using different vehicles to overcome their insolubility in water. Paclitaxel is dissolved in a 1:1 mixture of cremophor EL (CrEL) and ethanol. CrEL has a small volume of distribution, almost similar to that of the blood compartment, and a long terminal half-life of 80 h [15]. It was recently shown that the vehicle CrEL appeared to have a major impact on the pharmacokinetics of paclitaxel, being responsible for its nonlinear plasma distribution [16]. This non-linear disposition of paclitaxel implies that the total exposure to this agent increases disproportional to its dose. An increase in the concentration of CrEL causes a reduction in both the free fraction of paclitaxel and its accumulation in erythrocytes, probably due to drug trapping in the CrEL micelles, which act as the main carrier of paclitaxel in the blood compartment. Combination chemotherapy schedules with paclitaxel may carry a risk of unforeseen interactions of CrEL with other anticancer agents. Indeed, clinically significant interactions resulting in excessive toxicity have been reported when paclitaxel given as a 3 h infusion preceded a bolus infusion of doxorubicin [17].

For docetaxel, linear pharmacokinetics are observed. Docetaxel is formulated in polysorbate 80 (Tween 80), which has a rapid plasma elimination and is already undetectable in plasma after 1 h [15,18]. Due to this rapid decline from plasma, it is unlikely that this vehicle causes significant interactions in docetaxel-based combination chemotherapy [15].

TAXANE/METHOTREXATE COMBINATIONS

Preclinical studies

Methotrexate (MTX) is one of the oldest anticancer drugs in clinical use. Antimetabolites such as MTX interfere with DNA synthesis, that is necessary for cell proliferation. Due to their mode of action, most antimetabolites act at specific phases of the cell cycle. MTX inhibits dihydrofolate reductase, which results in depletion of intracellular tetrahydrofolate, and thereby impedes synthesis of thymidilate and purines required for DNA synthesis. It acts as a phase-specific agent by arresting cells in the S phase. In the search for folate analogues with increased antitumor activity, new dihydrofolate reductase inhibitors such as edatrexate have been developed [19].

Paclitaxel/Methotrexate

Various schedules of paclitaxel and MTX were tested *in vitro* in human breast cancer cells, using both growth inhibition and clonogenic assays to evaluate drug activity [20]. Simultaneous exposure to both agents for 3 days and exposure to paclitaxel for 6 h after which MTX was added for 3 days resulted in antagonistic effects. However, sequential exposure to MTX for 12 h followed by the addition of paclitaxel for 12 days clearly showed synergism. The significance of the sequence in combining both drugs was confirmed by an *in vitro* study with human breast, ovarian and lung cancer cell lines, which used the isobologram method to analyze the effect of the drug combinations [21]. In this study, simultaneous exposure to both agents for 24 h and exposure to paclitaxel for 24 h followed by MTX for 24 h also exhibited antagonism, whereas the reverse sequence yielded synergistic effects. Colony forming assays in a human bladder cancer cell line demonstrated that MTX (for 24 h) prior to a low dose of paclitaxel (for 24 h) resulted in maximal synergistic cytotoxicity [22]. Chou and collegues assessed the effect of combining edatrexate and a taxane on the inhibition of cell growth with the combination index-isobologram method [23]. In two human breast cancer cell lines, incubation with edatrexate for 3 h followed after 24 h by paclitaxel for 3 h appeared to be synergistic, while antagonism was noted with the reverse schedule.

Docetaxel/Methotrexate

Similar schedule-dependent synergistic or antagonistic effects were observed for the combination of edatrexate and docetaxel [23].

Thus, preclinical data suggest that for efficacy it may be best to administer MTX prior to the taxane. A possible explanation for the sequence-dependent synergism observed *in vitro* might be an MTX-induced synchronisation of cells in the S phase, after which cells are more susceptible to the cytotoxic action of the taxanes [23]. Indeed, another compound which

arrests cells at the S phase, such as gallium nitrate, also shows sequence-dependent synergism when given at least 12 h prior to paclitaxel [24]. These studies indicate that MTX should preceede taxane administration by at least 12 h, the time necessary for cells to enter the M phase [20,24]. In the reverse sequence, paclitaxel might reduce the cytotoxicity of MTX by arresting the cell cycle and preventing cells from entering the S phase, in which they are most susceptible to MTX.

Clinical studies

Nearly all studies combining taxanes and MTX used a 3-weekly cycle. Dosing schedules and response rates of these trials are depicted in Tables 1 and 2.

Paclitaxel/Methotrexate

Two trials in urothelial carcinoma investigated paclitaxel and MTX administered both on day 1, in combination with carboplatin or cisplatin, respectively [25,26]. Paclitaxel and MTX were administered iv immediately after each other. Toxicity mainly consisted of grade 3-4 neutropenia and grade 1-2 neurotoxicity and appeared tolerable. In contrast, the regimen of Huber and collegues administering MTX as a bolus 24 h prior to paclitaxel as a 24 h infusion was found to be fairly toxic, with febrile neutropenia preventing further dose escalation of paclitaxel beyond 135 mg/m^2 despite G-CSF support [6]. As paclitaxel plasma levels during the paclitaxel infusion were not altered by prior MTX infusion in this study, the observed excessive toxicity in this regimen is unlikely to be related to pharmacokinetic interactions [6]. Unfortunately, further pharmacokinetic data on the paclitaxel-MTX combination in humans are lacking, which is a major drawback of the reported studies. Taking into account the *in vitro* data on the synergism for the sequential administration of MTX preceding paclitaxel, the severe myelosuppression in the latter trial might be due to similar synergistic activity on normal bone marrow cells [21]. Another explanation for the observed toxicity may be the long duration of the paclitaxel infusion of 24 h. Indeed, a similar sequence of another folate analogue, edatrexate, followed after 24 h by paclitaxel given as a short 3-h infusion was well tolerated in two studies and did not require the support of growth factors [27,29].

Docetaxel/Methotrexate

Only 2 trials have investigated the combination treatment of docetaxel and MTX, both involving patients with solid tumors (Table 2) [5,30]. Administration of both drugs on the same day appeared to be feasible, and did not require the support of haematopoietic growth factors (30). A bolus infusion of MTX followed after 24 h by docetaxel given as a 1-h infusion was complicated by dose-limiting neutropenia and mucositis at relatively low doses of the drugs, even despite the additional use of haematopoietic growth factors [5]. Extensive pharmacokinetic analyses revealed no pharmacokinetic interactions for this sequence [5].

Table 1. Clinical studies combining methotrexate and paclitaxel

Author	q (weeks)	Paclitaxel regimen	MTX regimen	G-CSF	Tolerable dose of paclitaxel-MTX (mg/m^2) per cycle	Tumor type	Prior chemo-therapy	No. of evaluable patients	RR (%)
Edelman (25)	3	d1 3h	d1 +carbo	+	200-60	bladder	-	32	56
Tu (26)	3	d1 3h	d1 0.5h +cddp	+	200-30	bladder	+	25	40
Huber (6)	3	d2 24h	d1	-	85-23	solid	+	41	10
				+	135-40				
D'Andrea (27)[1]	3	d2 3h	d1 1h	-	175-350 [1]	breast	+/-	35	31
Diamandidis (28)[1]	3	d2 24h	d1,15	+	170-250 [1]	solid	+	40	33
Rigas (29)[1]	4	d2,16 3h	d1,15	-	350-240 [1]	solid	+	34	24

All studies administered MTX as a bolus infusion, unless stated otherwise

[1] Study using edatrexate in stead of MTX

Table 2. Clinical studies combining methotrexate and docetaxel

Author	Docetaxel regimen	MTX regimen	G-CSF	Tolerable dose of docetaxel-MTX per cycle (mg/m^2)	Tumor type	Prior chemo-therapy	No. of evaluable patients	RR (%)
Louwerens (5)	d2 1h	d1,15	+	75-80	solid	+	18	22
Zanetta (30)	d8	d1,8	-	90-80	solid	ng	28	14

All studies used a 3-weekly cycle

ng= data not given in publication

A study on the effect on MTX pharmacokinetics by the administration of docetaxel immediately or 24 h afterwards found a non-significant rise in the area under the concentration curve (AUC) and a somewhat lower plasma clearance of MTX [31]. Another pharmacokinetic study confirmed a lack of interaction of the concomitant administration of docetaxel and MTX [30].
The combination schedules of MTX followed by a taxane after an interval of 24 h show a striking excess of toxicity at relatively low doses of both agents, with no apparent benefit on tumor responses. As no pharmacokinetic interactions were observed, severe toxicity may be due to sequence-dependent synergistic cytotoxicity on normal tissue such as bone marrow and mucosa. Some of the preclinical studies as mentioned above support this explanation.

TAXANE/5-FU COMBINATIONS

Preclinical studies

5-FU is a pyrimidine antimetabolite, which is phosphorylated to 5-fluorouridine triphosphate (5-FUTP). Subsequent incorporation into RNA interferes with cellular RNA processes. Another activated 5-FU metabolite, 5-fluorodeoxyuridine monophosphate (5 FdUMP), inhibits thymidilate synthase, which is required for DNA synthesis. *In vitro* treatment of mouse T-lymphocytes and human breast cancer cell lines with 5FU resulted in an interruption at the G_1-S phase of the cell cycle [32,33].

Paclitaxel/5FU

Kano and collegues investigated various schedules of paclitaxel and 5-FU *in vitro* in four human cancer cell lines, evaluating dose-response effects with isobolograms [34]. Synchronous exposure to both agents for 24 h, or sequential exposure to 5-FU for 24 h followed by paclitaxel for 24 h showed an antagonistic interaction, while reversal of the sequence of exposure had an additive effect. Interestingly, prolongation of the interval of simultaneous exposure to 5 days resulted in an additive interaction. *In vitro* studies with human breast and epidermoid cancer cell lines indicated that pretreatment with 5-FU for 6 h or simultaneous treatment with 5-FU and paclitaxel for 24-72 h impaired overall cell killing activity compared with paclitaxel monotherapy [33]. The antagonistic effect of 5-FU on paclitaxel cytotoxicity was no longer apparent if tumor cells were pretreated with paclitaxel at least 24 h prior to 5-FU. A similar schedule-dependency with antagonism of 5-FU followed by paclitaxel and synergism for the reverse sequence was noticed in a clonogenic assay of human breast cancer cells [35]. The authors suggested that 5-FU appeared to interfere with paclitaxel cytotoxicity by preventing tumor cells from accumulating in the G_2-M phase of the cell cycle, in which paclitaxel exerts its cytotoxic effect. It is confusing that a similar reasoning was used to explain the synergistic effect of MTX followed by taxanes. Apparently, we lack an appropriate mechanism. Other *in vitro* studies using DNA fragmentation techniques

revealed that pretreatment or simultaneous exposure with 5-FU together with paclitaxel reduced the induction of apoptosis by paclitaxel, whereas it blocked paclitaxel-induced bcl-2 phosphorylation, c-raf-1 phosphorylation and p21 $^{WAF/CIP1}$ expression [36].
All preclinical data on the combination of 5FU and paclitaxel favour the sequence of paclitaxel prior to 5FU.

Docetaxel/5FU

Preclinical studies investigating the simultanous treatment of docetaxel and 5-FU in xenografts of coloncarcinoma in mice resulted in a synergistic cell kill [37]. To our knowledge, preclinical studies with cell lines on the influence of sequence in using 5-FU and docetaxel have not been performed.

Clinical studies

Tables 3 and 4 summarise data of clinical trials evaluating the addition of 5-FU to paclitaxel and docetaxel, respectively. A large variety of chemotherapy schedules has been studied. For the ease of survey, studies using the combination of 5-FU and taxanes with other cytotoxic agents were excluded. As a sequence-dependent synergism for the administration of taxanes followed by 5FU was observed in the preclinical studies, all clinical studies except one [50] used this prefered sequence. In contrast to the *in vitro* studies, many clinical trials added leucovorin to the 5FU-paclitaxel chemotherapy regimen.

