ORAL ADMINISTRATION OF TAXANES

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ORALE TOEDIENING VAN TAXANEN
(met een samenvatting in het Nederlands)

PROEFSCHRIFT

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Voor mijn moeder,
Ter nagedachtenis aan mijn vader

CONTENTS

CONTENTS (continued)

PREFACE

Preface

Over the last 10 years the taxanes paclitaxel and docetaxel have obtained a prominent place in cancer chemotherapy with activity against a broad range of human solid tumors. Both drugs are routinely administered intravenously. Oral administration of the drugs, however, is to be preferred for several reasons. In the first place, oral administration is more convenient to patients. The drugs can be taken at home without hospital admission. Furthermore, the oral route facilitates the use of more chronic treatment regimens. This seems important for paclitaxel as there are strong indications that activity is increased with prolonged exposure to the drug. Finally, oral administration reduces administration costs as it eliminates the need for hospitalization.

The very low oral bioavailability of the taxanes, however, has limited development of treatment by the oral route. In preclinical studies using mdr1a P-glycoprotein knock-out mice [1] it was shown that the low oral bioavailability of the taxanes is, at least in part, due to affinity of the drugs for the multidrug efflux pump P-glycoprotein, abundantly present in the gastro-intestinal tract [2]. In addition, first-pass elimination by the cytochrome P450 metabolic enzymes in gut and liver may also contribute. In wild-type mice it was subsequently shown that the low oral bioavailability of paclitaxel could be significantly increased by co-administration of cyclosporin A, an efficacious inhibitor of P-glycoprotein and cytochrome P450 3A4 mediated drug metabolism [3]. These promising preclinical results formed the basis for investigation of the feasibility of oral administration of taxanes in patients.

In the clinic it was first started with a proof of concept study of orally administered paclitaxel in combination with cyclosporin A. Co-administration of cyclosporin A resulted in a significant increase in the systemic exposure of paclitaxel and drug concentrations increased from negligible to potential therapeutic levels [4]. Based on these first promising clinical results development of an oral treatment strategy with taxanes was pursued. This thesis describes the clinical development and optimization of oral therapy with the taxanes paclitaxel and docetaxel by modulation of the pharmacokinetics of the drugs after oral administration in combination with blockers of P-glycoprotein and/or cytochrome P450 3A4.

In Chapter 1 results of the preclinical and clinical studies of oral paclitaxel and docetaxel are reviewed. In the second part of this chapter the performance of the analytical assays of paclitaxel, docetaxel and cyclosporin A, the necessary tools to determine the pharmacokinetics of these drugs, is described.

In Chapter 2 the development of a single dose administration schedule of oral paclitaxel in combination with cyclosporin A is described, starting with the proof of concept study. In order to further increase the systemic exposure to paclitaxel, dose-increment of cyclosporin A and dose-escalation of paclitaxel were performed. To obtain better insight into the mechanisms of uptake, disposition and excretion of orally administered paclitaxel, plasma, urine and feces were analyzed for the presence of paclitaxel and its major metabolites. During the course of our investigations it became clear that the paclitaxel co-solvent Cremophor EL could not be considered as an inert pharmaceutical vehicle and might be a limiting factor in the uptake of paclitaxel. The effect of this vehicle on the uptake and excretion of orally administered paclitaxel was investigated in more detail. In addition, the effect of co-administration of the more potent, non-immunosuppressive and furthermore specific P-glycoprotein inhibitor, GF120918, on the pharmacokinetics of oral paclitaxel was studied.

To achieve a greater overall daily systemic exposure to oral paclitaxel, a twice daily dose regimen of the drug in combination with cyclosporin A was investigated (Chapter 3). As repeated twice daily administration of cyclosporin A may result in toxicities, the effect of dose-reduction of this P-glycoprotein blocker was studied in order to determine the minimally effective dose that would result in a maximal increase in the systemic exposure to paclitaxel.

Similar to paclitaxel, a proof of concept study of oral docetaxel in combination with cyclosporin A was initiated (Chapter 4). Based on the promising results of the proof of concept study, a phase II activity study of weekly administered oral docetaxel in combination with cyclosporin A in patients with advanced breast cancer was started. An interim analysis of the latter is given.

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CHAPTER 1.1

Oral delivery of taxanes

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Abstract

Oral treatment with cytotoxic agents is to be preferred as this administration route is convenient to patients, reduces administration costs and facilitates the use of more chronic treatment regimens. For the taxanes paclitaxel and docetaxel, however, low oral bioavailability has limited development of treatment by the oral route. Preclinical studies with mdr1a P-glycoprotein knock-out mice, which lack functional P-glycoprotein activity in the gut, have shown significant bioavailability of orally administered paclitaxel. Additional studies in wild-type mice revealed good bioavailability after oral administration when paclitaxel was combined with P-glycoprotein blockers such as cyclosporin A or the structurally related compound SDZ PSC 833. Based on the extensive preclinical research, the feasibility of oral administration of paclitaxel and docetaxel in cancer patients was recently demonstrated in our Institute. Co-administration of cyclosporin A strongly enhanced the oral bioavailability of both paclitaxel and docetaxel. For docetaxel in combination with cyclosporin A an oral bioavailability of 90% was achieved with an interpatient variability similar to that after intravenous drug administration; for paclitaxel the oral bioavailability is estimated at approximately 50%. The safety of the oral route for both taxanes is good. A phase II study of weekly oral docetaxel in combination with cyclosporin A is currently ongoing.

Introduction

In the past years an increasing interest can be seen towards oral administration of cytotoxic agents with several new oral analogues or oral formulations of commonly used cytotoxic drugs [1]. Examples are etoposide and analogues, topotecan and related compounds, cyclophosphamide and trophosphamide, idarubicin, vinorelbine, miltefosine and several prodrugs of 5-fluorouracil (5-FU) [2]. Oral chemotherapy is to be preferred in the first place for its convenience for patients and its potential to improve patients' quality of life [3]. Oral drug treatment is convenient to patients as oral drugs can be taken at home eliminating the need for hospital admission. In addition, oral treatment avoids the discomfort of an injection and the risks of infection and extravasation that are associated with intravenous (i.v.) access lines. Rightly, patients' quality of life is increasingly becoming a central consideration in cytotoxic drug treatment especially in palliative treatment regimens. A further argument for oral treatment of cytotoxic drugs is that the oral route facilitates the use of more chronic treatment regimens. This is especially important for cell cycle specific agents and agents with a predominately cytostatic

effect such as angiogenesis inhibitors and signal transduction inhibitors. For these agents prolonged exposure to the drug may have pharmacodynamic advantages over intermittent i.v. administration [4]. Finally, in view of increasing costs of anticancer therapy, oral treatment of cytotoxic agents is attractive, as oral administration eliminates the need for hospitalization, physician and nursing assistance and infusion equipment.

Considerations in Oral Drug Administration

An obvious prerequisite for oral drug treatment with cytotoxic agents is sufficient bioavailability after oral administration. Bioavailability concerns the rate and extent to which a drug is absorbed into the systemic circulation. Important factors which limit the oral bioavailability of drugs are structural instability in the gastro-intestinal fluids, limited aqueous solubility and dissolution, and/or affinity for intestinal and liver cytochrome P450 metabolic enzymes and the multidrug efflux pump P-glycoprotein, which serve to protect the body from xenotoxins [5]. Another limitation associated with poor bioavailability is the substantial interpatient variability in oral pharmacokinetics. This is important as cytotoxic drugs have in general a narrow therapeutic window. It is evident that caution is warranted with oral cytotoxic treatment since either toxic or subtherapeutic dosing may easily occur. Another major impetus in oral treatment of cytotoxic drugs is patient non-compliance. Inability of patients to comply to adequate oral drug intake is thought to be a major source of therapy failure for many diseases. However, patient non-compliance may be less of an issue for oral cancer therapy, because the seriousness of the disease may provide adequate motivation for adherence to the prescribed regimen. Another factor that may complicate oral treatment with cytotoxic agents is the risk of local irritation of the gastro-intestinal tract by the cytotoxic drug and/or its formulation, which can result in side effects such as nausea, vomiting and diarrhea. Finally, the medical condition of the patient may preclude oral drug therapy, such as in obstructive disorders of the gastro-intestinal tract and motility disorders.

Preclinical Studies on Oral Delivery of Taxanes

The taxanes paclitaxel and docetaxel are potent anticancer drugs with proven activity against a broad range of human malignancies, including ovarian and breast cancer and non-small cell lung carcinoma [6,7]. The drugs are currently administered i.v. at different dosages and infusion schedules. Oral treatment has

not appeared feasible because of the low oral bioavailability of paclitaxel and docetaxel. Initial studies have reported an oral bioavailability of paclitaxel of less than 1% [8,9].

Recent studies using wild-type and mdr1a P-glycoprotein knock-out mice have shed new light on this issue [10-12]. P-glycoprotein is an energy-dependent multidrug efflux pump, which was initially discovered by its ability to confer multidrug resistance (MDR) [13]. This MDR phenotype is based on a drug accumulation defect in tumor cells caused by P-glycoprotein which functions as an outward directed drug efflux pump for a broad array of drugs, including many anticancer agents such as anthracyclines, vinca alkaloids, epipodophyllotoxins and taxanes [14,15]. Later high expression of P-glycoprotein was also discovered in normal tissues with an excretory function such as liver and kidney and in tissues that fulfill an important barrier function such as endothelial cells in the brain, the testis and the placenta and in the intestinal epithelium [16-19]. The normal physiological function of these P-glycoproteins is still a matter of conjecture, but the idea is that they serve to protect the organism against toxins. Human P-glycoprotein is encoded by the MDR1 gene. In mice, two P-glycoproteins, encoded by mdr1a and mdr1b, perform the same function as the single human protein [20-22]. The mdr1a knock-out mice are particularly useful for studying the role of P-glycoprotein in the intestine, because the mdr1a gene is the only murine P-glycoprotein expressed in this tissue. Because paclitaxel is a very good substrate of P-glycoprotein, the hypothesis was raised that the low oral bioavailability of paclitaxel results from P-glycoprotein activity in the gut.

This was investigated in wild-type and mdr1a P-glycoprotein knock-out mice receiving orally administered paclitaxel and i.v. paclitaxel [23]. After oral drug administration, the plasma area under the concentration-time curve (AUC) of paclitaxel was 6-fold higher in mdr1a P-glycoprotein knock-out than in wild-type mice. After i.v. administration of paclitaxel the AUC was 2-fold increased in P-glycoprotein knock-out mice compared to wild-type mice. It was also investigated whether the pattern of drug excretion had been altered in mdr1a knock-out mice compared to wild-type mice. After i.v. administration of paclitaxel, the fecal excretion of unaltered drug was 40% of the delivered dose in wild-type mice and was markedly reduced to only 1.5% of the administered dose in mdr1a knock-out mice. Also after oral administration of paclitaxel a large difference in fecal excretion was observed. In mdr1a P-glycoprotein knock-out mice, only 2% of the orally delivered dose was recovered in the feces as unchanged drug, whereas in wild-type mice almost 90% was excreted unchanged. Biliary secretion was not significantly different in wild-type mice and mdr1a P-glycoprotein knock-out mice. It was then concluded that P-glycoprotein in the epithelium of the gut limits the

bioavailability of orally administered paclitaxel. Intestinal P-glycoprotein also contributes to the elimination of parenterally administered paclitaxel by a direct secretion of drug into the intestinal lumen. These findings provided a rationale for attempts to improve the low and variable oral bioavailability of paclitaxel by concomitant administration of P-glycoprotein inhibitors.

This rationale was tested in wild-type mice receiving either oral paclitaxel as a single agent or oral paclitaxel in combination with the experimental P-glycoprotein inhibitor SDZ PSC 833 [24]. Combined treatment with SDZ PSC 833 resulted in an approximately 10-fold increase in the AUC of paclitaxel. An estimation of the oral bioavailability was made using the data of a previously performed study of i.v. administered paclitaxel [25]. AUCs obtained after i.v. administration of paclitaxel in Cremophor EL-free formulations were used as Cremophor EL causes non-linear pharmacokinetic behavior of paclitaxel. Although the oral formulation used in this study contained Cremophor EL, the systemic uptake of this compound from the gastro-intestinal tract was very low (plasma levels were undetectable). Treatment with SDZ PSC 833 increased the bioavailability from 20% to 210%, suggesting that, apart from the effect of SDZ PSC 833 on intestinal paclitaxel uptake by P-glycoprotein inhibition, the increased systemic exposure also results from the interaction of this agent with drug elimination pathways. Various mechanisms may contribute to this decreased clearance, e.g. both paclitaxel and cyclosporins are substrates for the cytochrome P450 isozymes [26,27], which may cause a metabolic interaction after simultaneous administration.

To further study on the feasibility of a clinically effective oral formulation of paclitaxel it was investigated whether co-treatment with a commonly applied and commercially available P-glycoprotein blocker, e.g. cyclosporin A, had a similar effect [28]. The effect of cyclosporin A on the pharmacokinetics of orally and i.v. administered paclitaxel was investigated in wild-type mice. Calculated relative to the AUC of i.v. administered paclitaxel (with Cremophor EL) in mice treated without cyclosporin A, the oral bioavailability of paclitaxel increased from 9% up to 67% with co-administration of cyclosporin A. The effect of cyclosporin A on the systemic exposure after orally administered paclitaxel was the result of both a significantly increased uptake and decreased clearance. Histological examination revealed that the enhanced absorption was not caused by gastro-intestinal toxicity. It was concluded that cyclosporin A is very effective in increasing the systemic exposure to orally administered paclitaxel. Importantly, these data enabled the development of a clinically useful oral formulation of paclitaxel in combination with oral cyclosporin A.

For docetaxel, high affinity for P-glycoprotein has also been shown, suggesting that the oral bioavailability of docetaxel may be enhanced by inhibition of intestinal P-

glycoprotein. Preclinical studies in mdr1a P-glycoprotein knock-out mice and wild-type mice with orally and i.v. administered docetaxel are currently ongoing. In addition, oral administration of docetaxel in wild-type mice with or without the P-glycoprotein inhibitor cyclosporin A, is currently being investigated. Preliminary results are promising in enhancing the oral bioavailability of docetaxel by concomitant administration of cyclosporin A.

Clinical Studies on Oral Delivery of Taxanes

Based on the extensive preclinical research, a proof of concept study of orally administered paclitaxel in cancer patients was initiated [29,30]. Patients received either one course of oral paclitaxel of 60 mg/m^2 as a single agent or oral paclitaxel 60 mg/m^2 in combination with 15 mg/kg oral cyclosporin A. In all subsequent courses patients received 3-weekly i.v. paclitaxel 175 mg/m^2 administered as a 3-hour infusion. The low oral dose of 60 mg/m^2 was selected for safety reasons because the results in mice indicated increased systemic exposure to paclitaxel after oral administration in combination with the P-glycoprotein inhibitor SDZ PSC 833 as compared with i.v. administration of paclitaxel [24]. On all occasions patients were premedicated with standard paclitaxel pretreatment. Co-administration of cyclosporin A resulted in an approximately 7-fold increase in the plasma AUC of paclitaxel (Figure 1).