Paclitaxel/5FU

Five studies investigating paclitaxel and 5-FU in a 3-weekly cycle noticed tolerable side-effects of mainly leucocytopenia and mild neurotoxicity [39-43]. Nicholson and collegues investigated a 4-weekly cycle of paclitaxel and 5-FU in 52 evaluable patients with metastatic breast cancer, of whom 47 had been pretreated with chemotherapy [44]. Toxicity was acceptable with mucositis (*n*=3) and neutropenic fever (5% of cycles). In a study applying continuous infusion of 5-FU, apart from neutropenia and mucositis, neurotoxicity grade 2 was also observed in 43% of these patients [45]. A regimen of 6 weeks of treatment with both agents followed by 2 weeks of rest administered as second-line therapy to 34 evaluable breast cancer patients was found feasible with grade 3-4 leukopenia in 36% of cycles [46]. Unfortunately, and in line with the studies combining paclitaxel and MTX, possible pharmacokinetic interactions have not been analysed in any of these studies and data on delivered dose-intensities are lacking.

Table 3. Clinical studies combining 5FU and paclitaxel

Author	q (weeks)	Paclitaxel regimen	5FU regimen	LV	Tolerable dose of paclitaxel-5FU per cycle (mg/m^2)	Tumor type	Prior chemo-therapy	No. of evaluable patients	RR (%)
Collichio (38)	2	d1 1h	d1-5, 8-12 ci[1]	-	135-3500	breast	-	16	38
Murad (39)	3	d1 3h	d2 3h	-	175-1500	gastric	-	29	66
Cascinu (40)	3	d1 3h	d1,8,15 b[2]	-	225-1500	gastric	+	15	13
Ciuleanu (41)	3	d1 3h	d1-5 2h	+	175-3000	nasophar	+	24	13
Takimoto (42)	3	d1 3h	d2-5 b	+	175-1480	solid	+/-	17	35
Madajewicz (43)	3	d1-4 ci	d5 23h	+	140-1000	solid	+	10	70
Nicholson (44)	4	d1 3h	d1-3 b	+	175-1050	breast	+	52	52
Vredenburg (45)	6	d1,22 3h	d1-42 ci	-	350-10500	breast	+	42	50
Klaassen (46)	8	d1,22 3h	d1,8,15,22, 29,36 24h	+	350-12000	breast	+	34	53
Bokemeyer (47)	8	d1,22 3h	d1,8,15,22, 29,36 24h	+	350-12000	gastric	-	22	32

[1] ci=continuous infusion

[2] b=bolus infusion

Table 4. Clinical studies combining 5FU and docetaxel

Author	q (weeks)	Docetaxel regimen	5FU regimen	LV	Tolerable dose of docetaxel-5FU per cycle (mg/m^2)	Tumor type	Prior chemo-therapy	No. of evaluable patients	RR (%)
Van den Neste (48)	3	d1 1h	d1-5 ci	-	85-3750	solid	+	39	13
Lortholary (49)	3	d1 1h	d1-5 ci	-	85-3750	breast	+	32	56
Eniu (50)	3	d2 1h	d1-4 2h	+	80-2400	gastric	-	26	31
Chun (51)	3	d1,8,15	d1-14 ci	-	75-2100	gastric	-	10	100
Ando (52)	3-4	d1 1h	d1-5 ci	-	50-2500	breast	+	18	50
Petit (53)	4	d1 1h	d1-5 ci	-	60-1500	solid	+	37	8

[1] ci=continuous infusion

Docetaxel/5FU

Most of the clinical studies examining the feasibility of combining docetaxel with 5-FU used a cycle of 3 weeks, and noticed tolerable toxicities of mainly neutropenia, stomatitis and diarrhoea [48,49,52]. The only study that administered a taxane (docetaxel) after the start of 5-FU did not show any apparent lack of efficacy [50]. In a dose-finding study in pretreated solid tumors the MTD of both drugs in a 3-weekly cycle was almost similar to those of the drugs used as single agent, grade 4 neutropenia being the dose-limiting toxicity (DLT) [48]. Another phase I study using a similar schedule found similar MTDs in pretreated breast cancer patients [49]. This study was the only one to mention that doses were administered on time and without dose reduction in 97% and 95% of cycles, respectively. Of note, two other studies found a lower MTD especially for docetaxel, despite an even longer schedule of 4 weeks instead of 3 or 3-4 weeks [52,53]. The Japanese study of Ando and collegues did not administer corticosteroid premedication and was the only one to report grade 3-4 diarrhoea as a DLT in 2 out of 6 patients at their highest dose level [52]. Petit and colllegues did not continue dose escalation because of grade 4 neutropenia lasting for more than 7 days and neutropenic fever in their heavily pretreated patients [53]. Pharmacokinetic analyses noticed no apparent relationship between the clearance and AUC of both docetaxel and 5-FU [48].

In general, the combination of 5-FU and a taxane seems feasible and the observed response rates at least do not suggest an antagonistic effect. Randomized phase II/III studies will be required to adequately assess efficacy.

TAXANE/CAPECITABINE COMBINATIONS

Preclinical studies

Capecitabine is an oral prodrug of 5-FU that is converted along a pathway with three enzymes to the active compound 5-FU. The final step of conversion into 5-FU is catalyzed by thymidine phosphorylase (TP), an enzyme that is more abudantly expressed in tumor tissue than in healthy cells. In studies with human colon and breast cancer xenografts in nude mice, both paclitaxel and docetaxel enhanced the level of TP in tumor cells [54]. These *in vitro* data support the use of the combination of capecitabine and taxanes. Indeed, simultaneous treatment of paclitaxel or docetaxel with orally administered capecitabine showed synergistic antitumor activity in the xenograft models, while only additive activity was noted with a taxane-5-FU combination.

Clinical studies

Six trials explored the clinical feasibility of oral capecitabine in combination with a taxane in a 3-weekly schedule (Table 5) [55-60]. The encountered DLT in the combination with paclitaxel was neutropenia, while other side-effects were similar to those following

Table 5. Clinical studies combining capecitabine and a taxane

Author	Taxane regimen	Capecitabine regimen	Tolerable dose of taxane-capecitabine per administration (mg/m^2)	Tumor type	Prior chemo-therapy	No. of evaluable patients	RR (%)
Villalona (55)	d1 3h	d1-14	P 175-1650	breast	+	19	56
Villalona (56)	d1 3h	d3-21	P 175-1331	solid	+	17	0
Batista (57)	d1	d1-14	P 175-2000	breast	+	64	63
Meza (58)	d1	d1-14	P 175-1650	breast	+	37	49
O'Shaughnessy (59)	d1 1h	d1-14	D 75-2500	breast	+	255	42
Pronk (60)	d1 1h	d1-14	D 100-1650	solid	+	33	15
Villalona (61)	d1,8,15	d5-18	D 36-1250	solid	+-	15	27

All studies used a 3-weekly cycle, except (61)
P=Paclitaxel
D=docetaxel

administration of the single agents [56]. Of notice, asthenia was the DLT for the combination with docetaxel [60]. Extensive pharmacokinetic analyses revealed no significant effects of paclitaxel or docetaxel on the AUC of capecitabine and its metabolites, and *vise versa* [56,60].

Combining capecitabine with a taxane therefore seems attractive, with acceptable toxicity and a promising efficacy. Data from a large randomized phase III trial in 511 metastatic breast cancer patients relapsing after anthracycline-based therapy showed superior activity with the combination of docetaxel and capecitabine versus docetaxel single agent therapy [59]. The combination resulted in a RR of 42% and a median survival of 13.7 months (95% CI 12.3-16.1), versus a RR of 30% and a median survival of 11.1 months (95% CI 9.8-12.4) for docetaxel alone.

TAXANE/GEMCITABINE COMBINATIONS

Preclinical studies

Gemcitabine is a nucleoside analogue that impairs DNA synthesis. It is phosphorylated intracellularly to active triphosphate metabolites, the intracellular concentrations are increased and prolonged by several self-potentiating mechanisms [62]. After *in vitro* exposure to gemcitabine, human lung cancer cells accumulated in G_0-G_1 and S phases [63,64].

Paclitaxel/Gemcitabine

Clonogenic survival assays of human tumor cell lines have shown less than additive cytotoxicity for any sequential exposure to gemcitabine and paclitaxel, and antagonism for concomitant exposure to both drugs [65]. Kroep and collegues investigated various combinations of both simultaneous and sequential administration of gemcitabine and paclitaxel in 4- and 24-h intervals in non-small cell lung cancer cell lines [66]. Multiple drug effect analysis from non-clonogenic assays in this study showed that any sequence resulted in not more than additive cytotoxicity [66]. However, as the administration of paclitaxel prior to gemcitabine was found to increase both the accumulation of gemcitabine triphosphate in the tumor cells, the incorporation of gemcitabine into RNA as wel as the apoptotic index, this sequence might be favourable. Studies of various schedules of combined treatment of paclitaxel and gemcitabine in xenograft models of adenocarcinoma in mice resulted in an enhanced delay of tumor growth, compared with monotherapy with either agent [67]. Although efficacy was dependent on schedule and sequence, lethal toxicity was frequently encountered. So far, no conclusive preclinical data support the use of a specific sequence of gemcitabine and a taxane. One could even state that the available preclinical data do not really support the use of such combinations in man.

Clinical studies

Nevertheless, in view of the relevant single agent activity of these agents, numerous studies have explored a large variety of dosing schedules of the combination regimen, especially in the treatment of metastatic breast cancer and non-small cell lung cancer (Tables 6 and 7). Although not all trials reported the sequence of administration, most used a schedule of a taxane followed by gemcitabine. A possible correlation of the dosing schedule and the MTDs of the multidrug regimen is obscured by the large variety of investigated schedules, in various tumor types with different extents of pretreatment. The fact that almost all of these studies were investigator initiated explains the apparent lack of a systematic approach.

Paclitaxel/Gemcitabine

A pharmacokinetic analysis of the combination of paclitaxel and gemcitabine was performed in patients with non-small cell lung cancer [93]. The accumulation of gemcitabine triphosphate (dFdCTP) in mononuclear cells was significantly higher with a C_{max} of 106 pmol/ 10^6 cells with paclitaxel at a dose of 200 mg/m^2, compared with 88 pmol/ 10^6 cells with paclitaxel at a dose of 150 mg/m^2. Moreover, the C_{max} of dFdCTP shifted from 2 hours after the administration of gemcitabine as a single agent or with paclitaxel 150 mg/m^2 to 4 hours after gemcitabine and paclitaxel 200 mg/m^2. Although the pharmacokinetics of either drug were not affected by the other agent, paclitaxel seems to increase the accumulation of the active metabolite of gemcitabine [91,93]. Combining gemcitabine and paclitaxel in a 2 or 3-weekly cycle appeared feasible and mainly resulted in neutropenia and mild neurotoxicity (Table 6). The addition of G-CSF does not seem to increase the MTDs of both paclitaxel and gemcitabine [75,83,87]. Two trials using a 3-weekly cycle reported difficulty in administering gemcitabine at day 15 due to myelotoxicity [84,85]. In schedules administering paclitaxel at day 8, the MTDs of paclitaxel were relatively low due to observed grade 4 neutropenia [86,87,92]. De Pas and collegues reported a higher delivered dose intensity for both agents at one dose level below the one of MTD (paclitaxel 100 mg/m^2 and gemcitabine 1500 mg/m^2) and subsequently recommended this dose level for further testing [91]. Unfortunately, only few other studies reported the actually delivered dose intensities [74,77,83,87]. Regarding the planned monthly doses in table 6, a 2-weekly administration of both drugs seems to achieve the highest dose per unit of time. Full papers of this schedule confirm its good tolerability [69,72,74].

Only recently, dose-limiting severe pulmonary toxicity has been described in detail as a side effect of the combination of gemcitabine and paclitaxel (90). Despite standard corticosteroid premedication, other studies have occasionally reported patients with severe lung edema [69,71,72].