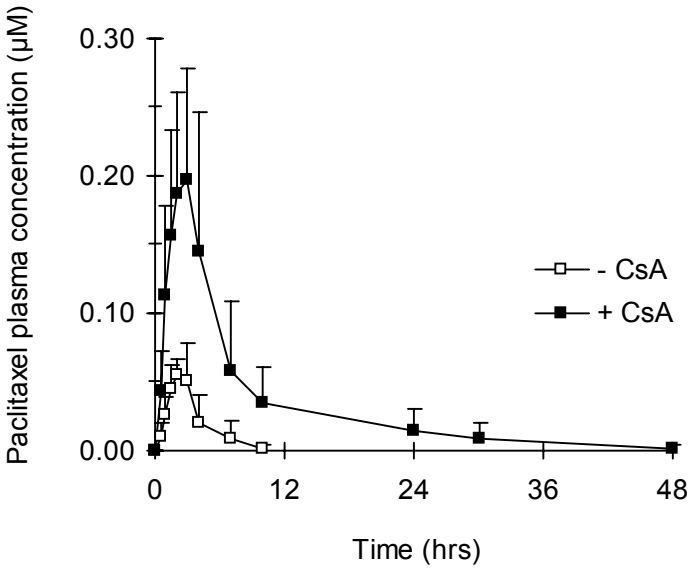

Figure 1. *Plasma concentration-time curves of oral paclitaxel with or without cyclosporin A (CsA) represented as means ± SD.*

The oral bioavailability of paclitaxel, calculated as the ratio of the AUC after oral drug administration divided by the AUC after i.v. administration with a correction for the difference in dose, was 4% for oral paclitaxel administered as a single agent and 28% when oral paclitaxel was combined with cyclosporin A. These oral bioavailabilities, however, are significant underestimations of the true oral bioavailability of paclitaxel, which is due to the non-linear pharmacokinetics of i.v. administered paclitaxel [25,31]. Re-calculation of the oral bioavailability of paclitaxel using the AUC value of i.v. paclitaxel at a lower dose [32], which is more realistic for comparison purposes, resulted in an apparant bioavailability of 6% without cyclosporin A and 47% of oral paclitaxel with cyclosporin A.
The increase in oral bioavailability is most likely caused by inhibition of intestinal P-glycoprotein by cyclosporin A. In addition, inhibition of paclitaxel metabolism by cyclosporin A may also have contributed as both paclitaxel and cyclosporin A are substrates for the cytochrome P450 3A4 metabolic system [26,27]. Co-administration of cyclosporin A resulted in a significant reduction of the formation of the paclitaxel metabolite 3'p-hydroxypaclitaxel, which is suggestive for cytochrome P450 3A4 inhibition (Figure 2).

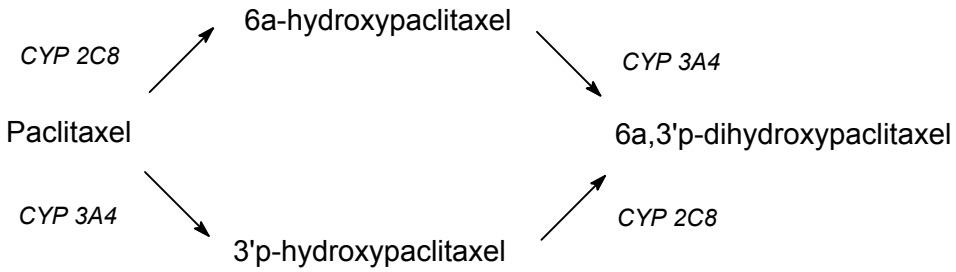

Figure 2. *Major metabolic pathways of paclitaxel.*

The oral combination of paclitaxel and cyclosporin A was very well tolerated and did not induce gastro-intestinal toxicities such as nausea, vomiting and diarrhea. Co-administration of a P-glycoprotein inhibitor might cause toxicities, which are due to inhibition of the physiological protective function of P-glycoprotein. P-glycoprotein inhibition could cause an increase of the paclitaxel levels in P-glycoprotein protected brain and cardiac tissue and may therefore enhance the risk of central neurotoxicity or cardiac toxicity [10,33,34]. In our clinical and animal studies, however, no signs of central neurotoxicity or cardiac toxicity were observed. The single oral dose of 15 mg/kg cyclosporin A resulted in peak and through values which were in the therapeutic range for immunosuppression and may be associated with toxicity, in particular renal toxicity. No renal toxicity nor any other

side-effects clearly related to the single administered cyclosporin A dose were observed. This study has demonstrated the proof of concept of efficient oral uptake of paclitaxel in cancer patients induced by concomitant administration of the P-glycoprotein blocker cyclosporin A.

Subsequently, it was investigated whether an increase in the cyclosporin A dose or fractionated cyclosporin A administration would result in an increase in paclitaxel AUC values [35]. Dose-increment of cyclosporin A to 30 mg/kg and changing the schedule to two administrations of 15 mg/kg separated by 2 hours did not result in a further increase in the AUC of paclitaxel. Apparently, P-glycoprotein inhibition was maximal at a single dose of cyclosporin A of 15 mg/kg. It remained, however, unclear whether cyclosporin A inhibited P-glycoprotein completely. In addition, incomplete distribution of cyclosporin A over the mucosa wall may also have contributed to the possible incomplete P-glycoprotein inhibition by cyclosporin A.

In an attempt to further increase the systemic exposure of orally administered paclitaxel and to determine the dose limiting toxicity and maximum tolerated dose, dose-escalation of oral paclitaxel was investigated [36,37]. Dose limiting toxicity was reached at the dose level of 360 mg/m^2 and consisted of acute nausea and vomiting. The maximum tolerated dose was then defined at 300 mg/m^2. Pharmacokinetic analysis of oral paclitaxel revealed that dose-escalation of oral paclitaxel from 60 to 300 mg/m^2 resulted in significant increases in the AUC of paclitaxel, however, these increases were moderate and not proportional with increases in dose. It was hypothesized that this non-linear absorption pharmacokinetic behavior of oral paclitaxel was due to the poor aqueous solubility of paclitaxel and consecutive limited dissolution in the gastro-intestinal tract. A similar non-linear pharmacokinetic absorption pattern due to poor aqueous solubility was observed for the oral anticancer drugs etoposide and the platinum complex JM216 [38,39]. At all investigated oral paclitaxel dose levels, plasma levels of the co-solvent Cremophor EL were undetectable. Apparently, Cremophor EL is not absorbed following oral administration of the paclitaxel i.v. formulation. This is important because systemic exposure to Cremophor EL can induce severe hypersensitivity reactions requiring extensive premedication [40-42]. Absence of systemic exposure to Cremophor EL after oral drug administration justifies paclitaxel treatment without premedication. In another study of orally administered paclitaxel in combination with cyclosporin A [43], oral paclitaxel was administered without premedication and no hypersensitivity reactions were observed. Furthermore, Cremophor EL is responsible for the non-linear pharmacokinetic behavior of i.v. paclitaxel [25,31,44-46]. It entraps paclitaxel in the plasma compartment, which results in a more than proportional increase in plasma paclitaxel levels with increasing doses. However, these higher total drug levels in plasma do not result in higher drug levels in tissue. This pseudo-non-linearity

of i.v. paclitaxel [46] has two important implications for the pharmacology of oral paclitaxel. Firstly, it will result in a significant underestimation of the true bioavailability of oral paclitaxel. This has been discussed above. Secondly, the pseudo-non-linearity of i.v. paclitaxel implies that after oral administration, when Cremophor EL is not systemically present, plasma levels of paclitaxel represent a higher fraction of free drug, which will result in enhancement of the availability of paclitaxel for the (tumor) tissues. Therefore, interpretation of differences between paclitaxel plasma levels after oral and i.v. administration, without and with Cremophor EL in the systemic circulation, respectively, should be done with great caution. At the maximum tolerated oral paclitaxel dose of 300 mg/m^2 a mass balance study was performed [47]. Excretion of the drug after i.v. administration was also investigated. After i.v. administration of paclitaxel, the major excretory route of paclitaxel and metabolites was feces, viz. 56% of the administered dose. The major compounds detected in feces were the metabolites, viz. 47% of the administered dose, of which the metabolite 6a-hydroxypaclitaxel accounted for 37%. Following oral paclitaxel administration in combination with cyclosporin A, the major excretion route of paclitaxel and metabolites was also with feces, viz. 76% of the administered dose. The major compound recovered in feces after oral drug administration was paclitaxel, accounting for 61% of the administered dose. In the preclinical studies of oral paclitaxel in wild-type mice and mdr1a P-glycoprotein knock-out mice fecal excretion of paclitaxel was significantly decreased from 87% in wild-type mice to 2% in the mdr1a knock-out mice [23]. This large decrease in fecal excretion of paclitaxel suggested almost complete (re)uptake of the drug from the gastro-intestinal tract in P-glycoprotein knock-out mice. Thus, according to the preclinical studies, only a small fraction of the paclitaxel dose excreted in the feces instead of the observed 61%, was expected. The most plausible explanation for this large amount of paclitaxel recovered in feces is excretion of unabsorbed drug, which is supported by the significant lower plasma AUC value of orally administered paclitaxel (300 mg/m^2) compared to i.v. administered paclitaxel (175 mg/m^2). Because of the non-linear oral pharmacokinetics of paclitaxel with only moderate further increases of the AUC with doses up to 300 mg/m^2 and the large amount of original drug recovered in feces after oral paclitaxel administration at a dose of 300 mg/m^2, an oral paclitaxel dose of 180 mg/m^2 is considered most appropriate for further investigation. The safety of the oral combination at this dose level is very good.

Based on the non-linear drug absorption a split dose regimen was investigated to achieve a greater overall daily systemic exposure [48]. Oral paclitaxel was administered in two doses seven hours apart at dose levels of 2x 60, 2x 90 and 2x 120 mg/m^2. In this study with oral paclitaxel, besides the AUC value, the pharmacokinetic parameter time above the threshold concentration of 0.1 μM (T>0.1

μM) was considered. Previous clinical work has suggested that time above this threshold concentration is related to the activity of the drug [32,49]. The pharmacokinetic data revealed that bi-daily dosing of oral paclitaxel also shows non-linear absorption pharmacokinetics as was observed after single dose administration of the drug. Comparison with the pharmacokinetic data after single dose administration revealed that fractionated administration of the drug resulted in higher AUC and T>0.1 μM values of paclitaxel. Therefore, a multiple dosing regime may be a realistic option to further increase the systemic exposure after oral administration of paclitaxel.

For docetaxel, a similar clinical proof of concept study was initiated as has been done for oral paclitaxel [50,51]. Patients received either one course of oral docetaxel 75 mg/m^2 as a single agent or oral docetaxel in combination with cyclosporin A. Patients continued on a 3-weekly schedule of 100 mg/m^2 i.v. docetaxel administered as a 1-hour infusion. Standard docetaxel pretreatment was given in all courses. Pharmacokinetic data showed that co-administration of oral cyclosporin A strongly enhanced the systemic exposure of orally administered docetaxel. Docetaxel administered as a single agent exhibited poor oral bioavailability of only 8%, whereas oral docetaxel in combination with cyclosporin A exhibited a bioavailability of 90%. Furthermore, the variance in the systemic exposure after oral drug administration was of the same order as after i.v. administration. Hence, oral administration did not result in a notable increase in the interpatient difference in systemic exposure. The oral combination of docetaxel and cyclosporin A was very well tolerated. Thus, oral docetaxel may become a realistic alternative to the current i.v. treatment of docetaxel. In addition, as recent clinical studies have shown that administration of i.v. docetaxel on a weekly schedule decreases the hematological toxicity profile of the drug while therapeutic activity is maintained [52-54], the feasibility of oral drug administration may stimulate and facilitate the use of weekly treatment schedules of docetaxel. The activity of weekly oral docetaxel in combination with cyclosporin A is currently investigated in our Institute in a phase II study in patients with advanced breast cancer.

Conclusions and Future Directions

Oral treatment with the taxanes paclitaxel and docetaxel is to be preferred as oral drug administration is convenient to patients, reduces administration costs and facilitates the use of more chronic treatment regimens. In addition, for paclitaxel, circumvention of systemic exposure to the co-solvent Cremophor EL is another advantage of oral therapy. Based on the extensive preclinical research we have

shown the feasibility of oral administration of the taxanes in cancer patients by concomitant administration of oral cyclosporin A.

For orally administered paclitaxel at a dose of 60 mg/m^2 a bioavailability of 47% was determined. However, true bioavailability of oral paclitaxel might be significantly higher due to the non-linear pharmacokinetics of i.v. paclitaxel. The maximum tolerated dose of oral paclitaxel was determined at 300 mg/m^2. However, because of the non-linear absorption pharmacokinetics of oral paclitaxel and the large amount of parent drug recovered after oral paclitaxel administration at a dose of 300 mg/m^2, administration of lower paclitaxel doses (180 mg/m^2) is considered most appropriate. Fractionated administration of oral paclitaxel appeared to result in an increase in the systemic exposure to paclitaxel compared to single dose administration and may therefore be a realistic option to increase the systemic exposure after oral administration of paclitaxel.

For orally administered docetaxel at a dose of 75 mg/m^2 a bioavailability of 90% was achieved with an interpatient variability similar to that after i.v. administration. The oral combination was well tolerated. Hence, oral administration of docetaxel is a realistic alternative to i.v. treatment of the drug. Furthermore, oral treatment may facilitate the use of weekly treatment regimens which currently become popular. The activity of weekly oral docetaxel in combination with cyclosporin A is currently investigated in a phase II study in patients with advanced breast cancer.

Finally, the concept of modulation of bioavailability by a P-glycoprotein inhibitor may well be applied for other (cytotoxic) drugs that show affinity for the multidrug efflux pump and are associated with poor or moderate oral bioavailability.

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CHAPTER 1.2

Performance of the analytical assays of paclitaxel, docetaxel and cyclosporin A in a routine hospital laboratory setting

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Abstract

The taxanes paclitaxel and docetaxel are important anticancer agents. To optimize therapy of these drugs, many studies have been performed by us with pharmacokinetic monitoring of the compounds. The numerous determinations of paclitaxel and docetaxel in our laboratory enabled us to monitor performance of the bioanalytical assays over a prolonged period of time. In addition, we analyzed the performance of the bioanalytical assay of cyclosporin A, a compound co-administered to enhance absorption of orally administered paclitaxel and docetaxel. Here, we report our experience with these assays over the past four years.
Paclitaxel and docetaxel were analyzed by validated high-performance liquid chromatography (HPLC) assays developed at our Institute. Cyclosporin A was analyzed with use of a specific fluorescence polarization immunoassay (s-FPIA) developed and validated by Abbott Laboratories. For acceptance of an analytical run we used the criteria for calibration and quality control samples issued by the conference on Analytical Methods Validation (1990). Quality control samples have been used to monitor performance of the assays.
In the past four years, all three analytical assays showed excellent performance. In this period, we performed 84 analytical runs of paclitaxel, 19 runs of docetaxel and 131 runs of cyclosporin A. Accuracies of the paclitaxel, docetaxel and cyclosporin A assays were 92-102%, 103-112% and 103-105%, respectively. Precisions of the paclitaxel and docetaxel assays were less than 10% for all concentrations. For the cyclosporin A assay, the coefficients of variation were always less than 12%. It can be concluded that the validated analytical assays of paclitaxel, docetaxel and cyclosporin A showed very good performance in a routine hospital laboratory setting for a prolonged period of time.

Introduction

Over the last 10 years the taxanes paclitaxel and docetaxel have obtained a prominent place in anticancer chemotherapy and are widely used in the treatment of breast, ovarian and lung cancer [1,2]. The drugs are routinely administered intravenously, either as single agent or in combination therapy, at different dosages and time schedules. To optimize therapy of paclitaxel and docetaxel, we have performed many studies, which were pharmacokinetically supported by bioanalysis of the compounds [3-13]. At our Institute high-performance liquid chromatographic (HPLC) assays, with solid-phase extraction (SPE) as sample pretreatment, were developed and validated for determination of paclitaxel [3,14,15] and docetaxel [16]

and their major metabolites. Further optimization of cancer treatment by paclitaxel and docetaxel is continued. We are currently exploring the oral route of administration of paclitaxel and docetaxel and have recently demonstrated profound enhancement of systemic exposure of the drugs by co-administration of the P-glycoprotein inhibitor cyclosporin A [17-19]. Based on the first promising results we have continued with further development of oral treatment of paclitaxel and docetaxel [20-22].

In order to pharmacokinetically support the clinical studies of paclitaxel and docetaxel we performed numerous determinations of these analytes in the last couple of years. In addition, many bioanalytical measurements of cyclosporin A were performed. The latter has been analyzed with use of a specific fluorescence polarization immuno-assay (s-FPIA) developed and validated by Abbott Laboratories [23-26]. This assay is generally used in therapeutic drug monitoring of cyclosporin A in transplant patients [24-26]. Robustness of an analytical method is a critical evaluation parameter [27,28], which can be obtained by long-term experience. Here, we present an overview of the performance of the bioanalytical assays of paclitaxel, docetaxel and cyclosporin A in a routine hospital laboratory setting over the past four years.

Materials and Methods

Paclitaxel Analysis

Development and validation of the HPLC bioanalytical assay of paclitaxel has been described previously [3,14,15]. The sample pretreatment involves a solid phase extraction (SPE) using 0.5 mL plasma, buffered with 0.5 mL of 0.2 M ammonium acetate pH 5.0, onto 1-mL Cyano Bond Elut columns. 2'-Methylpaclitaxel is used as internal standard. The eluent is evaporated under nitrogen and low heat, and reconstituted with the mobile phase, acetonitrile-methanol-water (AMW) (4:1:5, v/v/v) containing 0.01 M ammonium acetate pH 5.0. The samples are chromatographed on a reversed-phase octyl column. Detection of the analytes is performed by UV absorbance measurement at 227 nm.

Each paclitaxel run involved analysis of calibration samples, quality control samples and study samples, which were processed as a batch. Calibration curves consised of at least five concentrations measured in duplicate at concentrations between 10-10,000 ng/mL. Quality control samples were measured in duplicate at three concentrations in the low, medium and high calibration range. Paclitaxel stock solutions were made by dissolving 10 mg of paclitaxel reference material in 2.0 mL methanol. Paclitaxel calibration and quality controls samples were prepared by

making the appropriate dilutions in blank, human plasma. The internal standard stock solution consisted of 1 mg/mL 2'-methylpaclitaxel in methanol. The working solution of the internal standard was 10 µg/mL. Stock solutions, calibration and quality control samples were stored at -20ºC. Every six months fresh stock solutions were made. Study samples were obtained in EDTA or heparinized tubes and immediately centrifuged. Plasma was separated and directly stored at -20ºC until analysis. If the available study sample volume was less than the validated sample volume, the study sample was supplemented with blank human plasma to the validated volume. When the concentration of a study sample was above the highest calibration standard, the sample was diluted with blank human plasma and re-analyzed.

For acceptance of a paclitaxel analytical run the guidelines issued by a joint conference of the FDA, AAPS, AOAC, HPB, and FIP on Analytical Methods Validation (1990) were used [27,28]. For the calibration samples, the mean percentage deviation of the nominal value and the relative standard deviation of the responses must be less than 15%. For the lower limit of quantitation (LLQ) of the assay a deviation of 20% is acceptable for both parameters. When calibration samples fall out of these ranges they are excluded from the calibration curve. A minimum of five calibration concentrations should meet the above criteria to accept the run. The correlation coefficient of the calibration curve must be higher than 0.995. For the quality control samples, at least four of the six samples must be within the 20% of their respective nominal values; two of the six samples (not both at the same concentration) may be outside the 20% respective nominal value. The relative standard deviation of the responses must be less than 15%.

Performance of the paclitaxel analytical assay in time has been monitored by use of the quality control samples. The accuracies of the assay were calculated for each quality control concentration by use of dividing the mean measured concentration by the nominal concentration and multiplication by 100. The assay precisions were obtained by one-way analysis of variance (ANOVA) for each quality control concentration using the run day as the classification variable. The following formula was used to calculate the precision:

$$\text{Precision} = \frac{\sqrt{(MS_{BG} - MS_{WG})/n}}{GM} \times 100\%$$

where, MS_{BG} is the mean square of the between runs, MS_{WG} the mean square of the within runs, GM the grand mean of the measured quality control concentration, and n the number of determinations per run.

Docetaxel Analysis

Development and validation of the docetaxel HPLC bioanalytical assay has been described previously [16]. A volume of 1.0 mL of plasma is extracted with Cyano end-capped solid phase columns using an ASPEC XL system. 2'-Methylpaclitaxel is used as internal standard. The eluent is evaporated under nitrogen and low heat, and reconstituted in acetonitrile-methanol-water (AMW) (4:1:5, v/v/v). The samples are chromatographed on an APEX-octyl column with acetonitril-0.02 M ammonium acetate buffer pH 5.0 mixture (36.8:63.2, w/w) as the mobile phase. UV detection is performed at 227 nm.

Each docetaxel run involved analysis of calibration samples, quality control samples and study samples, which were processed as a batch. Calibration curves consisted of at least five concentration levels measured in duplicate at concentrations of 10-10,000 ng/mL. Quality control samples were measured in duplicate at three concentrations in the low, medium and high calibration range. Docetaxel stock solutions were prepared by dissolving 1 mg of reference material in 2.0 mL methanol. Docetaxel calibration and quality controls samples were prepared by making the appropriate dilutions in blank, human plasma. The 2'-methylpaclitaxel stock and working solutions were made as described for paclitaxel. Stock solutions, calibration and quality control samples were stored at -20ºC. Every six months fresh stock solutions were prepared. Study samples were obtained in EDTA or heparinized tubes and immediately centrifuged. Plasma was separated and stored at -20ºC until analysis. Study samples were supplemented with human blank plasma if the sample volume available was less than the validated sample volume. When exceeding the calibration range, samples were diluted with blank human plasma and re-analyzed.

Acceptance of a docetaxel analytical run was based on the same guidelines as used in paclitaxel analysis [27,28]. Performance of the docetaxel analytical assay in time has been evaluated by use of the quality control concentrations as described above for paclitaxel.

Cyclosporin A Analysis

Development and validation of the specific FPIA bioanalytical assay of cyclosporin A (Abbott Laboratories, Amstelveen, The Netherlands) has been described elsewhere [23-26]. The analysis requires 150 µL whole blood to which solubilization reagent (aqueous surfactant with 0.1% sodium azide) and precipitation reagent (zinc sulfate in methanol and ethylene glycol) are added. The samples are mixed, centrifuged and the supernatant is then analyzed in the TDxFLx analyzer (Abbott Laboratories) and thereafter automatically quantified. The reagents provided in the analyzer kit included cyclosporin A antibody (<25% mouse monoclonal in a buffer

containing stabilizer with sodium azide) and a <0.01% cyclosporin A monoclonal whole blood fluorescein tracer solution in buffer containing surfactant and a protein stabilizer with sodium azide.

Each analytical run (with a maximum of 20 samples) involved analysis of study samples and cyclosporin A quality control samples at concentrations of 150 ng/mL (Low), 400 ng/mL (Medium) and/or 800 ng/mL (High), which were processed as a batch. Prior to start of cyclosporin A analysis a calibration curve was run (0-1500 ng/mL, six concentrations) and stored as long as the quality control samples were within the accepted ranges of their nominal values. Accepted ranges for the quality controls are ± 20% (Low) and ± 15% (Medium and High) which were used for acceptance of the cyclosporin A run [23]. Study samples were obtained in EDTA or heparinized tubes and stored at 4ºC until analysis. When study samples were above the highest calibration standard (print output HI) the supernatant was diluted with dilution buffer (phosphate buffer with sodium azide) and re-analyzed.

Performance of the cyclosporin A assay has been monitored by use of the quality control samples. As performed for paclitaxel and docetaxel analysis, accuracies of the assay have been determined for each quality control concentration by dividing the mean measured concentration by the nominal concentration and multiplication by 100. Precisions could not be calculated using one-way analysis of variance (ANOVA) because of the single measurements of the quality control concentrations. We calculated the coefficients of variation in the quality control concentrations by dividing the standard deviations by the mean measured concentrations, multiplied by 100.

Results and Discussion

Development of the Paclitaxel Analytical Assay

In 1992 The Netherlands Cancer Institute participated in a large randomized multicentre European-Canadian trial which investigated the safety and antitumor efficacy of paclitaxel in high (175 mg/m^2) versus low (135 mg/m^2) dose and long (24 hours) versus short (3 hours) infusion in platinum pretreated ovarian cancer patients [29]. This was an unique opportunity to investigate the pharmacokinetic behavior of paclitaxel in the two different dose levels and two different infusion schedules. At that time, several HPLC methods, including various sample pretreatment procedures, had been reported for the analysis of paclitaxel in human plasma. However, these methods were relatively insensitive with lower limit of quantitations of 50-100 nM [30-33]. Furthermore, these assays could not detect paclitaxel metabolites. We developed and validated a more sensitive HPLC method

for quantification of paclitaxel in human plasma with a SPE procedure as sample pretreatment [3,14]. The lower limit of quantitation of this assay was 12 nM. During the implementation of this assay, however, recovery problems of paclitaxel arose. The major problems were 1) a large batch-to-batch difference in performance of the SPE columns, 2) loss of paclitaxel during the second wash step with methanol-0.01 M ammonium acetate pH 5 (20:80, v/v) and 3) a reduction in paclitaxel recovery due to the pharmaceutical vehicle Cremophor EL (Taxol® contains 6 mg/mL paclitaxel in ethanol/Cremophor EL 1:1 v/v) [34]. To avoid recovery problems we modified the assay with addition of 2'-methylpaclitaxel as internal standard. The modified assay was subsequently revalidated for quantification of paclitaxel [15]. In addition, the assay was validated for the three major human paclitaxel metabolites 6a-hydroxypaclitaxel, 3'p-hydroxypaclitaxel and 6a,3'p-dihydroxy-paclitaxel [15]. Methods for the quantification of these compounds in human plasma were not described previously, which was most likely caused by the lack of reference compounds. We were able to isolate and purify the metabolites in sufficient amounts from human feces [35]. The molar absorptivities, the extraction recoveries and the slopes of the calibration curves of 6a-hydroxypaclitaxel and 3'p-hydroxypaclitaxel were in the same range of that of paclitaxel. Therefore, these metabolites can be determined by use of the paclitaxel calibration curve [15]. The quantification of the 6a,3'p-dihydroxypaclitaxel metabolite using the paclitaxel calibration curve needs a correction factor of 1.14 as its extraction recovery is slightly lower than for paclitaxel [15].

Performance of the Paclitaxel Analytical Assay

Since the development of the assay for quantification of both paclitaxel and the three major metabolites, no modifications of the assay were necessary. We evaluated the analytical runs of paclitaxel from January 1997 up to January 2001. In this four-year period, more than 5200 study samples have been analyzed in 84 analytical runs. Six different reversed-phase octyl HPLC columns were used and four different batches of SPE columns.

Performance of the paclitaxel quality control samples is presented in Table 1. The mean measured values of the quality control samples very closely resemble their nominal values and accuracies of the paclitaxel assay yield values of 92-102%. For validation of an analytical assay, acceptance ranges of accuracy of 85-115% are applied [27,28]. Considering the limited amount of variables during validation of an assay, the obtained accuracies in a four year period of 92-102% can be considered as very good. Precisions of the assay were less than 10% for all quality control concentrations. For validation of an assay, values of less than 15% must be obtained [27,28]. Precisions of less than 10% during a four-year period can

therefore be considered as acceptable. It should be noted that precisions are calculated on quality controls spiked with preparations of different stock solutions, which we have considered as one batch.

Table 1. *Performance of the paclitaxel quality control samples.*

Nominal value (ng/mL)	Measured value (ng/mL)	Accuracy (%)	Precision (%)	Number of Runs
50	46 ± 4	92	8	6
100	98 ± 9	98	8	59
500	491 ± 39	98	7	82
750	742 ± 39	99	5	35
5000	5005 ± 359	100	6	59
7500	7634 ± 347	102	2	19

Figure 1 gives the individual values of the most frequently assayed quality control samples in the past four years. For all quality control concentrations scattered patterns around their nominal values are observed and it can therefore be concluded that there has not been an obvious trend in the paclitaxel analytical assay. However, trend detection is difficult because new quality controls have been made in time. The charts show that the quality control samples perfectly apply to the 20% ranges of the acceptance criteria [27,28]. However, in one run (September 1999), all quality control samples fell out of the 20% ranges. This has led to preparation of new quality controls, which then were within the 20% acceptance criteria.