Table 6. Clinical studies combining gemcitabine and paclitaxel

Author	q (weeks)	fixed dose	Paclitaxel regimen	Gemcitabine regimen	Tolerable dose of paclitaxel-gemcitabine per administration (mg/m^2)	Tolerable dose of paclitaxel-gemcitabine per month (mg/m^2)	Tumor type	Prior chemo-therapy	No. of evaluable patients	RR (%)
Ives (68)	1	+	d1 1h	d1	85-1000	267-3240	lung	-	28	14
Colomer (69)	2	+	d1 3h	d1 1h	150-2500	300-5000	breast	-	38	68
Athanassiacus (70)	2		d1 1h	d1	175-3000	350-6000	lung	-	26	35
Fontaine (71)	2	+	d 1 3h	d1	150-3000	300-6000	lung	-	10	20
Isla (72)	2	+	d 1 3h	d1	150-2000	300-4000	lung	-	89	32
Sanchez (73)	2	+	d1 3h	d1	135-2500	270-5000	breast	+	44	45
Rothenberg (74)	2		d1 3h	d1	150-3000	300-6000	solid	+	37	5
Marini (75)	2	+	d1 3h	d1 +GCSF	150-2500	300-5000	bladder	+	15	53
Giaccone (76)	3	+	d1 3h	d1,8	200-1000	267-2667	lung	-	49	24
Kosmidis (77)	3	+	d1 3h	d1,8	200-1000	267-2667	lung	-	64	38
Lazaro (78)	3	+	d1 3h	d1,8	175-1000	233-2667	lung	-	10	50
Domine (79)	3	+	d1 3h	d1,8	175-1250	233-3333	lung	+	20	50
Rinaldi (80)	3		d1 3h	d1,8	200-1300	267-3467	solid	+	25	16
Fleming (81)	3		d1 3h	d1,8	150-900	200-2400	solid	+	18	22
Garcia (82)	3		d1 1h	d1,8	225-1200	300-3200	bladder	-	12	67

Table 6. - Continued -

Author	q (weeks)	fixed dose	Paclitaxel regimen	Gemcitabine regimen	Tolerable dose of paclitaxel-gemcitabine per administration (mg/m^2)	Tolerable dose of paclitaxel-gemcitabine per month (mg/m^2)	Tumor type	Prior chemo-therapy	No. of evaluable patients	RR (%)
Iaffaioli (83)	3		d1 1h	d1,8 +GCSF	240-1000	320-2667	breast	+	30	53
							ovarian	+	13	46
Meluch (84)	3	+	d1 1h	d1,8,(15)	200-1000	267-2667	bladder	-	39	56
								+	15	47
Murad (85)	3	+	d1 3h	d1,8,(15)	175-1000	233-2667	breast	+	29	55
Poole (86)	3		d8	d1,8	175-1000	233-2667	ovarian	+	10	40
Androulakis (87)	3	+	d8 3h	d1,8 +GCSF	175-900	233-2400	lung	+	49	18
Dongiovanni (88)	3	+	d1,8,15	d1,8	80-1000	320-2000	lung	+	16	19
Einhorn (89)	4		d1,8,15 3h	d1,8,15	130-1000	390-3000	solid	+/-	24	17
Thomas (90)	4		d1,8,15 1h	d1,8,15	40-1000	120-3000	lung	-	8	38
de Pas (91)	4		d1,8,15 1h	d1,8,15	100-1500	300-4500	lung	-	30	43
Poole (92)	4		d8	d1,8,(15)	100-800	100-2400	ovarian	+	8	50

All studies administered gemcitabine as a 30 min infusion, unless stated otherwise

Docetaxel/Gemcitabine

Even more studies have explored numerous schedules combining docetaxel and gemcitabine (Table 6). To our knowledge, pharmacokinetic parameters of the gemcitabine-docetaxel combination therapy have not been published in detail [118]. No full papers have yet been published on a weekly or 2-weekly schedule. Most studies administering docetaxel at day 1 of a 3-weekly cycle used a fixed dose, despite the lack of a full paper of a dose finding trial on such a regimen [99,100,102-104]. As docetaxel induces neutropenia after 5-8 days, more recent studies investigated a 3-weekly cycle administering docetaxel at day 8 in order to enable the repeated infusion of gemcitabine at the same day [105-116]. Rischin and collegues noticed in their dose finding study a good tolerability, with neutropenic fever and prolonged grade 4 neutropenia as DLTs [112]. Other commonly noticed side effects were alopecia and asthenia. Data on the achieved dose intensities of the 3-weekly regimens ranged from 75-90% and from 73-94%, for docetaxel and gemcitabine, respectively [103,104,109,112-114,116]. Regarding Table 7, the support of G-CSF does not seem to enable a relevant higher dose of both agents. Although no direct comparisons have been made, most of the 3-weekly schedules administering docetaxel at day 8 seemed to give a somewhat higher dose of docetaxel, compared to those administering docetaxel at day 1. A 4-weekly cycle even using a very low dose of docetaxel at day 15 appeared not to be feasible, because of thrombocytopenia and elevated liver enzymes [125,127]. Likewise, two studies using a cycle of 4 weeks with docetaxel on day 1 reported a delivered dose intensity of only 64 and 74% for gemcitabine, due to required dose reductions at day 8 or 15 because of myelotoxicity [123,125]. As a consequence, 4-weekly schedules do not result in an adequate dose intensity of both agents.

While some studies with the docetaxel-gemcitabine combination have also ocassionally reported pulmonary toxicity [108,112,113,119,121,124,131,132], Dunsford and collegues described a high incidence of severe lung toxicity in 4 out of 7 patients with metastatic urothelial cancer, despite the use of dexamethason starting 24 h prior to treatment [100]. Another study was prematurely terminated because of severe lung toxicity in 5 out of 26 lung cancer patients [128]. The discrepancy in observed pulmonary toxicity cannot be explained by a difference in sequence, as Rizvi and collegues found no difference in clinical toxicity for either regimen [118].

When looking at the planned dose intensities of the various schedules in Table 7, a cycle of 2 or 3 weeks might be favoured in terms of a maximal dose intensity and good tolerability. For 3-weekly schedules, docetaxel may be preferentially administered at day 8. However, side effects still render the combination of these two agents not very interesting and in view of the lack of preclinical evidence to combine these agents, it may be worthwhile to consider halting further clinical development.

Table 7. Clinical studies combining gemcitabine and docetaxel

Author	q (weeks)	fixed dose	Docetaxel regimen	Gemcitabine regimen	Tolerable dose of docetaxel-gemcitabine per administration (mg/m^2)	Tolerable dose of docetaxel-gemcitabine per month (mg/m^2)	Tumor type	Prior chemo-therapy	No. of evaluable patients	RR (%)
Lueck (94)	1		d1	d1	40-800	160-3200	pancreas	-	20	?
Eckardt (95)	2		d1	d1	75-3000	150-6000	solid	ng	16	19
Obrocea (96)	2		d1	d1 0.5h	55-3500	110-7000	solid	+/-	23	13
				d1 10mg/min	50-1600	100-3200				
Kornek (97)	2	+	d1	d1 +GCSF	50-1500	100-3000	breast	-	34	59
Bildat (98)	3		d1	d1,5	75-900	100-2400	lung	-	22	27
Ventriglia (99)	3	+	d1	d1,15	65-1000	87-2667	lung	-	19	47
Dunsford (100)	3	+	d1	d1,15	60-1000	80-2667	bladder	-	5	0
Jensen (101)	3		d1	d1,8	80-900	107-2400	lung	+	23	39
Brandi (102)	3	+	d11h	d1,8	80-1000	106-2667	breast	+	30	60
Fountzilas (103)	3	+	d1	d1,8 +GCSF	75-1000	100-2667	breast	+	39	36
Hejna (104)	3	+	d1 1.5h	d1,10 +GCSF	80-1000	107-2667	lung	-	34	50
Schlösser (105)	3		d8	d1	85-900	113-2400	lung	-	9	22
Lizon (106)	3	+	d8	d1,8	75-1000	100-2667	lung	-	8	25
Miyazaki (107)	3	-	d8	d1,8	60-800	80-2133	lung	+/-	30	15
Quantin (108)	3		d8	d1,8	85-1000	113-2667	lung	+/-	16	38
Garcia (109)	3	+	d8	d1,8	75-1000	100-2667	lung	ng	37	46
Jaremtchuk (110)	3		d8	d1,8	90-1000	120-2667	solid	+	25	?
Pawinski (111)	3	+	d8	d1,8	85-1000	113-2667	solid	+	5	40

Table 7. - Continued -

Rischin (112)	3		d8	d1,8	85-1200	113-3200	solid	+/-	28	29
Georgoulias (113)	3	+	d8	d1,8 +GCSF	100-900	133-2400	lung	-	51	37
Mavroudis (114)	3	+	d8	d1,8 +GCSF	100-900	133-2400	breast	+	52	54
Dimopoulos (115)	3	+	d8	d1,8+GCSF	75-1000	100-2667	bladder	-	16	50
Stathospoulos (116)	3	+	d8	d1,8+GCSF	100-1000	133-2667	pancreas	-	54	13
Frasci (117)	3		d1,8	d1,8	40-1000	107-2667	breast	+	19	16
Rizvi (118)	3		d1,8	d1,8	40-800	106-2133	solid	-	23	30
Chen (119)	3	+	d1,8	d1,8	30-800	80-2133	lung	+	40	33
Carreca (120)	3	+	d2,9	d1,8	40-1000	106-2667	lung	+	15	60
Ryan (121)	4		d1	d1,8,15	60-600	60-1800	solid	+	21	29
Poole (122)	4		d1	d1,8,15	70-800	70-2400	solid	+/-	24	0
Laufman (123)	4	+	d1	d1,8,15	100-800	100-2400	breast	+	32	59
Garland (124)	4		d1	d1,8,15	80-1000	80-3000	lung	-	8	25
				10 mg/min						
				idem	80-800		lung	+	10	20
Spiridonidis (125)	4		d1 1h	d1,8,15	100-800	100-2400	solid	+	40	35
			d15 1h	d1,8,15	<45-800	<45-2400				
Spiridonidis (126)	4	+	d1 1h	d1,8	100-800	100-2400	lung	+	40	33
Pawinski (127)	4	+	d15 1h	d1,8,15	< 85-800	<85-2400	solid	+	11	9
Kourosiis (128)	4		d1,8,15	d1,8,15	40-1000	120-3000	lung	-	16	25
Hainsworth (129)	4	+	d1,8(15)	d1,8(15)	30-800	90-2400	lung	-	41	29
Jacobs (130)	4?	+	d1	d1,8,15	75-800	75-2400	pancreas	ng	9	33
Rubio (131)	3 or 4	+	d8	d1,8	75-1000	150-2000	lung	-	13	31

All studies administered docetaxel as an 1h infusion and gemcitabine as a 30 min infusion, unless stated otherwise

ng= data not given in publication

Table 8. Summary of taxanes and antimetabolites

Drug combination	Sequence	*In vitro* studies	Pharmacokinetic studies	Clinical studies
Paclitaxel and				
MTX	**MTX-P**	synergism	scarce	**toxic**
	P-MTX	antagonism	?	?
	concomitant	antagonism	?	effective
5FU	5FU-P	antagonism	?	?
	P-5FU	**synergism**	?	**effective and feasible**
	concomitant	antagonism	?	?
Capecitabine	C-P	?	no change	?
	P-C	?	no change	feasible
	concomitant	**synergism**	no change	**effective and feasible**
Gemcitabine	G-P	antagonism	?	feasible
	P-G	antagonism	no change	**feasible**
	concomitant	antagonism	no change	?
Docetaxel and				
MTX	**MTX-D**	? (edatx: synergism)	no change	**toxic**
	D-MTX	? (edatx: antagonism)	?	?
	concomitant	? (edatx: mixed results)	no change	feasible

Table 8. - Continued -

Drug combination	Sequence	*In vitro* studies	Pharmacokinetic studies	Clinical studies
5FU	5FU-D	?	?	?
	D-5FU	?	scarce	**effective and feasible**
	concomitant	synergism	?	?
Capecitabine	C-D	?	no change	?
	D-C	?	no change	?
	concomitant	synergism	no change	**effective and feasible**
Gemcitabine	G-D	?	**?**	feasible
	D-G	?	**?**	**feasible**
	concomitant	?	?	?