In the past four years, a total of 4 different batches of SPE columns were used. Extraction recoveries of paclitaxel were calculated in each run by comparing the area of paclitaxel in human plasma with the area of paclitaxel dissolved in AMW (Figure 2). Previously, we noticed a large SPE batch-to-batch variability in the recovery data of paclitaxel [34]. This has led to application of an internal standard in the assay. For the 4 used batches, we found mean recovery percentages of 79 ± 11% (66 runs), 81 ± 6% (6 runs), 78 ± 2% (2 runs) and 73 ± 8% (9 runs). We did not observe substantial differences between the 4 batches. For three runs, recovery values of paclitaxel were only 40%. These low recoveries were retrieved from the same SPE batch, which, however, also produced recoveries of 70-80%. One possible reason for this difference in recovery in one SPE batch is storage of open packaging causing loss of active sides of the sorbent under the influence of moisture and air. We therefore recommend careful storage of the SPE columns in closed packaging. Importantly, in these three runs, calibration and quality control

samples perfectly applied to the acceptance criteria with mean percentage deviations from their nominal values of less than 5%. These results show the appropriate application of an internal standard resulting in reliable data.

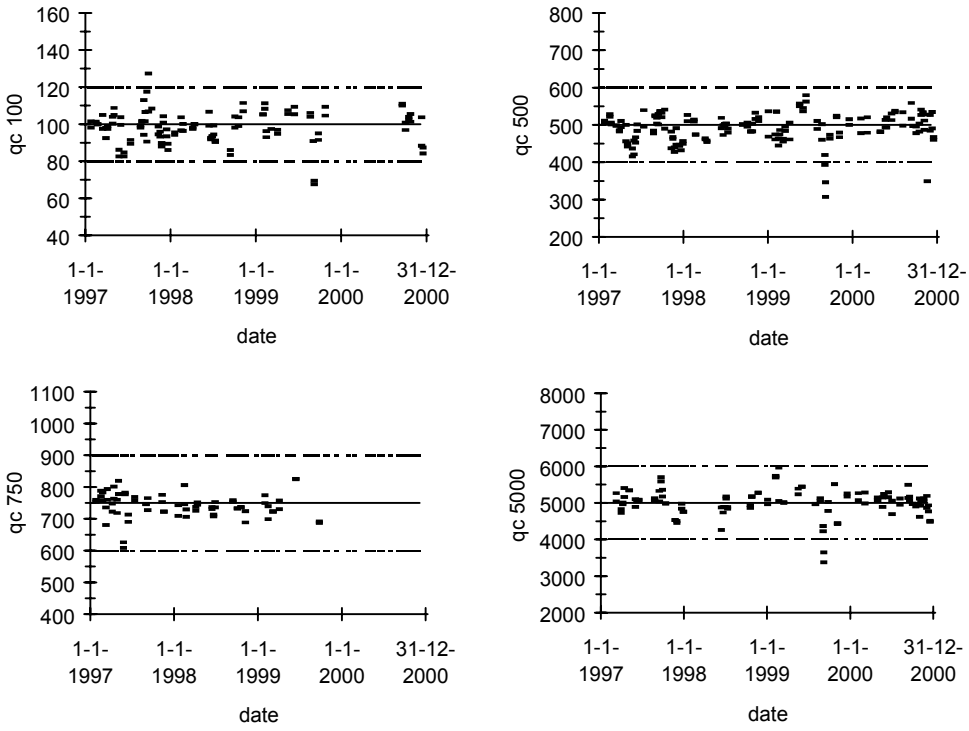

Figure 1. *Individual values of the paclitaxel quality control concentrations of 100, 500, 750 and 5000 ng/mL. The dotted horizontal lines represent the 20% acceptance ranges.*

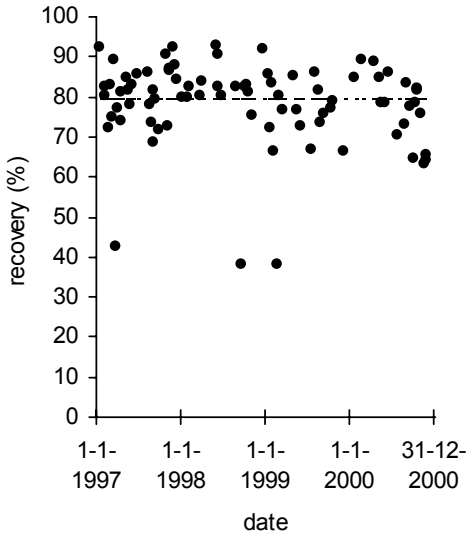

Figure 2. *Recovery data of paclitaxel in human plasma. The dotted horizontal line represents a 80% recovery.*

For preparation of the paclitaxel calibration and quality control samples we made fresh stock solutions of paclitaxel and 2'-methylpaclitaxel every 6 months. During the past four years we re-evaluated the stability of the stock solutions and the stability of paclitaxel in human plasma when stored at -20ºC. Diluted stock solutions were injected and the areas were compared. A percentage of less than 5% deviation was considered acceptable. The paclitaxel stock solution was found to be stable for at least 8 months and the 2'-methylpaclitaxel for at least 13 months. Stability of paclitaxel in human plasma was evaluated at concentrations of 50 and 5000 ng/mL. Three replicates were analyzed at 0, 6 and 14 months. Paclitaxel was found to be stable in human plasma for at least 14 months. Stability of the stock solutions and paclitaxel in human plasma have not been tested for longer periods.
In conclusion, the validated assay of paclitaxel and its three major metabolites in human plasma showed excellent performance over the past four years. Accuracies of 92-102% and precisions of less than 10% were achieved. Recovery data of paclitaxel underline the need of use of an internal standard in the assay. New stability data of the stock solutions of paclitaxel and 2'-methylpaclitaxel and paclitaxel in plasma allow us to prepare these solutions on a less regular basis.

Development of the Docetaxel Analytical Assay

In order to pharmacokinetically support clinical studies of docetaxel, we recently developed and validated an HPLC analytical assay for determination of docetaxel in human plasma [16]. Furthermore, the assay was also capable of detection of four hydroxylated docetaxel metabolites M1, M2, M3 and M4 for which a limited validation was performed [16]. Quantification of docetaxel metabolites in plasma had not been described earlier. Only the analysis of parent drug in human plasma had been reported [36]. We were able to isolate and purify the metabolites in sufficient amounts from human feces [37]. The HPLC system used, however, did not separate the metabolites M1 and M2; total concentrations of the products were determined. The molar absorbtivities, the extraction recoveries and the slopes of the calibration curves of the metabolites were all in the same range of that of docetaxel. Therefore, the metabolites can be quantified by the use of the docetaxel calibration curve, when these compounds are not available as references [16]. We have used this assay in clinical studies of docetaxel in which we were able to detect docetaxel metabolites in plasma [13,16].

Performance of the Docetaxel Analytical Assay

We have evaluated the analytical runs of docetaxel from November 1998 up to January 2001. The last docetaxel run before November 1998 was of March 1996.

In this more than two-year period we have analyzed almost 1500 study samples in 19 runs. One HPLC column was used and 5 different batches of SPE columns.
Performance of the docetaxel quality control samples is presented in Table 2. The mean measured values of the quality control samples closely resemble their nominal values and accuracies of the docetaxel assay yield values of 103-112%. Precisions of the assay were less than 10% for all quality control concentrations. As described above for paclitaxel analysis, these accuracies and precisions can be considered as very good.

Table 2. *Performance of the docetaxel quality control samples.*

Nominal value (ng/mL)	Measured value (ng/mL)	Accuracy (%)	Precision (%)	Number of runs
100	103 ± 10	103	9	19
500	560 ± 50	112	8	19
750	770 ± 53	103	6	19

Figure 3 gives the individual values of the docetaxel quality controls in the past two years. From the charts it can be seen that in three runs (April 1999) the quality control of 500 ng/mL reveals values exceeding the 20% acceptance ranges. This has led to the preparation of new quality control samples, which perfectly applied to the 20% acceptance criteria. For the other two quality control concentrations, measured values all fall within the 20% acceptance ranges.
In the past two years, a total of 5 different batches of SPE columns were used. Recoveries of docetaxel were calculated in each run by comparing the area of docetaxel in human plasma with the area of docetaxel dissolved in AMW (Figure 4). During validation of the assay we determined recoveries of docetaxel from several batches cyano SPE columns from two suppliers. The best results were obtained with end-capped columns from IST (Sopachem BV, Nieuwegein, The Netherlands) [16]. Only these columns have been used in further docetaxel analysis. Mean recovery percentages of the 5 batches were 85 ± 4% (3 runs), 76 ± 9% (4 runs), 70 ± 5% (2 runs), 64 ± 15% (6 runs) and 91 ± 9% (4 runs). The relative low recovery of 64% of one batch is merely caused by the low recovery of 40% obtained in one run (June 1999). In this run, it was observed that the needle of the SPE equipment was slightly bent resulting in reduced transfer of plasma to the SPE columns. Most likely this has caused the low recovery. Calibration and quality control samples in this run perfectly applied to the acceptance criteria, which shows the usefulness of an internal standard.

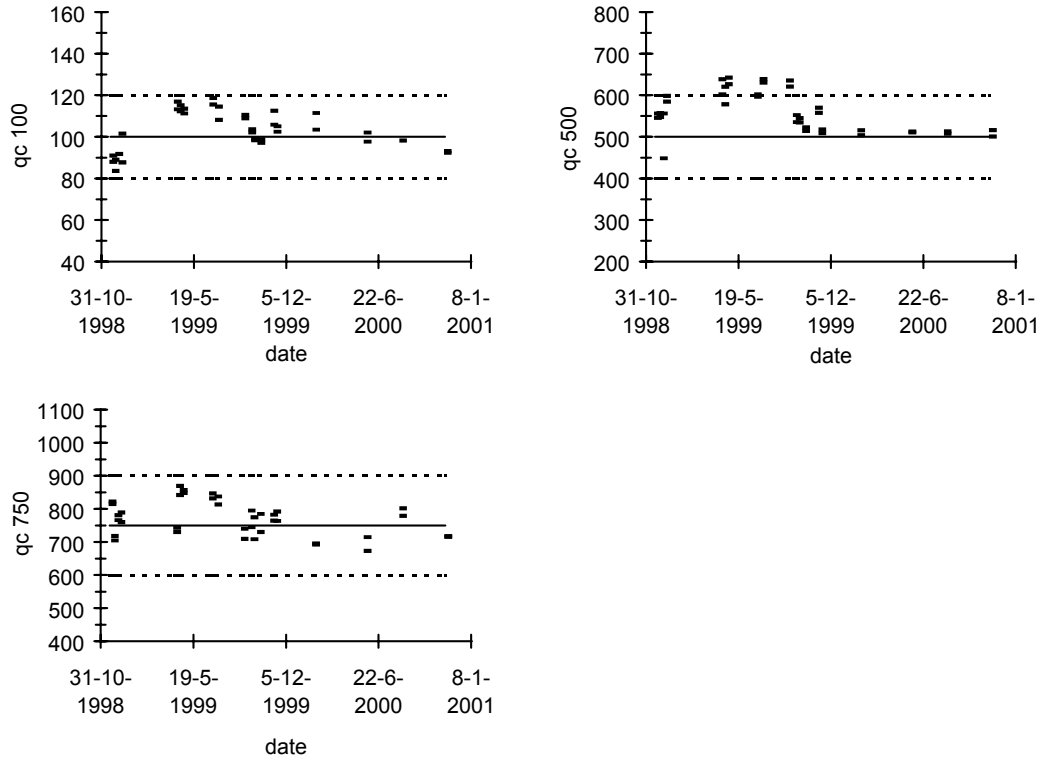

Figure 3. *Individual values of the docetaxel quality control concentrations of 100, 500 and 750 ng/mL. The dotted horizontal lines represent the 20% acceptance ranges.*

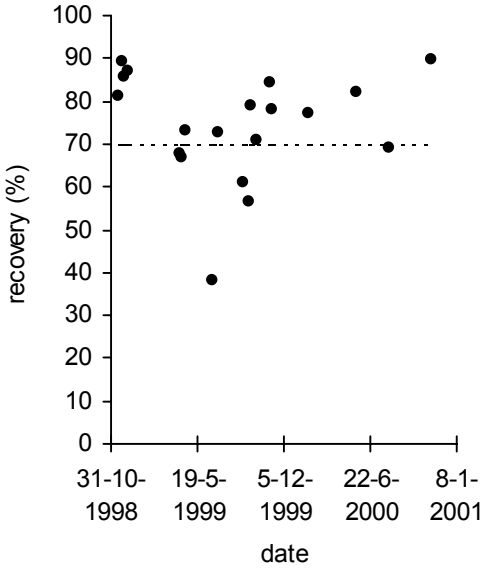

Figure 4. *Recovery data of docetaxel in human plasma. The dotted horizontal line represents a 70% recovery.*

For preparation of the docetaxel calibration and quality control samples we made fresh stock solutions of docetaxel and 2'-methylpaclitaxel every 6 months. We re-evaluated stability of the docetaxel stock solution, which was found to be stable for at least 18 months. Stability of the 2'-methylpaclitaxel stock solution was already performed for paclitaxel analysis and determined at at least 13 months. Longer periods have not been tested.
In conclusion, the validated assay of docetaxel and the four hydroxylated metabolites showed very good performance over the past two years. Accuracies of 103-112% and precisions of less than 10% were achieved. Variability in the recovery data of docetaxel stress the use of an internal standard in this assay.

Development of the Cyclosporin A Analytical Assay
The specific FPIA we used for quantification of cyclosporin A in whole blood has been validated and developed by Abbott Laboratories in order to monitor cyclosporin A therapy in transplant patients [23-26]. The assay is a modification of the non-specific FPIA for determination of cyclosporin A in whole blood with less cross-reactivity of the cyclosporin A metabolites [23-26]. The assay uses a competitive immunoassay methodology in which tracer-labeled antigen and patient antigen compete for binding sites on the antibody molecules. The precise relationship between polarization and concentration of the unlabeled drug is established by measuring the polarization values of calibrators with known concentrations of the drug.

Performance of the Cyclosporin A Analytical Assay
The use of cyclosporin A as an enhancer of the absorption of orally administered paclitaxel and docetaxel gave us a large amount of cyclosporin A blood samples to study performance of the assay. From April 1997 up to January 2001 more than 2000 study samples were analyzed in 131 runs.
Different from the paclitaxel and docetaxel HPLC assays, cyclosporin A FPIA analytical runs do not involve analysis of calibration samples in each run. Prior to start of analysis, a calibration curve is made, which is stored as long as the quality controls fall within their accepted ranges. Each cyclosporin A run involves analysis of study samples and single measurements of 1-3 of the quality control samples. Performance of the cyclosporin A quality controls is presented in Table 3. Accuracies of the quality control samples yield values of 103-105%. The coefficient of variation in the quality control samples was 11% for the 150 ng/mL quality control and less than 10% for the 400 and 800 ng/mL quality controls. Applying the 15% values for precision during validation of an analytical assay, these coefficients of variation fall within the predefined range.

Individual values of the cyclosporin A quality control samples are given in Figure 5. From these charts it can be seen that, in general, the cyclosporin A quality controls meet the requirements of acceptance. Furthermore, the charts show scattered patterns, indicating no obvious trend in the cyclosporin A assay.
However, it can be seen that in the period of February-March 1999 cyclosporin A quality control samples exceeded the 15% and 20% acceptance ranges. It can also be seen that in this period other quality control samples at the same concentrations yielded values close to the target values and met the requirements of acceptance. In this period, we used different lot numbers of the cyclosporin A reagent pack. Between the different lot numbers we observed large differences between the values of the quality controls, with acceptable values for one of the lot numbers, however, with unacceptable values for the other. A similar observation was made in May 1999. In the same week, two analytical runs of cyclosporin A were performed with two different reagent lot numbers. In one run, the three quality controls exceeded the acceptable ranges, while in the other run quality controls perfectly met the requirements of acceptance. The cyclosporin A assay guide [23] recommends recalibration of the assay when a new reagent lot is used. Our observations strongly underline this recommendation.
When cyclosporin A study sample concentrations were above the highest calibration standard (1500 ng/mL) the supernatant was diluted with dilution buffer and re-analyzed as performed for many other compounds analyzed by the TDxFLx system. However, the cyclosporin A assay guide [23] recommends re-analysis of these study samples after dilution with the Cyclosporine Monoclonal Whole Blood Calibrator A (0.0 ng/mL cyclosporin A) prior to performing the solubilization step. We analyzed the 800 ng/mL quality control diluted according to the cyclosporin A assay guide with control whole blood [23] and diluted with dilution buffer. In this run the undiluted 800 ng/mL quality control was also analyzed. The results are given in Table 4. The data clearly show that only dilutions in control whole blood result in an accurate determination of the concentration. Because dilution of study samples is performed when concentrations are above the 1500 ng/mL we have applied both dilution protocols to study samples with high expected values. At concentrations above the 1500 ng/mL differences between the two dilution protocols were very small (less than 3%) and can be considered as negligible.
In conclusion, the specific FPIA for determination of cyclosporin A in whole blood showed very good performance in a routine laboratory setting. Accuracies of the assay yielded values of 103-105% and the coefficients of variation were less than 12%.

Table 3. *Performance of the cyclosporin A quality control samples.*

Nominal value (ng/mL)	Measured value (ng/mL)	Accuracy (%)	Variance (%)	Number of runs
150	158 ± 18	105	11	94
400	420 ± 39	105	9	94
800	821 ± 53	103	6	86

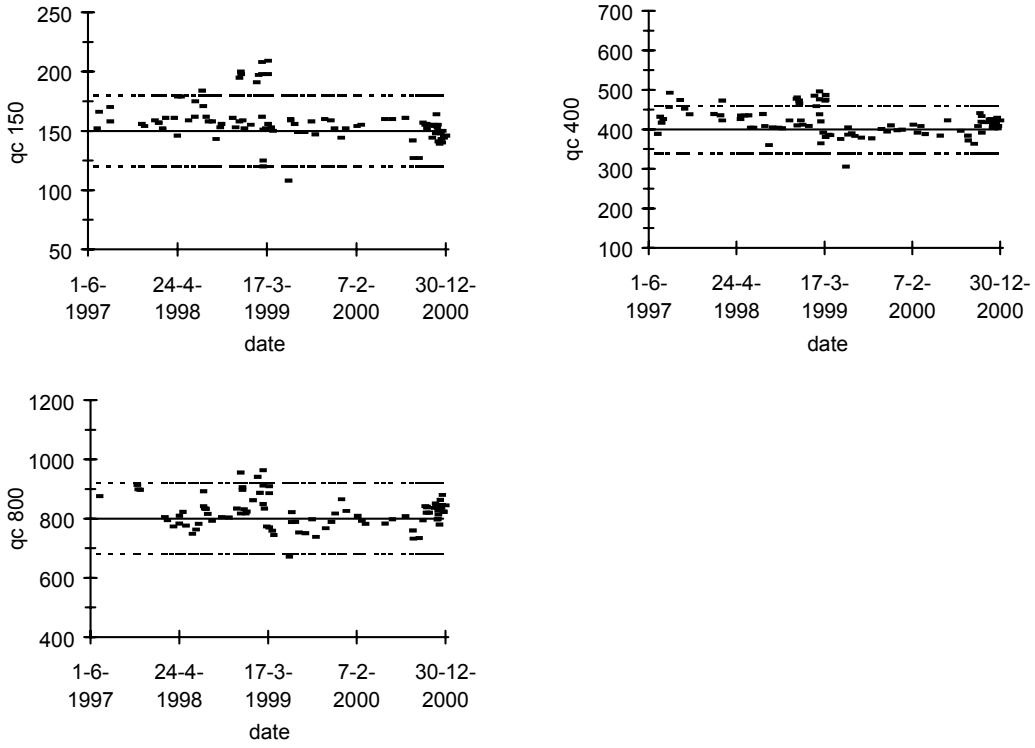

Figure 5. *Individual values of the cyclosporin A quality control concentrations. The dotted horizontal lines represent the 20% and 15% acceptance ranges.*

Table 4. *Cyclosporin A data of the 800 ng/mL quality control diluted with whole blood [23], diluted with dilution buffer, and undiluted.*

Dilution protocol	Dilution	Mean measured value (ng/mL)	CV (%)	N	DEV (%)
Whole blood	5	917	2.2	2	2.2
Dilution buffer	5	735	3.8	2	-18.1
Undiluted	-	897	-	1	

CV: coefficient of variation
DEV: deviation from undiluted sample

Conclusions

In the past four years, the analytical assays of paclitaxel, docetaxel and cyclosporin A showed excellent performance. In this period, we performed 84 analytical runs of paclitaxel, 19 runs of docetaxel and 131 runs of cyclosporin A. Accuracies of the paclitaxel, docetaxel an cyclosporin A assays were 92-102%, 103-112% and 103-105%, respectively. Precisions of the paclitaxel and docetaxel assays were less than 10% for all concentrations. For the cyclosporin A assay, the coefficients of variation were less than 12%. It can be concluded that the validated analytical assays of paclitaxel, docetaxel and cyclosporin A showed very good performance over a prolonged period of time in a routine hospital laboratory setting and can thus be considered as robust assays.

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CHAPTER 2.1

Co-administration of oral cyclosporin A enables oral therapy with paclitaxel

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Abstract

Intravenous (i.v.) paclitaxel is inconvenient and associated with significant and poorly predictable side-effects largely due to the pharmaceutical vehicle Cremophor EL. Oral administration may be attractive as it may circumvent the use of Cremophor EL. However, paclitaxel, as well as many other commonly applied drugs, has poor bioavailability due to high affinity for the mdr1 P-glycoprotein (P-gp) drug efflux pump, which is abundantly present in the gastro-intestinal tract. Consequently, inhibition of P-gp by oral cyclosporin A (CsA) should increase systemic exposure of oral paclitaxel to therapeutic levels. A proof of concept study was carried out in 14 patients with solid tumors. Patients received one course of oral paclitaxel of 60 mg/m^2 with or without 15 mg/kg CsA and with i.v. paclitaxel in subsequent courses. The pharmacokinetics of paclitaxel and its major metabolites were determined during the first 2 courses. In addition, levels of CsA, Cremophor EL and ethanol were measured. Bioavailability of oral paclitaxel in combination with CsA was 8-fold higher than after oral paclitaxel alone ($p < 0.001$). Therapeutic concentrations were achieved on average during 7.4 hours, which is comparable to an equivalent i.v. dose. The oral combination was well tolerated and did not induce gastro-intestinal toxicity or myelosuppression. Cremophor EL plasma levels after oral drug administration were undetectable. In conclusion, co-administration of oral CsA increased the systemic exposure of oral paclitaxel from negligible to therapeutic levels. The combination enables treatment with oral paclitaxel. Undetectable Cremophor EL levels after oral administration may have a very beneficial influence on the safety of the treatment with oral paclitaxel.

Introduction

Paclitaxel is an important new antitumor agent widely used in the treatment of advanced breast and ovarian cancer [1-3]. However, intravenous (i.v.) administration of paclitaxel is inconvenient to patients and associated with significant and unpredictable side-effects [4-6]. The current commercially available i.v. formulation consists of a mixture of ethanol and Cremophor EL (polyoxyethyleneglycerol triricinoleate 35) and it is now well established that the latter plays a major role in the hypersensitivity reactions observed after i.v. administration of paclitaxel [7,8]. Cremophor EL is also responsible for the non-linear tissue distribution of i.v. administered paclitaxel [9]. Oral administration of paclitaxel is to be preferred as it may circumvent the use of Cremophor EL. Paclitaxel, however has poor oral bioavailability due to its high affinity for the

multidrug transporter P-glycoprotein (P-gp), which is abundantly present in the gastro-intestinal tract [10-18]. P-gp in the mucosa of the small and large intestine may limit the oral uptake of paclitaxel and mediate direct excretion of the drug in the intestinal lumen. This became clear when we investigated the oral uptake of paclitaxel in mdr1a knock-out mice lacking functional P-gp in the gut [19]. In this mouse model the systemic exposure was 6-fold higher than in wild-type mice. High systemic availability could also be achieved in wild-type mice when paclitaxel was orally administered in combination with SDZ PSC 833 or with cyclosporin A (CsA), both efficacious P-gp inhibitors [10]. Based on these results we hypothesized that the systemic exposure in humans after oral administration of paclitaxel might be increased with orally administered CsA hopefully to therapeutic plasma drug concentrations. To investigate this, a proof of concept study in patients with solid tumors was initiated.

Patients and Methods

Patient Population

Patients with a histologic proof of cancer for whom no standard therapy of proven benefit existed were eligible for the study. Previous radiotherapy or chemotherapy other than taxoid therapy was allowed as long as the last treatment was at least four weeks prior to study entry and any resulting toxicities were resolved. Patients had to have acceptable bone marrow (WBC > $3.0x10^9$/L; platelets > $100x10^9$/L), liver (serum bilirubin $\leq$ 25 µmol/L; serum albumin $\geq$ 25 g/L) and kidney (serum creatinine $\leq$ 160 µmol/L or clearance $\geq$ 50 mL/min) functions and a WHO performance status $\leq$ 2. Patients were excluded if they suffered from uncontrolled infectious disease, neurologic disease, bowel obstruction or brain metastases. Further exclusion criteria were concomitant use of known P-gp inhibitors, CYP3A-substrates, H2-receptor antagonists or proton pump inhibitors. The trial was approved by the ethics committee of the Institute and all patients gave written informed consent.

Study Design

In the first part of the study, a small cohort of 4 evaluable patients was planned to receive paclitaxel orally as a single agent at a dose of 60 mg/m^2 during course 1 and paclitaxel intravenously at a dose of 175 mg/m^2 administered as a 3 hour infusion during course 2. In the second part of the study, 8 evaluable patients were planned to receive paclitaxel at two occasions which were randomized. At one occasion they would receive paclitaxel orally at a dose of 60 mg/m^2 combined with

a single oral dose of CsA of 15 mg/kg. This low oral dose was selected for safety reasons, because the results in mice indicated increased systemic exposure to paclitaxel after oral administration combined with CsA, as compared to after i.v. administration of paclitaxel alone. Paclitaxel (Paxene®) and CsA (Neoral®) were ingested as oral solutions with 100 ml of tap water. Paclitaxel was taken 10 minutes after CsA. At the other occasion paclitaxel would be administered as a 3 hour infusion at a dose of 175 mg/m^2 without CsA. The oral and i.v. dosages were administered at 9.00 a.m. after an overnight fast. A standard breakfast was served at two hours after paclitaxel administration. The i.v. formulation of paclitaxel (Paxene®, i.e. paclitaxel, 6 mg/ml, dissolved in Cremophor EL and ethanol 1:1 w/v, Baker Norton, Miami, FL) was used for both i.v. and oral administration. At all occasions, patients were premedicated with dexamethasone 20 mg orally 12 and 6 hours prior to, clemastine 1 mg i.v. 30 minutes prior to and ranitidine 50 mg i.v. shortly prior to paclitaxel administration. If in their best interest, all patients continued on a 3 weekly schedule of i.v. paclitaxel at a dose of 175 mg/m^2.

Pharmacokinetics

Pharmacokinetic monitoring of paclitaxel and its major metabolites 6a-hydroxypaclitaxel, 3'p-hydroxypaclitaxel and 6a,3'p-dihydroxypaclitaxel was performed during the first 2 courses. Whole blood samples of 5 ml each were collected at 15 time points up to 48 hours after paclitaxel administration. After centrifugation plasma was stored at -20ºC and analyzed within 4 weeks using a validated high performance liquid chromatographic assay [20,21]. Non-compartmental pharmacokinetic methods were applied to interpret the results [22]. The area under the concentration-time curve (AUC) of paclitaxel was calculated, using the trapezoidal rule with extrapolation to infinity. To compare the systemic exposure after oral and i.v. administration of paclitaxel (F), the ratio of the mean value of the AUC after oral and i.v. administration was calculated and corrected for the difference in dose. Other parameters to be assessed were the maximal plasma concentration of paclitaxel (Cmax), the time to maximal plasma concentration (Tmax), total plasma clearance after i.v. administration (CL), terminal half-life (t1/2) and volume of distribution at steady state (Vss). The terminal t1/2 was calculated as ln 2/k, where k is the rate constant of the terminal phase (h^{-1}) of the plasma concentration-time curve. Cmax and Tmax were determined graphically. Statistical analysis of the data was performed using SPSS/PC+ (SPSS/PC+ Advanced Statistics®, version 6.1, 1994; Chicago, Illinois, USA). Nonparametric tests (Mann-Whitney U-test) were used for comparison of the oral and i.v. results.

The AUCs of the metabolic products were determined using the trapezoidal rule without extrapolation to infinity. The Cmax and the Tmax are the highest measured values and the Tdet represents the duration that the metabolites could be detected in plasma. Additionally, relationships between metabolite concentrations and paclitaxel concentrations were evaluated by calculation of the ratios of the mean AUC of the metabolites and the mean AUC of paclitaxel.
Concentrations of CsA (in whole blood), Cremophor EL (in plasma) and ethanol (in plasma) at different time points were measured according to validated methods. Concentrations of CsA and Cremophor EL were measured at the time points corresponding with the time points of the paclitaxel sampling, and ethanol concentrations were measured at 3 separate time points: 15 minutes, 30 minutes and 1 hour after oral administration of oral paclitaxel with CsA. Cremophor EL was quantified using a high-performance liquid chromatographic assay, as described previously [23] with minor modifications. CsA was measured with a fluorescence polarization immuno assay [24] (TDxFLx, Abbott Laboratories, Amstelveen, The Netherlands) and ethanol was quantitatively determined by gas chromatography.