CONCLUSIONS

Table 8 summarises preclinical, clinical and pharmacokinetic data on combination treatment of taxanes and antimetabolites.

Preclinically observed synergism for the sequence of MTX prior to a taxane might explain excessive bone marrow toxicity found in some clinical studies. However, despite *in vitro* observed antagonism, simultaneous exposure resulted in high response rates and good tolerance in patients with breast cancer and urothelial cancer, although the results do not look strikingly different from the reported single agent activities. Thus, MTX/taxane combinations may not be ideal for pursuing further studies. As the antagonistic effect of 5-FU prior to paclitaxel was evident from *in vitro* studies, all clinical studies used the reverse sequence. Various schedules appeared feasible and effective in both gastric and breast cancer patients. Again, acticity data are not very different from the single agent data, with the clear exception for the combination of capecitabine with docetaxel in breast cancer. Despite a lack of preclinical data suggesting synergism for any combination schedule of gemcitabine and a taxane, many studies using multiple schedules have noted efficacy, especially in metastatic breast cancer and non-small cell lung cancer, although not strikingly dissimilar from single agent activity. An optimal schedule has, however, not yet been established. Regarding data on actually delivered dose intensities, a 2- or 3-weekly cycle might be favoured and most feasible. Possible severe pulmonary toxicity warrants cautious monitoring of patients treated with this combination.

Combining two chemotherapeutc agents is not simply a matter of putting antitumor activities together. Drug interactions may result in synergism, not only of efficacy but also of toxic side-effects. Adding two drugs may also cause antagonism in drug efficacy due to unwanted interference in cytotoxicity or pharmacokinetics. It is therefore dissappointing that, in the vast majority of reviewed clinical studies, the preclinical evidence of schedule dependency was simply ignored and sequence of drug administration was not made part of the clinical protocol. Besides interference with the pharmacokinetics of an antimetabolite by a taxane, or *vise versa*, the vehicle CrEL might also have a major impact on pharmacokinetics of drugs other than paclitaxel itself. If one compares the number of studies done on antimetabolites plus taxanes as summarised in Tables 1-7 with the number of studies that include an adequate assessment of pharmacokinetics, the lack of such assessments becomes striking. This suggests that investigators are not adequately aware of the possible pharmacokinetic interactions and the necessity at least to exclude negative interactions. Clearly, trial design isues are not appropriately taken care of. This is a major concern since, in the case of these combinations, the increase and duplication of likely unnecessary trials may not have benefitted our patients. A better use of registries of trials seems warranted to avoid duplications.

For agents acting at a specific phase of the cell cycle, the sequence of administration may determine the efficacy and toxicity of a combination. Because of observed discrepancy between the *in vitro* data and clinical studies, we would like to stress the urge for adequate dose-finding clinical trials together with pharmacokinetic data analysis before examining any new combination chemotherapy in more detail in phase II studies. With the exception of the combination of capecitabine with docetaxel, other combinations of antimetabolites with taxanes were either not very promising or very toxic. Further studies should only be started after carefull consideration of the data available.

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CHAPTER 8

Randomized-Crossover Evaluation of Body-Surface Area-Based Dosing Versus Flat-Fixed Dosing of Paclitaxel

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Submitted

ABSTRACT

Purpose:

Despite dose calculation using body-surface area (BSA), pharmacokinetics of most anticancer drugs show wide interindividual variability (IIV). Here, we evaluated the role of BSA in paclitaxel disposition.

Methods:

Paclitaxel pharmacokinetics were initially studied retrospectively in 40 patients (dose, 70 to 200 mg/m^2). Subsequently, 12 patients were treated in a randomized crossover design with paclitaxel (3-hour infusion at a 3-week interval) at 175 mg/m^2 in cycle 1 (A) and a flat-fixed dose of 300 mg in cycle 2 (B), or *vice versa*. Blood samples were collected up to 24 h after dosing, and analyzed for total (Cp) and unbound (Cu) paclitaxel.

Results:

IIV in Cu clearance was significantly reduced after adjusting for BSA (196 ± 45.4 L/h/m^2 *vs* 346 ± 98.5 L/h; $P<.0001$; $n=40$). The area under the curves (AUC) of Cu were similar in both dosing groups, with mean values (A *vs* B) of 1.34 ± 0.158 *vs* 1.30 ± 0.329 μM.h, respectively, suggesting that BSA could explain 53.3% ($P=.022$) of IIV. Cu and Cp clearance was also significantly related to various body-size measures, including BSA ($R\geq.617$; $P\leq.033$), weight ($R\geq.621$; $P\leq.031$), and lean-body mass ($R\geq.630$; $P\leq.028$). We hypothesize that this is caused by the association of paclitaxel in the circulation with Cremophor EL, the distribution of which is linked to total blood volume, and thus to BSA.

Conclusion:

This study indicates that paclitaxel disposition is significantly related to BSA. This provides a pharmacokinetic rationale for BSA-based dosing of this drug.

INTRODUCTION

In medicine, most drugs for adult patients are administered at a flat-fixed dose. Only the dosage of some drugs with a small therapeutic index, such as aminoglycosides, cyclosporine, phenytoin and sympaticomimetics, are based on body weight of the patient and are adjusted by monitoring either serum drug levels or clinical outcome. In contrast, in oncology the dosage of nearly all cytotoxic drugs is based on body-surface area (BSA) of the patient [1]. The optimal dose of a cytotoxic drug is expected to result in two important clinical endpoints: a maximum antitumor effect and a minimum of toxicity. Studies on the appropriate rate of input (*viz*, dose and schedule) of antitumor agents are difficult, because the desired tumor responses cannot be observed immediately and may vary due to differences in drug sensitivity, while possible toxic effects may be severe and life-threatening. Therefore, pharmacokinetic variables such as drug clearance, area under the curve (AUC) and volume of distribution may serve as surrogate endpoints. Gurney has described a possitive

correlation of several pharmacokinetic parameters, especially AUC, with the toxicity of anticancer drugs, although a correlation with the tumor response is less often found [1]. However, for most cytotoxic agents no significant correlation has been noticed between BSA and drug clearance or AUC [1-2]. As normalization of drug dose to BSA seems unlikely to have a relevant impact on tumor response or toxicity of most anticancer drugs, this common way of dose calculation has been questioned [1-4].

The antineoplastic agent paclitaxel is widely used to treat a variety of solid tumors, particularly ovarian and breast cancer [5]. Dose calculation of paclitaxel is based on BSA, using a dosing schedule of 135 to 225 mg/m^2, that is usually administered as a 3-hour infusion every 3 weeks. Despite this dose adjustment based on BSA, a wide interpatient variability persists for total paclitaxel clearance [6]. In the present report, we studied paclitaxel disposition as a function of body-size measures retrospectively in a cohort of 40 adult cancer patients as well as prospectively in 12 patients treated in a randomized-crossover design with BSA-based *vs* flat-fixed dosing to provide a pharmacokinetic rationale for appropriate dosing strategies for this agent.

PATIENTS AND METHODS

Historic Patient Population

Records were collected of patients with a confirmed diagnosis of advanced solid tumor for which paclitaxel monotherapy was a viable therapeutic option or for which other treatment options were not available. Paclitaxel was administered as a 3-hour intravenous infusion (median, 3.00 hours; range, 2.72 to 3.75 hours) at dose levels of 70 (*n* = 10 patients), 100 (*n* = 14), 150 (*n* = 1), 175 (*n* = 13), and 200 mg/m^2 (*n* = 2). The eligibility criteria and full clinical and toxicological profiles have been documented in detail elsewhere [7-9].

Eligibility Criteria Prospective Evaluation

Eligible patients had histologically or cytologically documented solid cancer for which paclitaxel was a therapeutic option or for which no effective therapy was known. In order to observe any potential influence of BSA-based dosing on the pharmacokinetics and/or toxicity of paclitaxel, the BSA of potential patients was established to be ≤ 1.65 or ≥ 1.85 m^2 (based on a mean BSA value with a percent SD of 1.73 mg/m^2 $\pm$ 5%). This procedure was chosen because *(i)* it would provide information on the need for potential dosage adjustments at extreme BSA values, and *(ii)* it would avoid inclusion of patients receiving similar total doses following the fixed- or BSA-based dosing regimen. Patients were required to have a WHO performance status ≤ 2, age ≥ 18 years, an adequate bone marrow function [absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$ /L, platelets $\geq 100 \times 10^9$ /L, hemoglobin ≥ 6.0 mmol/L], an adequate liver function [bilirubin < 1.5 $\times$ the upper limit of institutional normal values (ULN),

AST and ALT < 2.5 × ULN], a normal renal function (creatinine clearance ≥ 60 ml/min), and no previous chemotherapy or radiotherapy during the preceeding 4 weeks prior to treatment. All patients gave written informed consent before study entry. The study was approved by the ethical commitee of the University Hospital Rotterdam (Rotterdam, the Netherlands).

Treatment Plan Prospective Evaluation

Paclitaxel formulated in a mixture of Cremophor EL and ethanol USP (Taxol; Bristol-Myers Squibb, Woerden, the Netherlands) was administered as a 3-hour intravenous infusion diluted in 500 mL of isotonic sodium chloride solution on the first day of a 3-week cycle. Standard intravenous premedication consisted of dexamethasone (8 mg), clemastine (2 mg) and ranitidine (50 mg), all given 30 minutes before start of the paclitaxel infusion. At study entry, patients were randomized using a random-number generator to receive one cycle of paclitaxel at a flat-fixed dose of 300 mg followed by a second cycle of paclitaxel at a BSA-based dose of 175 mg/m^2, or *vice versa*, with each patient serving as his or her own control. No dose reductions were allowed. Patients who could not receive the two cycles of paclitaxel went off study and were to be replaced. In case of absence of progressive disease, patients were offered to continue treatment outside this study with paclitaxel at the standard BSA-based dose in consecutive cycles of 3 weeks.

Pretreatment and Follow-Up Evaluation

At study entry and before each chemotherapy cycle, a history and physical examination were performed and weight, height, complete blood cell count with differential (including hemoglobin, platelets, WBC and ANC), and a clinical chemistry analysis (including sodium, potassium, calcium, albumin, total serum proteins, creatinine, bilirubin, γ-glutamyltransferase, alkaline phosphatase, AST, and ALT) were measured. Various measures of body-size, including BSA (in m^2), lean-body mass (in kg), ideal-body weight (in kg), adjusted ideal-body weight (in kg), and body-mass index were calculated as described elsewhere [10]. Blood cell counts and a chemistry analysis were also obtained weekly while on study. Tumor measurements were performed every two cycles, and responses and toxicity were evalutated according to the WHO criteria and the NCI Common Toxicity Criteria (version March 1998), respectively.

Sampling Procedure

Venous blood samples of approximately 5 mL were collected in both cycles at the following time points: before infusion, at 1 and 2 hours during infusion, at 5 minutes before the end of infusion, and at 5, 15, 30 minutes and 1, 2, 4, 8, 10 and 21 hours after the end of infusion. Blood samples were taken from a vein in the arm opposite to the one used for drug infusion, and collected in tubes containing lithium heparin as anticoagulant. Plasma was

separated by centrifugation at 3000×*g* for 10 minutes at 4ºC, and stored frozen at -80ºC until analysis.