Results

In total 14 patients were enrolled in the study. Patient characteristics are outlined in Table 1. Three patients went off-study before they had received paclitaxel i.v. in a second course, because of rapid disease progression. Five patients received oral paclitaxel at a dose of 60 mg/m^2 without CsA during the first course and three of them received i.v. paclitaxel during course 2 and subsequent courses. Five other patients received oral paclitaxel at a dose of 60 mg/m^2 in combination with CsA at a dose of 15 mg/kg at the first course and in four patients this was followed by i.v. administration of paclitaxel during course 2 and subsequent courses. The remaining four patients started with i.v. paclitaxel during the first course, followed by oral paclitaxel and CsA during the second course. During all subsequent courses paclitaxel was administered i.v.
Table 2 summarizes the main pharmacokinetic parameters. The mean AUC in patients who received oral paclitaxel in combination with CsA was 1.7 μM.h (± 0.9), which is approximately 8-fold higher than the mean AUC of 0.2 μM.h (± 0.1) in patients who received oral paclitaxel without CsA ($p < 0.001$, Figure 1). The mean AUC in the five patients that started with oral paclitaxel + CsA was not significantly different from the mean AUC in the four patients who received oral paclitaxel + CsA at the second course. The dose-corrected ratio of mean AUC values of oral paclitaxel and i.v. paclitaxel was 0.036 and of oral paclitaxel + CsA and i.v.

paclitaxel 0.282, respectively. However, because of the nonlinear pharmacokinetics of paclitaxel caused by Cremophor EL effects, this calculation results in an underestimation of the true bioavailability [9,25]. In a dose-finding study performed by Huizing et al., a mean AUC of i.v. paclitaxel at a dose of 100 mg/m^2 of 5.8 μM.h was reported [26]. Re-calculation of the above ratios applying the dose-adjusted AUC found by Huizing et al. provided values of 0.059 for the ratio oral paclitaxel/i.v. paclitaxel and 0.474 for the ratio oral paclitaxel + CsA/i.v. paclitaxel. The mean time of the paclitaxel plasma concentration above a previously defined level of 0.05 μM was 1.2 h (± 0.9) after oral paclitaxel and 7.4 h (± 4.4) after oral paclitaxel plus CsA ($p < 0.001$). The mean duration of plasma levels above 0.1 μM was 3.7 h (± 2.2) after oral paclitaxel with CsA and this threshold was not reached after oral paclitaxel alone. CL after i.v. paclitaxel was 13 L/h/m^2 (± 3) in 3 patients who had received oral paclitaxel without CsA at the first course and 12 L/h/m^2 (± 2) in the 8 other patients (not statistically significant, NS). The terminal t1/2 of i.v. paclitaxel in the two groups of patients was 17.7 h (± 2.7; n=3) and 16.3 h (± 10.5; n=8; NS). The difference in Vss was also not statistically significant in the two groups of patients and was 69 L/m^2 (± 27; n=3) and 86 L/m^2 (± 62; n=8). The i.v. pharmacokinetic data are in good agreement with earlier observations [1,26].

Table 1. *Patient characteristics.*

No. of patients	14
Male/Female	2/12
Median age, years (range)	56 (34-69)
Median Performance Status (range)	1 (0-2)
Primary tumor sites	
Ovary	4
Breast	3
Unknown primary	2
Neuroectoderm	1
Rectum	1
Lung	1
Colon	1
Stomach	1
Prior treatment	
No pretreatment	1
Surgical therapy	1
Chemotherapy	1
Surgical therapy and radiotherapy	1
Surgical therapy and chemotherapy	8
Surgical therapy, radiotherapy and chemotherapy	2

Table 2. *Pharmacokinetic parameters of paclitaxel after oral administration (60 mg/m^2) without and with administration of CsA and after i.v. administration (175 mg/m^2). Data are presented as means ± SD.*

PK parameter	PART I		PART II	
	oral (n=5)	i.v. (n=3)	oral + CsA (n=9)	i.v. (n=8)
AUC (μM.h)	0.2 (± 0.1)	16.4 (± 4.3)	1.7 (± 0.9)[a]	17.1 (± 3.7)
Cmax (μM)	0.1 (± 0.0)	4.5 (± 0.9)	0.2 (± 0.1)[a]	4.7 (± 1.0)
Tmax (h)	2.4 (± 0.6)	3.0 (± 0.1)	2.4 (± 0.8)	3.1 (± 0.2)
T> 0.1 μM (h)	-	15.0 (± 3.8)	3.7 (± 2.3)	17.2 (± 3.5)
T> 0.05 μM (h)	1.2 (± 0.9)	22.2 (± 3.3)	7.4 (± 4.4)[a]	28.1 (± 8.9)

[a] $p < 0.001$ compared to oral paclitaxel without CsA.

Plasma metabolite concentrations after i.v. paclitaxel as well as after oral paclitaxel with CsA showed large interpatient variability. After oral administration of paclitaxel alone, metabolites could not be detected. The mean pharmacokinetic parameters of the metabolites after oral administration of paclitaxel with CsA and after i.v. administration are represented in Table 3. After oral administration with CsA, the mean peak plasma concentration ratios of 6a-hydroxypaclitaxel, 3'p-hydroxypaclitaxel and 6a,3'p-dihydroxypaclitaxel to paclitaxel were 0.78, 0.14 and 0.26, respectively. After i.v. administration, these values were 0.08, 0.03 and 0.04. For the AUCs these ratios were 0.87, 0.16 and 0.44 after oral administration with CsA and 0.06, 0.03 and 0.04 after i.v. administration. Significant increases in metabolite/paclitaxel ratios were observed after oral administration compared to i.v administration ($p < 0.001$). All three metabolites could be detected in plasma for only a limited period of time.

Whole blood CsA concentrations were measured in 7 patients. Maximum CsA concentrations ranged from 2.1 mg/L to 4.7 mg/L (mean 3.0) and were reached at 3 to 4 hours after intake. The concentrations 10 hours after intake ranged from 0.3-1.3 mg/L (mean 0.7). Cremophor EL levels in plasma after oral administration of paclitaxel with or without CsA were lower than the lower limit of quantitation of the assay of 0.01% [24]. Ethanol concentrations were measured in 7 patients and the highest detected ethanol concentration in plasma was 0.1‰, which was found in 3 patients 13 minutes after paclitaxel intake.

Paclitaxel in the oral formulation with or without CsA had a bitter taste, but was very well tolerated. No significant side-effects were seen after one course of oral paclitaxel with or without CsA. A pattern of toxicity common to paclitaxel developed after 2 to 3 i.v. courses. CTC grade 2 myalgia was observed in 7 out of 14 patients (50%) and grade 1 neurotoxicity also in 7 patients (50%). Granulocytopenia grade 3

developed in 2 patients (14%). All patients developed alopecia, which was grade 1 in 8 patients (57%) and grade 2 in one patient (7%). Stomatitis grade 2 was seen in 1 patient and flushing grade 2 in another patient. Mild nausea grade 1 (4 patients) and vomiting grade 1 (3 patients) were observed, but only after i.v. paclitaxel. At present, 5 of the 14 patients are still on study. A total of 61 courses of paclitaxel have been administered, 14 of which were oral. The median number of courses per patient was 4 (range 1-8).

Table 3. *Pharmacokinetic parameters of the metabolites 6a-hydroxypaclitaxel (6a-HP), 3'p-hydroxypaclitaxel (3'p-HP) and 6a,3'p-dihydroxypaclitaxel (6a,3'p-DHP) (mean ± SD).*

	N	Tdet (h)	Tmax (h)	Cmax (μM)	AUC (μM.h)	AUCtmet / AUCpac
6a-HP						
oral pac + CsA	9	11.7 (± 7.9)	4.2 (± 1.8)	0.18 (± 0.11)	1.25 (± 1.23)	0.87
i.v. pac	8	7.4 (± 9.0)	3.2 (± 0.2)	0.37 (± 0.34)	1.05 (± 1.29)	0.06
3'p-HP						
oral pac + CsA	9	6.8 (± 6.8)	4.1 (± 1.3)	0.03 (± 0.02)	0.22 (± 0.22)	0.16
i.v. pac	8	7.6 (± 8.8)	3.3 (± 0.2)	0.14 (± 0.11)	0.56 (± 0.71)	0.03
6a,3'p-DHP						
oral pac + CsA	9	10.7 (± 8.3)	6.4 (± 2.0)	0.06 (± 0.04)	0.62 (± 0.59)	0.44
i.v. pac	8	5.7 (± 9.5)	3.7 (± 0.5)	0.18 (± 0.31)	0.67 (± 1.38)	0.04

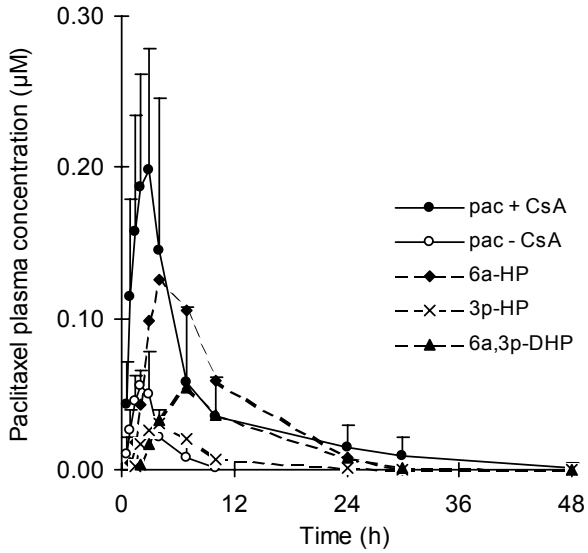

Figure 1. *Plasma concentration-time curves of paclitaxel and its three major metabolites after oral administration (means ± SD).*

Discussion

The results presented above prove that the co-administration of a P-gp inhibitor significantly increases the systemic exposure of orally administered paclitaxel. Paclitaxel administered orally as a single agent without CsA exhibits poor apparent bioavailability of only 4% of the exposure after i.v. administration. Co-administration of CsA increased the systemic exposure of paclitaxel up to 28%. However, the true oral bioavailability may be significantly underestimated, because i.v. paclitaxel clearly shows pronounced non-linear pharmacokinetics due to the presence of Cremophor EL [9,25]. Re-calculation of these figures using the AUC of i.v. paclitaxel at a lower dose [26], which is more realistic for comparison purposes, resulted in an apparent bioavailability of 47% after administration of oral paclitaxel with CsA. An important pharmacokinetic parameter is the time-period of exposure above a certain paclitaxel threshold concentration. Earlier data indicate a strong positive relationship between the duration of the paclitaxel plasma concentration above 0.05 µM or 0.1 µM and pharmacologic activity [25,26]. The frequently applied i.v. dose of paclitaxel of 175 mg/m^2 resulted in a time-period above 0.05 µM of 28.1 (± 8.9) hours. Even at the low oral dose of 60 mg/m^2 applied in our study, plasma concentrations higher than 0.05 µM were achieved during 7.4 (± 4.4) hours. Our preclinical data obtained in wild-type and P-gp mdr1a knock-out mice combined with these first clinical results reveal that CsA increases the absorption of paclitaxel by effectively blocking P-gp in the gut. A second mechanism which may contribute to the increased systemic exposure is an inhibition of paclitaxel metabolism by CsA, as paclitaxel and CsA are both substrates for the cytochrome P450 (CYP) 3A4-isozymes [27,28]. The three main metabolites of paclitaxel are 6a-hydroxypaclitaxel, 3'p-hydroxypaclitaxel and 6a,3'p-dihydroxypaclitaxel and are formed via CYP 2C8, CYP 3A4 and both CYP 2C8 and 3A4, respectively (Figure 2) [29].

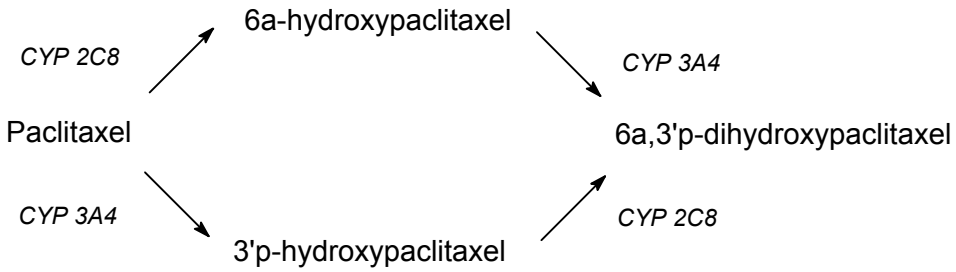

Figure 2. *Major metabolic pathways of paclitaxel.*

All metabolites showed reduced *in vitro* cytotoxicity as compared to paclitaxel [30]. Competition for CYP 3A4 by cyclosporin A may result in altered ratios between the metabolite levels. This hypothesis was supported by our data. Oral administration of paclitaxel with CsA resulted in an increase in the AUC ratio metabolite/paclitaxel for all three metabolites. However, a relative larger increase (15-fold) in the AUC ratio 6a-hydroxypaclitaxel/paclitaxel is observed in comparison to the AUC ratios of 3'p-hydroxypaclitaxel/paclitaxel and 6a,3'p-dihydroxypaclitaxel/paclitaxel (a 5- and 11-fold increase, respectively). Increased metabolism of paclitaxel following oral administration can be explained by the relatively higher amount of paclitaxel passing the liver (first-pass effect). Additionally, metabolism of paclitaxel in the intestinal wall may contribute to the increased metabolite levels. Increased metabolism following oral administration may indeed result in diminished levels of the active drug and possibly reduced efficacy. However, in our opinion, the achieved gain in increased uptake outweighs the possible loss by the increased metabolism. A plausible explanation for the relative larger increase in 6a-hydroxypaclitaxel levels may be that competitive inhibition of CYP 3A4 by CsA results in relatively less formation of 3'p-hydroxypaclitaxel and 6a,3'p-dihydroxypaclitaxel. Consequently, metabolism of paclitaxel by CYP 2C8 is favoured, resulting in increased formation of 6a-hydroxypaclitaxel. Thus, as CsA interferes with CYP mediated metabolism of paclitaxel, decreased elimination by inhibition of the metabolic enzymes may contribute to the observed increase in systemic exposure. In addition, Sparreboom et al. [19] showed that direct intestinal excretion of paclitaxel, another important route of drug elimination, is significantly diminished in absence of P-gp. At present, it is unknown whether involvement of other factors, including drug release from the pharmaceutical formulation (dissolution), modification of biliary excretion and drug degradation in gastro-intestinal fluids contribute to the extent of the systemic exposure of orally administered paclitaxel.