Analytical Assays

Concentrations of total paclitaxel (the total of bound and unbound fractions) in plasma samples were determined by reversed-phase high-performance liquid chromatography with detection at 230 nm as described earlier [11]. Measurement of unbound paclitaxel was performed by equilibrium dialysis using a [*G*-^{3}H]paclitaxel tracer [12]. Coinciding levels of Cremophor EL were measured by a colorimetric dye-binding microassay [13].

Pharmacologic Calculations

Concentration-time profiles of unbound paclitaxel and total paclitaxel were analyzed by compartmental methods using the Siphar v4.0 software package (InnaPhase, Philadelphia, PA) as described previously [7,8]. The area under the curve (AUC) was extrapolated to infinity, and determined based on the best-fitted curve and used for calculation of the absolute clearance (in L/h), defined as the ratio of dose delivered (in mg) and AUC. The apparent clearance (in L/h/m^2) was calculated by dividing the absolute clearance of paclitaxel by a patient's individual BSA value. All paclitaxel concentration-time curves were best described by a 3-compartment model, without any demonstration of saturable behavior (R^2 = 0.989 ± 0.006; range, 0.967 to 0.997; root mean squared error = 18.5 ± 5.52%; range, 10.1 to 31.6%; *n* = 64). Noncompartmental analysis was used for calculation of Cremophor EL parameters [14].

Statistical Evaluation

All pharmacologic parameters are reported as mean values ± SD. Least-squares linear-regression analysis was performed to evaluate relationships between paclitaxel clearance and each of the studied body-size measures. Interindividual variability in parameters was evaluated by the coefficient of variation, defined as the ratio of SD and the observed mean value. For all tests, a $P<.05$ was considered as statistically significant, and all analyses were carried out using NCSS v5.X (J.L. Hintze, East Kayesville, UT; 1992) or SISA binomial (D. Uitenbroek, Hilversum, the Netherlands; 2001; http://home.clara.net/sisa/binomial.htm). In the retrospective analysis, the potential effect of paclitaxel dose on pharmacokinetic parameters was estimated by a one-way ANOVA, followed by the Duncan's multiple range test.

In order to detect a clinically relevant difference (δ) of more than 30% in AUC variability between the BSA-adjusted and the flat-fixed dosing regimen, with a two-tailed significance level of 0.05 and a statistical power of 0.80, a total of 12 patients was required for the prospective evaluation. The standardized difference was calculated as 2δ/sd, where sd is the standard deviation of the changes expected, which was estimated from data obtained in the

retrospective analysis. Since multiple measurements were performed at different times on the same patients, comparisons between the sets of observations were based on within subject differences. Therefore, variation between subjects, which is usually considerable, does not affect the ability to distinguish between the sets of observations, which here relate to the two dosing strategies. Differences in pharmacokinetic and pharmacodynamic parameters between cycles were evaluated using a two-tailed, paired Student's t-test after testing for normality. The significance of the relationship between the absolute clearance of paclitaxel (unbound and total drug) and the various measures of body size were evaluated by analyzing the cycles in which patients received fixed doses.

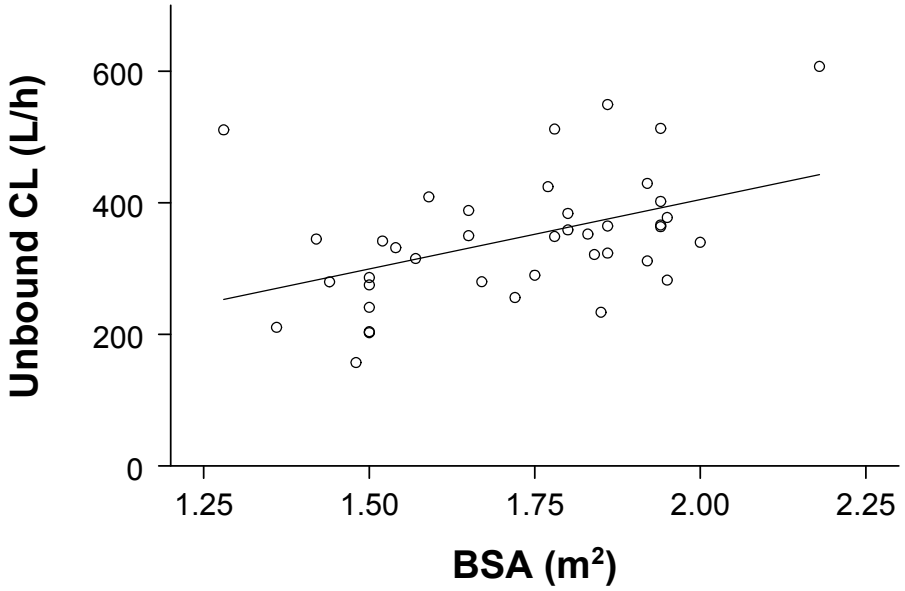

Figure 1. Relationship between absolute clearance (CL) of unbound paclitaxel and body-surface area (BSA) in retrospective evaluation (n = 40).

RESULTS

Retrospective Evaluation

The entire population consisted of 10 males and 30 females, with a median age of 50 years (range, 29 to 71 years), and a median BSA of 1.78 m2 (range, 1.28 to 2.18 m^2). The mean absolute and apparent clearance values of unbound paclitaxel were 346 ± 98.5 L/h

(range, 157 to 607 L/h) and 196 ± 45.4 L/h/m^2 (range, 106 to 295 L/h/m^2), respectively (Table 1). The coefficient of variation in clearance was substantially lower after correction for BSA (28.5 vs 23.1%), and the absolute clearance of paclitaxel was significantly related to BSA (R = .451; P = .003), suggesting that differences in BSA between patients contribute to explaining interindividual pharmacokinetic variability (Fig 1).

Table 1. Summary of Unbound Paclitaxel Data from Retrospective Evaluation*

Dose level (mg/m^2)	*n*	AUC (μM.h)	CL (L/h)	CL (L/h/m^2)
70	10	0.473 ± 0.133	303 ± 66.2	183 ± 40.2
100	14	0.616 ± 0.116	361 ± 99.5	197 ± 39.7
150	1	0.818	430	224
175	13	0.987 ± 0.290	375 ± 105	211 ± 50.8
200	2	1.46, 1.72	204, 241	136, 161
All dose levels	**40**		**346 ± 98.5**	**196 ± 45.4**

Abbreviations: *n*, number of patients at each dose level; AUC, area under the plasma concentration-time curve; CL, plasma clearance.

* Data are expressed as mean values ± SD.

Prospective Evaluation

To confirm a role of BSA in paclitaxel disposition, an additional group of 12 patients was studied in a randomized-crossover design with two treatment cycles based on BSA-corrected dose or flat-fixed dose regimens. Seven patients started with a flat-fixed dose followed by a BSA-based dose, and 5 patients were randomized to receive the reverse sequence. Baseline demographic data and median values for the various body-size measures were similar between patients randomized for a flat-fixed dose or a BSA-based dose in cycle 1 (Tables 2 and 3). The most prominent tumor types were breast (*n* = 4) and lung cancer (*n* = 3), and half of the patient had received 2 or more prior chemotherapy regimens.

The exposure to unbound paclitaxel, total paclitaxel and Cremophor EL was similar in both dose groups, with overall mean AUC values of 1.34 ± 0.16 *vs* 1.30 ± 0.33 μM.h (*P* = .67), 17.7 ± 3.0 *vs* 17.3 ± 5.2 μM.h (*P* =.71), and 57.5 ± 13.9 *vs* 55.5 ± 17.7 μL.h/mL (*P* =.53), respectively (Table 4).

Table 2. Patient Demographics at Baseline by Randomized Group in Cycle 1

Characteristic	Fixed dose Median	Range	BSA-based dose Median	Range
Entered on trial*	7		5	
Sex, male/female*	3/4		2/3	
Age, years	56	42 – 66	58	34 – 72
Serum creatinine, μmol/L**	74	48 – 119	76	62 – 87
Serum albumin, g/L	41	29 – 47	43	30 – 44
Total serum protein, g/L	78	64 – 87	76	74 – 85
Serum bilirubin, μmol/L	6	4 – 11	8	4 – 15
AST, units/L	27	11 – 45	46	15 – 61
ALT, units/L***	22	16 – 138	52	28 – 59

* Data indicate number of patients; **One patient had a creatinine clearance of 41 mL/min, but was accepted by exemption based on the limited role of the renal funtion in paclitaxel metabolism; ***Another patient had an elevated value of ALT (grade 2), but normal values of AST and serum bilirubin.

Table 3. Summary of Body-Size Measures by Randomized Group in Cycle 1

Characteristic	Fixed dose* Median	Range	BSA-based dose** Median	Range
Height, cm	170	152 – 181	173	158 – 178
Weight, kg	74	48 – 91	77	51 – 87
Body-surface area, m^2	1.88	1.46 – 2.10	1.95	1.50 – 2.05
Lean-body mass, kg	71	44 – 92	78	48 – 88
Ideal-body weight, kg	62	45 – 76	69	50 – 74
Adjusted ideal-body weight, kg	65	48 – 78	73	51 – 75
Body-mass index	25	18 – 30	24	19 – 29

*Data from 7 patients; **Data from 5 patients.

Table 4. Pharmacokinetic Parameter Estimates by Randomized Group*

Parameter	Group 1 Cycle 1	Cycle 2	Group 2 Cycle 1	Cycle 2
Dose	300 mg	175 mg/m2	175 mg/m2	300 mg
Unbound paclitaxel				
Cmax, μM	0.35 ± 0.08	0.34 ± 0.02	0.35 ± 0.02	0.39 ± 0.14
AUC, μM.h	1.26 ± 0.30	1.32 ± 0.19	1.36 ± 0.11	1.35 ± 0.40
CL, L/h	290 ± 62.1	277 ± 47.0	270 ± 43.6	278 ± 76.9
CL, L/h/m2	163 ± 30.7	156 ± 28.6	151 ± 11.7	154 ± 27.6
Total paclitaxel				
Cmax, μM	4.65 ± 1.24	4.82 ± 0.89	5.20 ± 0.87	5.27 ± 2.43
AUC, μM.h	16.8 ± 3.55	17.0 ± 2.42	18.8 ± 3.59	18.0 ± 7.33
CL, L/h	22.0 ± 6.02	21.6 ± 4.06	20.1 ± 5.17	21.9 ± 7.84
CL, L/h/m2	12.3 ± 2.60	12.1 ± 1.80	11.1 ± 1.83	12.0 ± 3.05
Cremophor EL				
Cmax, μL/mL	4.26 ± 1.04	4.39 ± 1.53	4.40 ± 0.63	4.46 ± 1.15
AUC, μL.h/mL	52.5 ± 13.6	54.1 ± 12.4	62.3 ± 16.0	59.8 ± 23.4
CL, mL/h	508 ± 143	499 ± 157	451 ± 180	502 ± 285
CL, mL/h/m2	282 ± 47.4	277 ± 60.0	248 ± 79.0	275 ± 132

Abbreviations: C_{max}, peak plasma concentration; AUC, area under the plasma concentration-time curve; CL, plasma clearance.

* Data are expressed as mean values ± SD.

The coefficient of variation in AUC was substantially lower in the BSA-based dose group (unbound paclitaxel, 11.8 *vs* 25.3%; total paclitaxel, 16.7 *vs* 29.9%; Cremophor EL, 24.2 *vs* 32.0%), suggesting that BSA could explain 53.4%, 44.2% and 24.4% of interindividual variability in exposure to unbound paclitaxel, total paclitaxel and Cremophor EL, respectively. Furthermore, the absolute clearances of unbound and total paclitaxel were significantly related to various measures of body-size, except for height (Table 5, Figs 2 and 3).

None of the patients developed non-hematologic toxicity grade >2, and there were no episodes of neutropenic fever or treatment-related deaths. Overall, hematologic toxicity in the first cycle was mild, with a median ANC nadir of 3.3×10^9/L (coefficient of variation, 70.2%) and 3.2×10^9/L (coefficient of variation, 94.6%) in the BSA-based dose group and the flat-

fixed dose group, respectively. Apart from one heavily-pretreated patient who experienced grade 3 thrombocytopenia during both treatment cycles, no thrombocytopenia was noticed in any of the other patients.

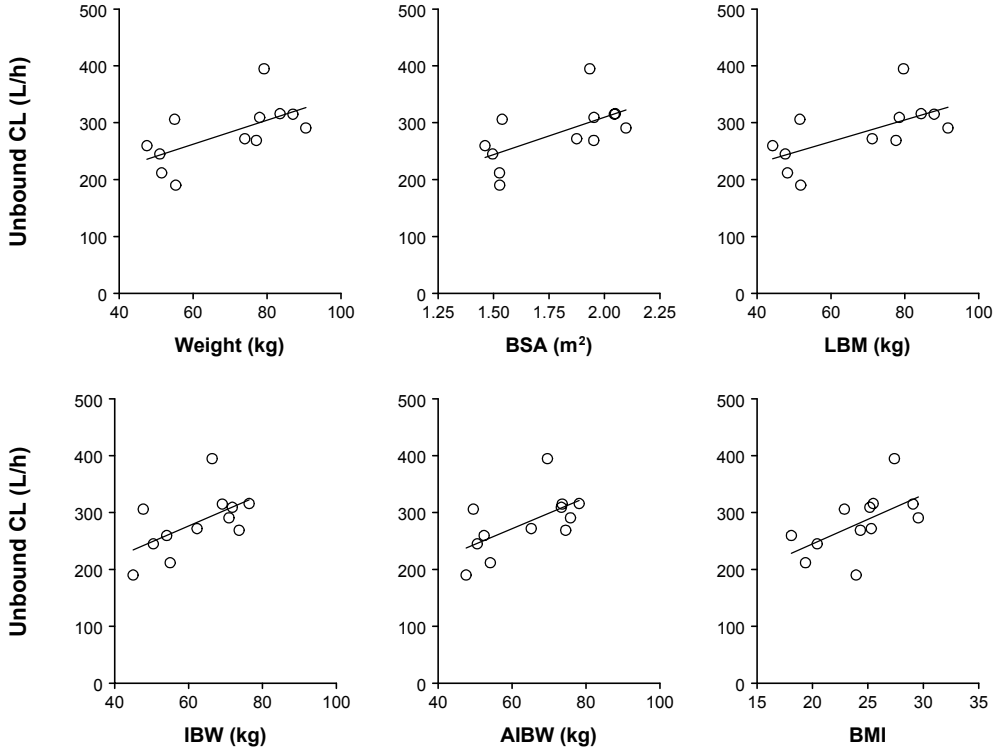

Figure 2. Relationships between absolute clearance (CL) of unbound paclitaxel and weight, body-surface area (BSA), lean-body mass (LBM), ideal-body weight (IBW), adjusted ideal-body weight (AIBW), and body-mass index (BMI) in prospective evaluation (*n* = 12).

Table 5. Relationships between Paclitaxel Clearance and Body-Size Measures*

	Unbound paclitaxel		Total paclitaxel	
Body-size measure	R	*P*	R	*P*
Height	.523	.081	.538	.071
Weight	.621	.031	.679	.015
Body-surface area	.617	.033	.673	.017
Lean-body mass	.630	.028	.688	.013
Ideal-body weight	.572	.052	.604	.038
Adjusted ideal-body weight	.604	.038	.645	.024
Body-mass index	.579	.049	.668	.018

* Data from cycles in which patients received fixed doses (seven in cycle 1 and five in cycle 2).

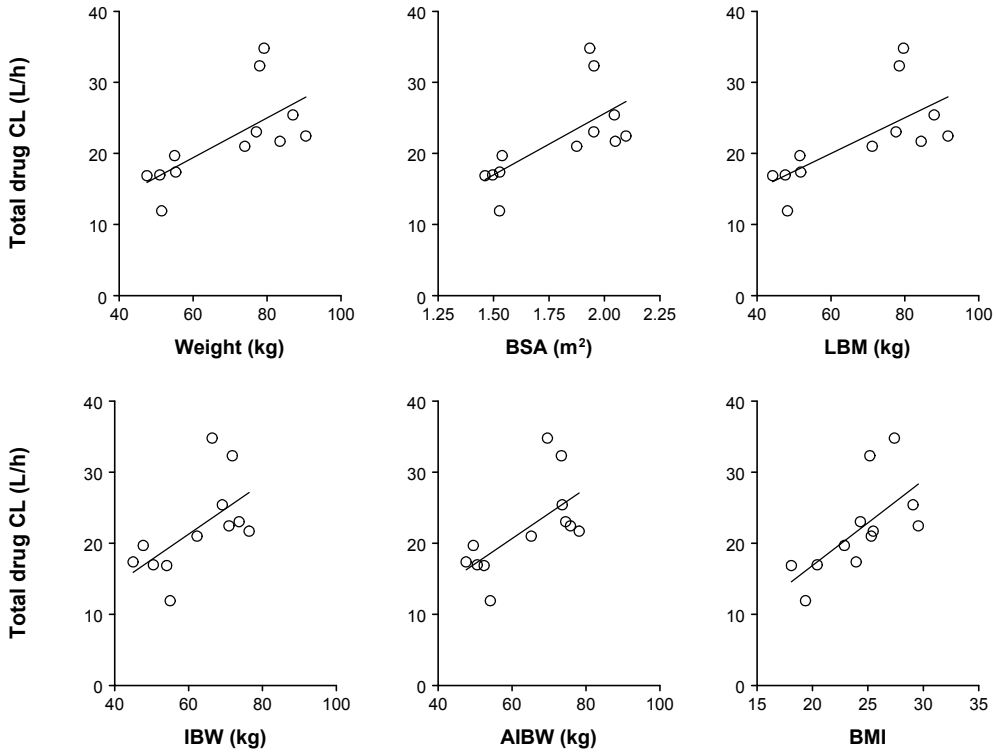

Figure 3. Relationships between absolute clearance (CL) of total paclitaxel and weight, body-surface area (BSA), lean-body mass (LBM), ideal-body weight (IBW), adjusted ideal-body weight (AIBW), and body-mass index (BMI) in prospective evaluation (*n* = 12).

DISCUSSION

In this study we have investigated paclitaxel disposition as a function of body-size measures by a retrospective analysis, as well as by a comparative randomized-crossover study using the convential method for dose calculation based on BSA (175 mg/m^2) and a flat-fixed dosing regimen (300 mg). Interestingly, we observed that the interindividual variability in exposure to unbound and total paclitaxel following administration as a 3-hour infusion in a 3-week regimen is approximately 50% reduced by BSA-based dosing as compared to flat-fixed dosing.

To achieve a therapeutic response with a predictable and acceptable degree of toxicity, one would have to obtain a certain level of drug exposure. To minimize any variation in this level, the dose of most anticancer agents is currently based on the BSA of individual patients. However, the contribution of other patient-related factors to variability in drug

exposure is usually much larger than that of body-size measures alone, so that the additional value of BSA in dose calculations may be questioned [1,2]. Indeed, a review of the available literature reveals that the clearance of most drugs in clinical oncology, including anticancer agents that have been available for many years (eg, cisplatin [15], epirubicin [16], irinotecan [10], and topotecan [17]], as well as investigational agents (eg, ET-743 [18], and ZD9331 [19]; reviewed in ref. 3) is not related to BSA.

Previously, Grochow and colleagues have correlated pharmacokinetic variables of total paclitaxel (dosed on the basis of BSA in mg/m^2) with the body-size measures, including height, weight, and BSA [3]. In a total of 16 patients treated in a phase I trial with paclitaxel administered in a 3-week regimen, only height was significantly associated with the clearance of paclitaxel. Unfortunately, the data generated in this trial were based on measurement of the total plasma concentration of paclitaxel using different dose groups. This is of particular importance in view of the profound nonlinear paclitaxel disposition in humans [6,20], which suggests that correlation analyses based on total plasma levels alone are not appropriate when different dose groups are included. Since the AUC of unbound paclitaxel is a linear function of the paclitaxel dose administered [7,8], we focused here on the unbound paclitaxel fraction.

The level of drug exposure varies with individual rates of absorption, distribution, metabolism and excretion. The disposition of paclitaxel in patients depends on the duration of infusion and the dose and time-varying concentrations of its vehicle Cremophor EL, due to a preferential affinity of paclitaxel for Cremophor EL in blood [21]. It has been demonstrated that Cremophor EL has an extremely small volume of distribution [mean (± SE), 2.53 ± 0.124 L/m^2; *n* = 67] [14], approximating the volume of the blood compartment [22]. In our present study, Cremophor EL clearance was not significantly related to BSA, which is most likely caused by the profound interindividual kinetic variability that is larger than normal for most drugs. However, we have recently found in a larger group of patients that BSA is a significant covariate on clearance in a population model for Cremophor EL pharmacokinetics (Mats Karlsson and Alex Sparreboom, manuscript in preparation). As total blood volume is related to BSA [23], we hypothesize that the impact of BSA on variability in paclitaxel pharmacokinetics is caused by the association of paclitaxel in the circulation with Cremophor EL micelles, of which the distribution is linked to total blood volume, and thus to BSA. To lend further support to this hypothesis, we are currently evaluating the relationship between BSA and clearance of other chemotherapeutic agents formulated for clinical use in a Cremophor EL-containing vehicle (eg, teniposide).

Paclitaxel is eliminated mainly by hepatic metabolism through CYP3A4 and CYP2C8 activity [24], as well as by MDR1 P-glycoprotein-mediated intestinal secretion [25]. The metabolic capacity of the liver has not been associated with body-size measures [4], suggesting that interindividual differences in enzyme activity contribute to pharmacokinetic variability independent of a patient's BSA. Furthermore, genetic polymorphism in the population results

in a large variability in CYP3A4 [26], CYP2C8 [27], and P-glycoprotein activity [28], and is therefore likely to have a major role in paclitaxel pharmacokinetics. In fact, altered liver functions in the elderly might explain the previously observed change in clearance of unbound paclitaxel in this age group as compared to adult patients [29]. Ongoing trials currently explore the role of metabolic capacity in paclitaxel disposition and treatment outcome using genotyping and phenotyping approaches for CYP3A4, CYP2C8 and P-glycoprotein as a potential measure for dose calculation of paclitaxel in addition to BSA.

In conclusion, this study shows that paclitaxel disposition has a unique feature in that the interindividual variability in exposure is significantly reduced by adjusting the dose to BSA. As hepatic metabolism is the principal elimination route for paclitaxel, it is of particular interest to investigate any correlation between metabolic capacity and variability in paclitaxel pharmacokinetics. At present, arguments to abandon the current way of dose calculation based on BSA are lacking in the case of paclitaxel.

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CHAPTER 9

Summary

SUMMARY

In this thesis the results of clinical studies with new chemotherapeutic agents and pharmacokinetic studies on taxanes in breast cancer patients are reported.
In metastatic breast cancer, endocrine and cytotoxic treatment often result in objective tumor responses, associated with relevant relief of symptoms. At present, however, metastatic breast cancer is still considered to be incurable even despite agressive multi-modality treatment options. Chemotherapy for metastatic breast cancer should therefore aim at a maximum of palliation and prolongation of life, at the cost of a minimal of toxicity.

In **chapter 2** the results of a phase II study with miltefosine as a topical treatment of skin metastases of breast cancer are described. Miltefosine (hexadecylphosphocholine) exerts its cytotoxicity by interfering with the metabolism of cell membrane phospholipids. It can be administered topically, which is attractive for its easy self-administration and lack of systemic side effects. Twenty breast cancer patients with progression of skin metastases were treated with a 6% solution of miltefosine, which was topically administered once daily during the first week and twice daily thereafter. Sixteen out of 20 patients also had metastatic disease at other sites. Concomitant systemic treatment when ongoing for at least 2 months prior to study entry was permitted. Miltefosine therapy was discontinued in 2 patients due to nausea and in 1 patient due to skin toxicity. Apart from grade 1-2 skin reactions and mild nausea and vomiting in 11 and 2 patients, respectively, no systemic toxicity was noticed. In 18 patients evaluable for response, four partial responses were noted (response rate 22%), while 7 patients had stable disease. A higher response rate was suggested in patients in whom the skin lesions were smaller than 1.5 cm^2 and in patients without progressive disease at other sites. The median duration of respons was 2.5 months and median time to progression for all patients was 1.9 months. We concluded that miltefosine may effectively control small skin metastases, without serious toxicity.