The single oral dose of 15 mg/kg of CsA resulted in Cmax and trough values that are in the therapeutic range for immunosuppression and may be associated with toxicity, in particular renal dysfunction. However, the available studies of CsA, pharmaceutically formulated in Neoral®, are limited [31] and more importantly, this side-effect is mainly associated with CsA when given on a chronic treatment basis. No renal toxicity, or any other side-effect clearly associated with the single CsA administration was observed. The CsA concentrations found in our study were higher than we expected, possibly due to competition for CYP-mediated metabolism by paclitaxel. Cremophor EL levels could not be detected after oral administration of paclitaxel as a single agent, nor when co-administered with CsA. This may be very beneficial for the safety profile of oral paclitaxel, as Cremophor

EL plays a pivotal role in the hypersensitivity reactions associated with i.v. paclitaxel administration [4-8]. The maximum measured ethanol levels of 0.1‰ are not clinically relevant. Besides a bitter taste, paclitaxel in the oral formulation at a dose of 60 mg/m^2 was very well tolerated without induction of gastro-intestinal or bone marrow toxicity. The main side-effects were alopecia and myalgia CTC grade 1 or 2, which developed after 2-3 courses of i.v. paclitaxel. Regarding the nearly uneventful oral administration of the dose of 60 mg/m^2, oral doses can be escalated or given bi-daily in order to prolong exposure at therapeutic levels. The ultimate goal is to test whether at least equal activity of oral paclitaxel can be obtained, as compared to i.v. paclitaxel, but with better safety. However, co-administration of a P-gp inhibitor may increase paclitaxel levels in brain and heart tissue and may therefore enhance the risk of central neurotoxicity or cardiac toxicity [32]. Neither in our clinical study nor in the animal studies did we observe signs of central neurotoxicity or cardiac toxicity, at least not at the dosages that were used. Furthermore, oral administration opens the opportunity to explore therapeutic activity and safety on a chronic daily treatment schedule.

Finally, the concept of modulation of P-gp may well be applied to other drugs, including non-cytotoxic agents, which have a high affinity for P-gp and are associated with poor oral bioavailability, e.g. HIV protease inhibitors [33]. The knowledge currently gained by the extensive analysis of mdr1a knock-out mice has proven to be extremely valuable to the development of new strategies to further optimize drug treatment [34,35]. Improvement of systemic exposure of oral paclitaxel and other drugs, as well as development of an optimal pharmaceutical formulation for oral administration are currently investigated in our institute. Based on these early results, oral administration of paclitaxel in combination with CsA may be a realistic alternative to the current treatment modalities.

In summary we have demonstrated for the first time in cancer patients the proof of concept of efficient oral uptake of paclitaxel, made possible by concomitant administration of the P-gp blocker CsA.

Acknowledgements

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CHAPTER 2.2

A phase I and pharmacokinetic study of oral paclitaxel

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Abstract

Purpose: To investigate dose-escalation of oral paclitaxel in combination with dose-increment and scheduling of cyclosporin A (CsA) in order to improve the systemic exposure to paclitaxel and to explore the maximum tolerated dose (MTD) and dose limiting toxicity (DLT).
Patients and Methods: A total of 53 patients received, on one occasion, oral paclitaxel in combination with CsA, co-administered to enhance the absorption of paclitaxel, and on another occasion intravenous paclitaxel at a dose of 175 mg/m^2 as a 3-hour infusion.
Results: The main toxicities observed after oral intake of paclitaxel were acute nausea and vomiting, which reached DLT at the dose level of 360 mg/m^2. Dose-escalation of oral paclitaxel from 60 to 300 mg/m^2 resulted in significant, but less than proportional increases in the plasma area under the concentration-time curve (AUC) of paclitaxel. The mean AUC values ± SD after 60, 180 and 300 mg/m^2 of oral paclitaxel were 1.65 ± 0.93, 3.33 ± 2.39 and 3.46 ± 1.37 μM.h, respectively. Dose-increment and scheduling of CsA did not result in a further increase in the AUC of paclitaxel. The AUC of intravenous paclitaxel was 15.39 ± 3.26 μM.h.
Conclusion: The MTD of oral paclitaxel was 300 mg/m^2. However, because the pharmacokinetic data of oral paclitaxel, in particular at the highest doses applied, revealed non-linear pharmacokinetics with only a moderate further increase of the AUC with doses up to 300 mg/m^2, the oral paclitaxel dose of 180 mg/m^2 in combination with 15 mg/kg oral CsA is considered most appropriate for further investigation. The safety of the oral combination at this dose level was good.

Introduction

Paclitaxel is an important antitumor agent widely applied in the treatment of advanced ovarian and breast cancer [1,2]. The intravenous (i.v) administration is, however, inconvenient to patients and associated with a number of unpredictable side-effects. Severe hypersensitivity reactions have been observed after i.v. infusion of paclitaxel and it is now well established that the pharmaceutical vehicle Cremophor EL contributes largely to this effect [3-6]. Oral administration of paclitaxel is very attractive, because it is convenient and practical for patients and it may circumvent systemic exposure to the vehicle Cremophor EL. Furthermore, oral administration may enable development of chronic treatment schedules resulting in sustained plasma concentrations above a pharmacological relevant threshold level.

Paclitaxel, however, has poor oral bioavailability due to its affinity for the membrane bound drug efflux pump P-glycoprotein (P-gp), which is abundantly present in the gastro-intestinal tract [7-10]. P-gp in the mucosa of the small and the large intestine limits the oral uptake of paclitaxel and mediates direct excretion of the drug into the intestinal lumen [10]. In addition, presystemic elimination in the liver by the cytochrome P450 (CYP) isozymes 3A4 and 2C8 may play an important role in the low oral bioavailability of paclitaxel [11-13].
Preclinical and clinical proof of concept studies carried out at our Institute revealed that co-administration of oral cyclosporin A (CsA), an efficacious inhibitor of P-gp as well as CYP 3A4 mediated drug metabolism, resulted in an approximately 8-fold increase in the systemic exposure of oral paclitaxel [14-16]. In this study we investigated dose-escalation of oral paclitaxel in combination with dose-increment and scheduling of CsA in order to improve the systemic exposure to paclitaxel and to explore the maximum tolerated dose (MTD) and dose limiting toxicity (DLT).

Patients and Methods

Patient Population
Patients with a histologic proof of cancer for whom no standard therapy of proven benefit existed were eligible for the study. Previous radiotherapy or chemotherapy other than taxoid therapy was allowed as long as the last treatment was at least four weeks prior to study entry and any resulting toxicities were resolved. Patients had to have acceptable bone marrow (white blood cells > 3.0 x 10^9/L; platelets > 100 x 10^9/L), liver function (serum bilirubin $\leq$ 25 µmol/L; serum albumin $\geq$ 25 g/L), and kidney function (serum creatinine $\leq$ 160 µmol/L or clearance $\geq$ 50 mL/min), and a World Health Organization (WHO) performance status $\leq$ 2. Patients were excluded if they suffered from uncontrolled infectious disease, neurologic disease, bowel obstruction or symptomatic brain metastases. Further exclusion criteria were concomitant use of known P-gp inhibitors and chronic use of H2-receptor antagonists or proton pump inhibitors. The study protocol was approved by the Medical Ethics Committee of the Institute, and all patients gave written informed consent.

Study Design
Patients received, on one occasion, oral paclitaxel and, on another occasion i.v. paclitaxel at a dose of 175 mg/m^2 administered as a 3-hour infusion. If it was considered to be in their best interest patients continued on a 3-weekly schedule of i.v. paclitaxel. The treatment schedule of oral paclitaxel in this study is outlined in

Table 1. At the first 2 treatment levels, oral and i.v. administration of paclitaxel were randomized during course 1 and 2. At all higher dose levels (3-9) patients received oral paclitaxel during course 1 and i.v. paclitaxel during course 2.

Table 1. *Treatment schedule of oral paclitaxel and oral cyclosporin A (CsA).*

Level	Oral paclitaxel dose	CsA dose
1	60 mg/m^2	15 mg/kg
2	60 mg/m^2	30 mg/kg
3	60 mg/m^2	2x 15 mg/kg
4	120 mg/m^2	15 mg/kg
5	180 mg/m^2	15 mg/kg
6	210 mg/m^2	15 mg/kg
7	250 mg/m^2	15 mg/kg
8	300 mg/m^2	15 mg/kg
9	360 mg/m^2	15 mg/kg

Drug Administration

The i.v. formulation of paclitaxel (Paxene®, paclitaxel 6 mg/ml, dissolved in Cremophor EL and ethanol 1:1 w/v, Baker Norton Pharmaceuticals, Miami, FL, USA) was used for both i.v. and oral administration of paclitaxel. Prior to oral paclitaxel administration patients received oral CsA (Neoral®, Novartis, Basel, Switzerland). At dose levels 1-3 patients ingested CsA as an oral solution 10 minutes prior to paclitaxel administration. At dose level 3 CsA was administered bid 10 minutes prior to and 2 hours after oral paclitaxel administration. Due to the bitter taste of the oral solution patients at subsequent dose levels (4-9) received CsA in capsules, administered 30 minutes prior to oral paclitaxel administration. Oral paclitaxel was administered after an overnight fast and a standard breakfast was served 2 hours after paclitaxel administration.

To prevent hypersensitivity reactions, patients were premedicated with dexamethasone 20 mg orally 12 and 6 hours prior to, clemastine 2 mg i.v. and cimetidine 300 mg i.v. 30 minutes prior to both i.v. and oral paclitaxel administration. Because Cremophor EL levels after oral administration of paclitaxel (Paxene®) seemed undetectable in plasma, three patients at dose level 8 and all patients at dose level 9 did not receive premedication prior to oral paclitaxel administration. To prevent nausea and vomiting following oral intake of paclitaxel, which occurred more frequently at dose levels 6-7 and higher, four patients at dose level 8 and all patients at dose level 9 received 1 mg oral granisetron (Kytril®) 1 hour prior to CsA administration. As nausea and vomiting continued, two patients at

dose level 8 and all patients at dose level 9 received additionally a light breakfast at least 2 hours prior to oral paclitaxel administration.

Patient Evaluation

Pretreatment evaluation included a complete medical history and complete physical examination. Before each course, an interim history including concomitant medications taken, toxicities and performance status were registered and a physical examination was performed. Hematology was checked twice weekly after course 1 and 2 and weekly after subsequent courses. Blood chemistries including liver and renal function, serum electrolytes, total protein and albumin and glucose levels, were checked weekly. All toxicities observed were graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTC) [17]. Dose limiting toxicities (DLT) were defined as grade 4 granulocytopenia of a duration of > 5 days, grade 4 thrombocytopenia of any duration or any grade 3/4 non-hematological toxicity except untreated nausea and vomiting. Tumor measurements were performed every other cycle, but initially after the first 2 i.v. courses. Responses were evaluated according to the WHO criteria [18].

Pharmacokinetics

Pharmacokinetic monitoring was performed during course 1 and course 2. For paclitaxel plasma concentrations blood samples of 5 ml each were collected in heparinized tubes at 15 time points up to 48 hours after both i.v. and oral paclitaxel administration. Pharmacokinetic parameters of i.v. paclitaxel at dose level 3 and subsequent dose levels were determined by a limited sampling model using 2 plasma concentration-time points at 1 and 8 hours after the end of paclitaxel infusion [19]. Blood samples were centrifuged, plasma was separated and samples were stored at -20ºC until analysis. Paclitaxel concentrations in plasma were determined using a validated high performance liquid chromatography (HPLC) assay [20]. Urine was collected in 24-hour aliquots after all oral paclitaxel administrations and after i.v. administration at dose levels 1-3. Urine samples were stabilized with a mixture of 5% Cremophor EL/ethanol 1:1 v/v and stored at -20ºC until analysis. Paclitaxel concentrations in urine were determined using a validated HPLC assay [21]. For CsA whole blood concentrations blood samples withdrawn for paclitaxel analysis were used. Whole blood samples were stored at 4ºC and analyzed within one week using a fluorescence polarization immuno assay [22]. Plasma samples for ethanol concentrations were obtained every 15 minutes up to 1 h following oral paclitaxel administration and analyzed by gas chromatography. Plasma concentrations of Cremophor EL were measured at 4 time points up to 4 hours after oral paclitaxel intake using a validated HPLC assay [23].

Non-compartmental pharmacokinetic methods were applied to process the results [24]. The area under the concentration-time curve (AUC) was estimated by the trapezoidal rule with extrapolation to infinity using the terminal rate constant k. The apparent bioavailability of oral paclitaxel was calculated as the ratio of the mean AUC values after oral and i.v. administration with a correction for the difference in dose. Other parameters to be assessed were the maximal concentration (Cmax), the time to maximal concentration (Tmax), the time above the previously defined threshold concentrations of 0.05 µM and 0.1 µM (T>0.05 µM, T>0.1 µM) and the terminal half-life (t1/2). Cmax and Tmax were determined graphically, T> 0.05 µM and T> 0.1 µM were determined using linear interpolation and t1/2 was calculated as ln2/k. The percentage of the administered dose recovered in the urine (U_{excr}) was calculated as the amount excreted in the urine divided by the actual administered dose times 100%. Statistical analysis of the data was performed using the nonparametric Jonckheere-Terpstra-test [25], the Mann-Whitney U-test and the Spearman correlation coefficient. The a priori level of significance was p=0.05.

Results

Patient Characteristics

A total of 53 patients (21 males and 32 females) was enrolled onto the study. At study entry, the median age of the patients was 54 years (range 25 to 78) and the median WHO performance status was 1 (range 0 to 2). Primary tumor types included breast (17), ovarian (5), gastric (6), non-small-cell lung cancer (NSCLC) (4), colorectal cancer (4), adenocarcinomas of unknown primary site (8), and other tumors (9). Four patients were cytotoxic therapy naive, all other patients had received prior surgical therapy, radiotherapy and/or chemotherapy. Two patients were not evaluable because they went off study before they had received oral paclitaxel. Six patients were considered not evaluable for pharmacokinetic analysis because of vomiting within 2 hours after ingestion of oral paclitaxel.