Anthracyclines and taxanes are among the most effective agents in metastatic breast cancer. After failure on these agents, only few other agents are of any use. Gemcitabine (2'2'-difluorodeoxycytidine) is a new cytotoxic agent that has shown efficacy with excellent tolerability in patients with a variety of solid tumors, including those with breast cancer. We describe in **chapter 3** a phase II study of gemcitabine as a systemic treatment for patients with metastatic breast cancer, previously treated with both an anthracycline- and taxane containing regimen. Twenty-three patients were treated with gemcitabine 1200 mg/m^2 as a 30-min infusion on day 1, 8 and 15 every 4 weeks. Seventy-four percent of the patients had visceral metastases. All patients were heavily pretreated, with two or more lines of chemotherapy in 91% of the patients and three or more lines in 57%. No complete or partial responses were observed in this heavily pretreated patient population, while only six patients

(26%) had stable disease with a median duration of 4.0 months. The median time to progression was 1.9 months and the median survival time was 7.8 months. Neutropenia grade 3-4 was observed in four patients (18%). Non-hematological toxicity grade 3 included nausea and vomiting in 14%, skin toxicity in 9% and elevation of transaminases in 23% of the patients. We concluded that single-agent gemcitabine is ineffective in metastatic breast cancer after prior anthracycline and taxane treatment, but because of non-overlapping toxicity and mode of action and in view of literature data on the use in less heavily pretreated patients, gemcitabine could still be evaluated in combination schedules in first- or second line therapy.

In view of encouraging results and tolerable toxicity of continuous infusion of 5-fluouracil (5-FU) in advanced gastrointestinal and breast cancer, innovative oral 5-FU agents such as capecitabine have been developed. **Chapter 4** reviews its use as monotherapy or in combination schedules in metastatic breast cancer. An intermittent dosing schedule of capecitabine twice daily at a dose of 2,510 mg/m^2 day on day 1-14 in a 3-weekly cycle appeared to be well tolerated, and resulted in a high dose-intensity. Its main side effects consist of diarrhea, nausea, the hand-foot syndrome and sometimes neutropenia. A large phase II study investigating capecitabine in 135 advanced breast cancer patients, pretreated with anthracyclines and taxanes, observed three complete and 24 partial responses (response rate 20%), with a mean duration of 8.0 months. Preliminary results of a study comparing capecitabine with paclitaxel in 42 breast cancer patients failing anthracyclines indicate that the efficacy of capecitabine is comparable to that of paclitaxel with response rates of 36% and 21%, respectively. Another study of capecitabine as first-line therapy for advanced breast cancer in women aged ≥ 55 years reported a response rate of 25%, which tended to be better than a combination schedule of cyclophosphamide/methotrexate/5-FU (CMF). Promising results are reported in studies combining capecitabine with paclitaxel or docetaxel in patients with metastatic breast cancer. Capecitabine is clearly an active agent for the treatment of breast cancer, and is currently being registered in various countries.

Weekly administration of paclitaxel has demonstrated sustained efficacy together with a favourable toxicity profile lacking severe myelotoxicity, and therefore seems an attractive chemotherapeutic option for elderly women with metastatic breast cancer. However, the exposure to a cytotoxic agent may be altered with aging due to impaired renal and hepatic functions and/or differences in body composition. **Chapter 5** reports the results of a pharmacokinetic analysis of paclitaxel in breast cancer patients aged 70 years or older treated with a weekly schedule, and compares the results with a control group of patients aged less than 70 years of age treated likewise. Paclitaxel was administered as a 1 h infusion at a dose of 80 mg/m^2 to 8 patients aged 70-84 years, and at a dose of 100 mg/m^2 to 15 patients aged 22-69 years. Serial blood samples were obtained up to 24 h after the end of

infusion. The apparent clearance of unbound paclitaxel was 124 ± 35.0 in the elderly group vs 244 ± 58.8 L/h/m^2 in the patients less than 70 years of age, and appeared to be inversely related to the patient's age (r^2=0.857; P<0.00001). The total plasma clearance of cremophor EL was 150 ± 60.7 vs 115 ± 39.2 mL/h/m^2 (p=0.04). These data indicate that the clearance of unbound paclitaxel is approximately 50% reduced in elderly patients as compared to adult patients, resulting in a significant increase in systemic exposure with age. The observed increase of clearance of Cremophor EL in elderly patients may (in part) explain the altered clearance of unbound paclitaxel. The clinical relevance of these observations warrants further evaluation.

In search of improving therapeutic options in oncology new combinations of known anticancer agents are being explored. The results of a dose-finding and pharmacokinetic study of the combination of docetaxel and methotrexate (MTX) in patients with breast cancer and other solid tumors is described in **chapter 6**. A total of 22 patients were treated with MTX as an i.v. push at a dose of 30, 40 or 50 mg/m^2 on day 1 and 15 together with docetaxel as a 1 h infusion at a dose of 75 or 85 mg/m^2 on day 2 in a 3-weekly schedule. Due to observed dose-limiting toxicity of severe mucositis and neutropenia in all 3 patients at the first dose level, subsequent patients received supportive care consisting of oral leucovorin (60 mg daily, day 1-2) and subcutaneous lenograstim (263 ug daily, day 4-10). However, considerable toxicity involving mucositis, fatigue, myalgia and myelotoxicity were also frequently observed at the dose levels above MTX at 40 mg/m^2 and docetaxel at 75 mg/m^2. A partial response was observed in three out of nine patients with advanced breast cancer and one patient with urothelial cancer. Pharmacokinetic data revealed no interaction between both agents or their metabolites and could not explain the observed excess of toxicity. The sequential administration of MTX followed by docetaxel after 24 h results in significant toxicity without evidence of increased efficacy.

To put the observed toxicity reported in chapter 6 into perspective, we reviewed preclinical and clinical studies on combinations of taxanes and the antimetabolites MTX, 5-FU, capecitabine and gemcitabine in **chapter 7**. Different outcomes of preclinical and clinical studies reveal that combining two chemotherapeutc agents is not simply a matter of putting anti-tumor activities together. Drug interaction may result in synergism, not only of efficacy but also of toxic side effects. Combining two drugs may also implicate antagonism in drug efficacy, due to unwanted interference in cytotoxicity or pharmacokinetics. For agents acting at a specific phase of the cell cycle, the sequence of drug administrations may determine the efficacy and toxicity of a combination therapy. Because of the observed discrepancy between the results of *in vitro* studies and clinical studies, we would like to emphasize the need for adequate pharmacokinetic data analysis in combination chemotherapy before examining any new combination chemotherapy in more detail in phase II studies.

The relevance of pharmacology studies for our understanding of appropriate drug treatment is further stressed in **chapter 8**. Paclitaxel is frequently used in advanced breast cancer and other solid tumors, using a dose calculation based on the body-surface area (BSA) of the individual patient. This chapter reports on a retrospective analysis and a randomized crossover study to evaluate the role of BSA in paclitaxel disposition. Paclitaxel pharmacokinetics were retrospectively analysed in 40 patients treated with paclitaxel at a dose of 70-200 mg/m^2 as a 3 h infusion. The interindividual variability in clearance of unbound paclitaxel was significantly reduced after adjusting for BSA, being 196 ± L/h/m^2 vs 346 ± 98.5 L/h (P<0.0001). Twelve patients were treated in a randomized crossover study with paclitaxel as a 3 h infusion in a 3-weekly schedule at a dose of 175 mg/m^2 in course 1 (A) and at a fixed dose of 300 mg in course 2 (B), or vice versa. Blood samples were collected up to 24 h after dosing. The exposure to unbound paclitaxel, total paclitaxel and Cremophor EL were similar in both dose groups. The coefficient of variation was substantially lower in the BSA-based dose group, suggesting that BSA could explain 53.4% of interindividual variability in exposure to unbound paclitaxel (11.8 vs 25.3%, P=0.022). We hypothesize that this is caused by the association of paclitaxel with Cremophor EL, the distribution of which is linked to the total blood volume and hence to BSA. Other studies are under way to explore any correlation between the metabolic capacity of the liver and the variability in paclitaxel exposure. So far, arguments to abandon the current way of dose calculation using BSA are lacking for paclitaxel.

In hormone-refractory advanced breast cancer, chemotherapy definitely has a role to play in view of the fact that it can induce a relevant relief of symptoms and a (albeit modest) gain in survival. Current evidence does not support the standard use of high dose schedules at the cost of much toxicity. Therefore future advances in systemic therapy are to be made with new targeted agents and with cytotoxic agents with an improved safety profile and/or more convenient schedule of administration. When combining two or more agents, a thorough analysis of pharmacokinetic interaction should precede any further clinical testing. Furhermore, pharmacologic studies can help us in refining our treatment approaches.

CHAPTER 9

Samenvatting

SAMENVATTING

Dit proefschrift bevat studies naar de werkzaamheid van enkele nieuw ontwikkelde antikanker medicijnen bij patienten met uitgezaaide borstkanker. Tevens is het beloop van bloedspiegels van paclitaxel, een effectief en veel gebruikt antikanker middel bij borstkanker, nader onderzocht in enkele farmacologische studies.
Borstkanker is in Nederland de meest voorkomende vorm van kanker bij vrouwen. Ondanks diverse behandelingsmogelijkheden is er bij het optreden van uitzaaiingen sprake van een ongeneeslijke ziekte. Een behandeling met hormonale therapie of met chemotherapie heeft hierbij een goede kans om, weliswaar tijdelijk, de ziekte terug te dringen en symptomen te verminderen. Chemotherapie is geindiceerd bij het falen van hormonale therapie of bij zeer uitgebreide ziekte. Omdat genezing niet mogelijk is, moeten de voordelen (klachten-vermindering) en de nadelen (bijwerkingen) van de behandeling steeds goed tegen elkaar worden afgewogen.

Hoofdstuk 2 beschrijft een studie naar de werkzaamheid van miltefosine bij borstkanker met uitzaaiingen (metastasen) naar de huid. Miltefosine werkt celdodend door beschadiging van fosfolipiden in de celmembraan, en kan direkt in de vorm van een lotion op de huidmetastasen worden gesmeerd. Twintig patiënten met toename van huidmetastasen van borstkanker werden behandeld met miltefosine 6% oplossing op de huidlesies. Gelijktijdige behandeling met andere antikanker medicijnen was toegestaan, mits deze de voorafgaande 2 maanden niet veranderd was. De bijwerkingen van miltefosine waren niet ernstig, met misselijkheid en huidirritatie in respectievelijk 2 en 11 patiënten. Van de 18 patiënten die geëvalueerd konden worden voor een respons, werd er bij 4 een afname van meer dan 50% van de huidmetastasen gezien (een partiële respons) en bij 7 een stabilisatie. Drie van de 4 partiële responsen traden op bij huidlesies kleiner dan 1.5 cm^2. Behandeling van huidmetastasen met miltefosine wordt goed verdragen, is makkelijk door de patiënt zelf toe te passen en blijkt met name effectief bij kleine huidlesies.

Anthracyclines en taxanen zijn momenteel de meest effectieve celdodende medicijnen bij uitgezaaide borstkanker. Als deze middelen falen, resteren er weinig andere cytotoxische middelen met een goede werkzaamheid tegen deze ziekte. Gemcitabine (2'2'-difluorodeoxycytidine) is een nieuw chemotherapeuticum dat effectief is bij sommige soorten kanker en relatief weinig bijwerkingen heeft. In enkele studies bleek gemcitabine bij niet voorbehandelde patiënten met gemetastaseerde borstkanker effectief. **Hoofdstuk 3** vermeldt een studie naar de effectiviteit van gemcitabine bij patiënten met gemetastaseerde borstkanker, die niet meer op anthracyclines en taxanen reageerden. Gemcitabine werd als een 30 minuten infuus in een ader (intraveneus) in een dosering van 1200 mg/m^2 op dag 1, 8 en 15 in een vierwekelijks schema toegediend aan 23 patiënten. De groep patiënten was

uitgebreid voorbehandeld, en had veelal ziekteactiviteit in meerdere orgaansystemen. Mogelijk mede daardoor werd geen afname van ziekteactiviteit (respons) gezien, en trad slechts bij 6 patiënten (26%) een stabilisatie van ziekte op. De behandeling werd redelijk verdragen, met graad 3 misselijkheid bij 14%, huidtoxiciteit bij 9% en graad 3-4 afname van witte bloedcellen bij 18% van de patiënten. Gemcitabine lijkt derhalve ineffectief in de behandeling van uitgezaaide borstkanker na falen op anthracycline- en taxaanbevattende chemotherapie, maar zou mogelijk wel een rol kunnen spelen in eerdere stadia van behandeling.

5-fluouracil (5-FU) is een al langer bestaand antikanker middel, dat als enkelvoudige therapie als bolusinfusie bij uitgezaaide bortskanker weinig effectief is. Wegens veelbelovende resultaten en een mild bijwerkingenprofiel van een continue intraveneuze toediening van 5-FU bij o.a. maagdarmkanker en borstkanker zijn nieuwe 5-FU analoga, zoals capecitabine, ontwikkeld die dagelijks via de mond (oraal) kunnen worden ingenomen. **Hoofdstuk 4** geeft een overzicht van studies met capecitabine als enkelvoudig middel of in combinatie met andere cytostatica bij patiënten met gemetastaseerde borstkanker. Een toedieningsschema van twee maal daags capecitabine in een dosering van 2510 mg/m^2 per dag op dag 1-14 iedere 3 weken wordt goed verdragen, met als belangrijkste bijwerkingen diarree, misselijkheid, rode pijnlijke handpalmen en voetzolen en een daling van het aantal witte bloedcellen. Meerdere studies met in totaal meer dan 300 patiënten tonen aan dat capecitabine effectief is bij de behandeling van borstkanker, met responspercentages van 20-40%, afhankelijk van de mate van voorbehandeling en uitgebreidheid van ziekte. Ook bij patiënten voorbehandeld met anthracyclines en taxanen werd nog een respons gezien bij 20-27%. Capecitabine is momenteel in meerdere landen geregistreerd voor de behandeling van gemetastaseerde borstkanker. De waarde van capecitabine in combinatie met andere antikankermiddelen wordt momenteel onderzocht.

Alhoewel borstkanker op oudere leeftijd veel voor komt, zijn er weinig gegevens over de mogelijkheden en beperkingen van behandeling met chemotherapie bij ouderen. Door een verminderde functie van lever en nieren en een veranderde lichaamssamenstelling kan de blootstelling aan toegediende antikanker medicijnen bij bejaarden anders verlopen dan bij jongere patiënten. In **hoofdstuk 5** werd de blootstelling aan paclitaxel onderzocht bij 8 patiënten met uitgezaaide borstkanker ouder dan 70 jaar en vergeleken met een controle groep van 15 patiënten van 22 tot 69 jaar oud. Paclitaxel werd toegediend in een ader als een 1 uurs infuus in een dosering van 80 (bij de bejaarden) of 100 mg/m^2 (bij jongere patiënten) op dag 1, 8 en 15 in een vierwekelijks schema. De concentratie van paclitaxel in het bloed werd gemeten op diverse tijdstippen tot 24 uur na de paclitaxel toediening. De berekende klaring (een maat voor de hoeveelheid van een stof die per tijdseenheid door het lichaam kan worden afgebroken en/of verwijderd) bleek voor de vrije fractie van paclitaxel

bijna met de helft gereduceerd in de bejaarde patiënten vergeleken met de andere groep, en omgekeerd evenredig met de leeftijd van patiënten. De verminderde klaring resulteert in een grotere blootstelling aan paclitaxel bij ouderen. Mogelijke verklaringen hiervoor worden besproken. Of dit relevante gevolgen heeft voor de werkzaamheid en bijwerkingen van paclitaxel bij ouderen, zou verder onderzocht dienen te worden.

In het onderzoek naar verbetering van behandelmogelijkheden van kanker worden naast nieuwe medicijnen ook nieuwe combinaties van bestaande middelen bestudeerd. Docetaxel, een taxaan, en methotrexaat (MTX) zijn effectieve antikanker middelen, o.a. werkzaam tegen borstkanker. **Hoofdstuk 6** beschrijft een studie, waarin oplopende doseringen van beide middelen in een driewekelijks schema gecombineerd werden bij patiënten met uitgezaaide solide tumoren, waaronder borstkanker. Een totaal van 22 patiënten werd behandeld met MTX op dag 1 (en 15) gevolgd door docetaxel op dag 2. Boven een dosering van 40 mg/m^2 MTX en 75 mg/m^2 docetaxel traden frequent hevige slijmvliesbeschadiging, spierpijn, moeheid en koorts bij verlaagde afweer op, ondanks aanvullende beschermende maatregelen met groeifactoren en leucovorin. Bij 4 partienten werd een significante afname van de kanker geconstateerd. De combinatiebehandeling met MTX en docetaxel in deze volgorde resulteert in forse bijwerkingen, zonder een duidelijke verbetering in antitumor effect.

Teneinde een verklaring te vinden voor de forse bijwerkingen in de combinatiebehandeling beschreven in hoofdstuk 6, hebben we in **hoofdstuk 7** de beschikbare literatuur samengevat van laboratorium- en patiëntstudies met een combinatiebehandeling van een taxaan met de antikanker middelen MTX, 5-FU, capecitabine en gemcitabine (alle behoren tot de klasse van antimetabolieten). Interactie van 2 middelen kan resulteren in synergisme, niet alleen van werkzaamheid maar ook van bijwerkingen. Andersom kan interactie ook resulteren in tegengestelde (antagonistische) effecten, met verminderde effectiviteit door onderlinge beinvloeding op het niveau van aangrijpen op de tumorcel of de mate van expositie aan het middel (farmacokinetiek). Bij middelen die aangrijpen op een specifiek deel van de celcyclus kan de volgorde en tijdsduur waarin middelen worden toegediend een rol spelen in de effectiviteit en bijwerkingen van de combinatie. Vanwege de geconstateerde verschillen tussen de resultaten van laboratorium- en patiëntstudies pleiten wij voor een gedegen studie naar expositieparameters ten tijde van patiëntstudies naar optimale doseringen, voordat nieuwe combinaties bij grotere groepen patiënten verder onderzocht worden op hun werkzaamheid.

Het belang van goed farmacologisch onderzoek voor een optimalisatie van medicijn behandeling wordt verder benadrukt in **hoofdstuk 8**. Net als vele andere cytostatica wordt paclitaxel gedoseerd naar het berekende lichaamsoppervlak (body-surface area, BSA) van

de individuele patiënt, om een zo constant mogelijke blootstelling aan het middel te bewerkstelligen. Dit hoofdstuk omvat een retrospectieve analyse en een gerandomiseerde crossover studie om na te gaan wat de rol van BSA voor de blootstelling van de patiënt aan paclitaxel is. In de retrospectieve studie waren 40 patiënten behandeld met paclitaxel in een dosering van 70 tot 200 mg/m^2. De variabiliteit tussen patiënten onderling in klaring van de vrije fractie van paclitaxel bleek significant minder na aanpassing voor de BSA (196± 45.4 L/h/m^2 vs 346 ± 98.5 L/h, P<0.0001). Vervolgens werden 12 patiënten in een gerandomiseerde crossover studie behandeld met paclitaxel in een 3 uurs infuus in een driewekelijks schema in een dosering van 175 mg/m^2 (A) in kuur 1 en 300 mg (absoluut) (B) in kuur 2, or vice versa. De blootstelling (gemeten aan de 'area under the concentration-time curve' AUC) aan vrij paclitaxel, totaal paclitaxel en het oplosmiddel Cremophor EL waren vergelijkbaar in beide doseringsgroepen A en B. De coefficient van variatie in AUC was aanzienlijk lager in de BSA-gebaseerde doseringsgroep (A), met 11.8 vs 25.3% voor de vrije fractie paclitaxel, 16.7 vs 29.9% voor totaal paclitaxel en 24.2 vs 32% voor Cremophor EL. Dit suggereert dat de BSA ruim 50% van de interpatiënt variatie in blootstelling aan ongebonden paclitaxel kan verklaren. Een hypothese hiervoor is dat de blootstelling aan de vrije fractie van paclitaxel nauw samenhangt met de verdeling van het oplosmiddel Cremophor EL, dat nauwelijks de bloedbaan verlaat en derhalve gerelateerd is aan het totale bloedvolume en de lichaamsoppervlakte.

De behandeling van het gemetastaseerd borstkanker omvat vele modaliteiten, waaronder lokale radiotherapie en eventuele hyperthermie, naast hormonale en chemotherapeutische behandelingen en algemene symptoombestrijding met pijnstillers, bisfosfonaten e.d.. Effectieve cytostatica als anthracyclines en taxanen hebben een nadeel van aanzienlijke bijwerkingen, en zijn vroeg of laat niet meer werkzaam. Onderzoek naar nieuwe middelen zal zich in de nabije toekomst richten op het verbeteren van bestaande soorten chemotherapeutica, met een selectiever aangrijpingspunt, makkelijkere toedieningsvormen en een milder bijwerkingenpatroon, naast het onderzoek naar middelen die meer gericht zijn op specifieke eigenschappen van de tumorcel. Het belang van goed farmacologisch onderzoek, teneinde de medicinale behandeling te verfijnen en te optimaliseren, kan hierbij niet genoeg benadrukt worden. Het is de hoop dat de nieuwe meer tumorcel-selectieve therapieen de kwaliteit en de lengte van de geinduceerde ziekteremissie kunnen verbeteren.

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Carolien

CURRICULUM VITAE

De auteur van dit proefschrift werd op 21 november 1965 geboren te Arnhem. In 1984 behaalde zij het VWO diploma aan het christelijk atheneum Adriaen Pauw in Heemstede. In datzelfde jaar werd aangevangen met de studie Geneeskunde aan de Vrije Universiteit te Amsterdam. Zij behaalde het artsexamen in 1991. Aansluitend was zij tot december 1992 werkzaam als persoonlijk assistent van prof. dr H.M. Pinedo op de afdeling Geneeskundige Oncologie van het Academisch Ziekenhuis van de Vrije Universiteit te Amsterdam. Vanaf januari 1993 was zij werkzaam als arts-assistent op de afdeling Interne Geneeskunde in hetzelfde ziekenhuis, alwaar in januari 1994 gestart werd met de opleiding tot internist bij prof. dr. J. van der Meer. In januari 2000 werd zij als internist geregistreerd. Ondertussen startte zij in september 1998 met het aandachtsgebied medische oncologie bij prof. dr G. Stoter op de afdeling Interne Oncologie van het Academisch Ziekenhuis Rotterdam, locatie Daniel den Hoed Kliniek. Als junior-internist was zij hier tot december 2001 werkzaam. In deze periode werd het onderzoek verricht dat heeft geleid tot dit proefschrift. Registratie tot internist-oncoloog vond plaats in november 2001. Vanaf januari 2002 is zij werkzaam als internist-oncoloog op de afdeling Geneeskundige Oncologie van het Academisch Ziekenhuis van de Vrije Universiteit, thans genaamd VU Medisch Centrum, te Amsterdam.