Toxicities

Toxicities observed following oral administration of paclitaxel were generally mild (grade 1-2). The principal hematological toxicities after oral intake of paclitaxel were leukocytopenia and granulocytopenia (data listed in Table 2). Thrombocytopenia grade 2 was observed in one patient at dose level 250 mg/m^2. Anemia was observed in 35 patients, which was often pre-existing and never exceeded grade 2 in severity. The non-hematological toxicities after oral intake of paclitaxel are listed in Table 3. Main toxicities observed following oral intake of paclitaxel were acute

nausea and vomiting, which occurred more frequently at dose levels 210-250 mg/m^2 and higher. Vomiting occurred mostly only once within 30 minutes after intake of oral paclitaxel. Toxicities clearly associated with CsA administration were not observed. During subsequent treatment with i.v. paclitaxel granulocytopenia, arthralgia/myalgia, neurotoxicity and allergic reactions were observed, which were typically related to paclitaxel and its formulation. Toxicities observed after i.v. administration of paclitaxel were generally mild with the exception of one patient who experienced an acute allergic reaction grade 4 despite premedication. This patient developed hypotension, bronchospasm, tachycardia, sweating and flushes which were reversed with adrenaline, dexamethasone, clemastine, and salbutamol within 1 hour.

Antitumor Activity

Partial responses were observed in three patients, which were documented after the third course (i.e. one oral and two i.v. courses). One patient with 5FU refractory advanced gastric cancer developed a substantial volume reduction of a large supraclavicular lymph node after a first course with oral paclitaxel of 180 mg/m^2. A partial response was documented after 2 additional i.v. courses. Another patient with advanced breast cancer showed significant reduction of cutaneous metastases after a first oral course of 210 mg/m^2. A partial response was documented after the third course. A third patient developed a partial remission of advanced platinum resistant ovarian cancer after the oral and 2 i.v. courses of paclitaxel.

Table 2. *Hematological toxicities after oral administration of paclitaxel (pac) (NCI CTC).*

Pac dose (mg/m^2)	60	60	60	120	180	210	250	300	360
CsA dose (mg/kg)	15	30	2x 15	15	15	15	15	15	15
No. of patients	9	7	6	3	6	4	4	7	5
Leukocytopenia									
grade 1	0	0	2	1	0	0	1	1	0
grade 2	0	0	0	0	1	2	0	0	0
grade 3	0	0	0	1	1	0	2	0	0
grade 4	0	0	0	1	0	0	0	0	0
Granulocytopenia									
grade 1	0	0	0	0	0	1	0	0	0
grade 2	0	0	0	0	0	0	0	0	0
grade 3	0	0	0	0	1	1	1	1	0
grade 4	0	0	0	2	1	0	1	0	0

Table 3. *Non-hematological toxicities after oral administration of paclitaxel (pac) (NCI CTC)*

Pac dose (mg/m^2)	60	60	60	120	180	210	250	300	360
CsA dose (mg/kg)	15	30	2x 15	15	15	15	15	15	15
No. of patients	9	7	6	3	6	4	4	7	5
Nausea									
Grade 1/2	2	3	1	0	2	1	1	1	2
Vomiting									
Grade 1/2	1	2	1	0	0	1	1	1	4
Diarrhea									
Grade 1/2	0	0	0	0	0	1	1	1	2
Grade 3	0	0	0	0	0	0	0	0	1
Gastric pain									
Grade 1/2	1	0	0	0	2	0	0	0	1
Arthralgia/myalgia									
Grade 1/2	3	1	2	1	1	0	1	2	3
Grade 3	0	0	0	1	0	0	0	0	0
Mucositis									
Grade 1/2	0	0	1	0	0	1	0	1	3
Neurotoxicity									
Grade 1	2	1	0	0	0	0	1	0	0
Alopecia									
Grade 1/2	0	0	0	0	1	1	0	1	3
Fatigue									
Grade 1/2	2	0	2	1	0	0	0	3	1
Skin									
Grade 1/2	1	0	1	0	0	1	0	1	2

Pharmacokinetics

Pharmacokinetic parameters of orally administered paclitaxel are outlined in Table 4. Dose-escalation of oral paclitaxel from 60 to 300 mg/m^2 in combination with CsA 15 mg/kg resulted in a significant increase in both AUC and T>0.1 μM of paclitaxel (Jonckheere-Terpstra Test, p=0.008, p=0.040, respectively). Mean AUC values for the oral paclitaxel doses of 60, 180 and 300 mg/m^2 were 1.65 ± 0.93, 3.33 ± 2.39 and 3.46 ± 1.37 μM.h, respectively. Mean T>0.1 μM values were 3.7 ± 2.3, 7.9 ± 6.7 and 8.1 ± 4.1 h, respectively. The apparent bioavailabilities of oral paclitaxel at doses of 60, 180 and 300 mg/m^2, calculated as the dose-corrected ratio of mean AUC values of oral and i.v. paclitaxel, were 31%, 21% and 13%, respectively. Increasing the CsA dose to 30 mg/kg or splitting the dose to 2x 15 mg/kg did not result in a significant further increase in the AUC and T>0.1 μM of paclitaxel compared to the single dose of 15 mg/kg. Figure 1 shows the mean plasma concentration-time curve of oral paclitaxel at a dose of 180 mg/m^2 in combination with oral CsA at a dose of 15 mg/kg.

Table 4. *Pharmacokinetics of oral paclitaxel (pac) (data listed as mean ± (SD)).*

Pac dose (mg/m^2)	CsA dose (mg/kg)	No. Patients	AUC (μM.h)	Cmax (μM)	Tmax (h)	T> 0.1 μM (h)	T> 0.05 μM (h)	t1/2 (h)	U_{excr} (% dose)
60	15	9	1.65 (0.93)	0.24 (0.08)	2.4 (0.8)	3.7 (2.3)	7.3 (4.4)	9.5 (5.5)	1.9 (1.3)
60	30	7	1.69 (0.44)	0.16 (0.04)	3.9 (1.6)	3.5 (2.0)	7.5 (2.0)	14.1 (6.5)	2.5 (1.8)
60	2x 15	6	1.53 (0.50)	0.19 (0.06)	2.6 (1.0)	2.7 (1.3)	5.9 (3.5)	16.4 (5.0)	1.4 (0.6)
120	15	3	2.55 (2.29)	0.31 (0.13)	3.7 (0.7)	7.9 (8.0)	13.0 (12.7)	10.4 (7.1)	1.5 (0.3)
180	15	6	3.33 (2.39)	0.34 (0.23)	3.2 (0.4)	7.9 (6.7)	14.6 (12.3)	16.0 (10.3)	1.7 (1.6)
210	15	3	2.59 (0.86)	0.28 (0.06)	3.8 (0.9)	6.6 (2.5)	11.5 (4.1)	16.0 (4.4)	1.4 (0.4)
250	15	3	3.27 (2.94)	0.21 (0.12)	4.4 (2.4)	7.0 (9.3)	13.6 (11.1)	18.6 (6.4)	1.0 (0.7)
300	15	6	3.46 (1.37)	0.33 (0.14)	3.7 (0.5)	8.1 (4.1)	14.3 (6.9)	17.9 (8.9)	1.1 (1.1)
360	15	2	1.46 - 9.31[a]	0.19 - 0.46[a]	4.0 – 7.3[a]	3.9 - 29.1[a]	10.3 - 41.5[a]	11.0 - 11.8[a]	0.3 - 1.4[a]

[a]2 patients were evaluated at this dose level, the second patient vomited 5 minutes after oral intake of paclitaxel and received a rechallange of both CsA and paclitaxel 2 hours later.

The pharmacokinetic data of i.v. paclitaxel (175 mg/m^2 as a 3-hour infusion) were in good agreement with earlier observations [26-28]. The mean AUC and T>0.1 μM values were 15.39 ± 3.26 μM.h and 17.1 ± 4.9 h, respectively (n=39). The mean urinary excretion of i.v. paclitaxel calculated as fraction of the administered i.v. dose was 6.6 ± 3.1% (n=19). Pharmacokinetic parameters of CsA are outlined in Table 5. Dose-escalation of oral paclitaxel did not produce significant differences in the pharmacokinetics of CsA.

Table 5. *Pharmacokinetics of CsA (Data listed as mean (±SD)).*

Pac dose (mg/m^2)	CsA dose (mg/kg)	No. Patients	AUC (mg.h/L)	Cmax (mg/L)	Tmax (h)
60	15	9	24.36 (9.95)	3.10 (0.88)	3.2 (0.9)
60	30	7	42.70 (13.62)	3.60 (1.03)	4.3 (2.3)
60	2x 15	6	52.66 (19.86)	3.85 (1.49)	5.6 (2.7)
120	15	3	28.61 (14.09)	2.38 (0.57)	2.9 (1.6)
180	15	6	22.20 (7.65)	2.19 (0.58)	2.6 (1.3)
210	15	3	16.44 (2.53)	1.74 (0.25)	1.7 (0.3)
250	15	3	13.45 (8.69)	1.15 (0.38)	3.0 (1.5)
300	15	6	17.63 (2.84)	1.84 (0.31)	1.9 (1.3)
360	15	2	17.34 - 21.10[a]	2.70 – 1.22[a]	0.85 - 0.92[a]

[a]2 patients were evaluated at this dose level, the second patient vomited 5 minutes after oral intake of paclitaxel and received a rechallange of both CsA and paclitaxel 2 hours later.

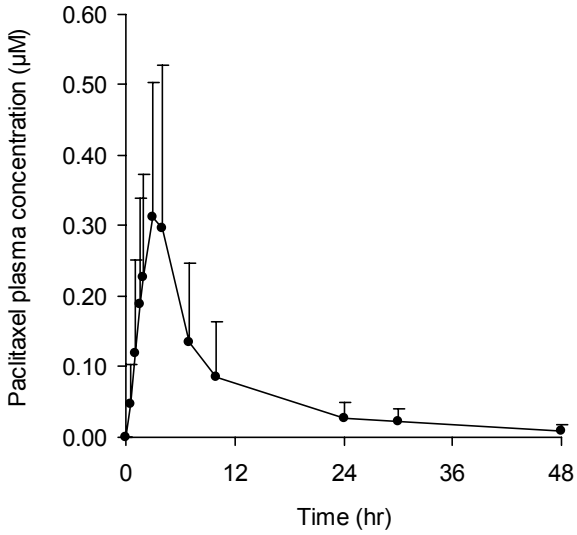

Figure 1. *Plasma concentration-time curve of oral paclitaxel at a dose of 180 mg/m^2 in combination with 15 mg/kg oral cyclosporin A (n=6). Data are represented as means ± SD.*

Maximal blood ethanol concentrations were reached within 1 hour after oral administration of paclitaxel in the i.v. formulation. Paclitaxel doses of 60 and 120 mg/m^2, corresponding with 5 and 10 ml/m^2 ethanol, respectively, resulted in maximal ethanol concentrations < 0.1‰ v/v. Paclitaxel doses of 180, 210, 250, 300 and 360 mg/m^2 with 15, 17.5, 21, 25 and 30 ml/m^2 of ethanol, respectively, resulted in mean (± SD) maximal ethanol concentrations of 0.31‰ (± 0.21), 0.32‰ (± 0.11), 0.28‰ (± 0.02), 0.46‰ (± 0.12) and 0.45‰ (± 0.01), respectively. Maximal ethanol concentrations were significantly correlated with maximal paclitaxel concentrations (p=0.019, r= 0.361). Cremophor EL levels in plasma after oral administration of paclitaxel (Paxene®) were undetectable at all investigated paclitaxel dose levels (< 0.01% v/v).

DISCUSSION

Preclinical and clinical proof of concept studies carried out at our Institute clearly revealed that co-administration of oral CsA, an efficacious inhibitor of P-gp as well as CYP 3A4 mediated drug metabolism, resulted in a significantly enhanced systemic exposure to oral paclitaxel [14-16]. The most plausible explanation for the observed increase of the oral uptake of paclitaxel is inhibition of P-gp in the gut wall by CsA. In addition, inhibition of CYP 3A4 mediated paclitaxel metabolism may play a significant role as we observed altered paclitaxel metabolism following CsA co-administration [16]. The first promising clinical results at low paclitaxel dosages of 60 mg/m^2 encouraged us to further increase the systemic exposure of orally administered paclitaxel by dose-escalation of paclitaxel and dose-increment and scheduling of CsA and to explore the maximum tolerated dose (MTD) and dose limiting toxicity (DLT) of oral paclitaxel.

Toxicities observed following oral administration of paclitaxel were generally mild (grade 1-2). The principal hematological toxicities after oral intake of paclitaxel were leukocytopenia and granulocytopenia. Four patients experienced grade 4 granulocytopenia (dose levels 120, 180 and 250 mg/m^2), which was short-lasting, uncomplicated and did not reach DLT. The main non-hematological toxicities after oral intake of paclitaxel were nausea, vomiting and arthralgia/myalgia. One patient experienced myalgia grade 3 (dose level 120 mg/m^2) and one patient experienced diarrhea grade 3 (dose level 360 mg/m^2). However, these toxicities were uncomplicated, short-lasting and not considered as DLT. To prevent symptoms of nausea and vomiting, four patients at dose level 300 mg/m^2 and all patients at dose level 360 mg/m^2 received 1 mg oral granisetron (Kytril®) 1 hour prior to CsA administration. Two patients at dose level 300 mg/m^2 and all patients at dose level

360 mg/m^2 received additionally a light breakfast at least 2 hours prior to oral paclitaxel intake in an attempt to further reduce occurrence of nausea and vomiting. An interval of at least 2 hours was chosen to exclude the influence of food on the pharmacokinetics of oral paclitaxel. At the dose level of 300 mg/m^2 the administration of a light breakfast 2 hours prior to oral paclitaxel administration had no measurable influence on the pharmacokinetic parameters of paclitaxel. Despite anti-emetic therapy and the additional light breakfast, four of five patients at the dose level of 360 mg/m^2 continued to experience acute nausea and vomiting. Apparently, an oral paclitaxel dose of 360 mg/m^2 produced acute gastro-intestinal toxicity resulting in acute nausea and massive vomiting. We considered the dose level of 360 mg/m^2 as DLT. The MTD of oral paclitaxel was determined at 300 mg/m^2. Toxicities clearly associated with CsA administration were not observed. Partial responses (PR) to paclitaxel were observed in three patients, which were documented after the third course. In at least two of these three patients there were strong indications of activity of oral paclitaxel.

Pharmacokinetic analysis of oral paclitaxel revealed that dose-escalation of oral paclitaxel from 60 to 300 mg/m^2 resulted in significant increases in both AUC and T>0.1 μM; however, these increases were moderate and not proportional with the increases in dose. This non-linear pharmacokinetic behavior of oral paclitaxel is most likely due to a maximum in absorption from the gastro-intestinal tract. Non-linear absorption pharmacokinetics have been observed for oral drugs that (1) have poor aqueous solubility and limited dissolution or (2) are absorbed via saturable transport mechanisms [29]. In this study paclitaxel was administered as a solution (Paxene®, i.v. formulation containing 6 mg paclitaxel per ml Cremophor EL/ethanol, 1:1 w/v), which suggests that dissolution of the drug was not involved in absorption of orally administered paclitaxel. However, it is possible that, after ingestion, paclitaxel was released from its pharmaceutical formulation and precipitated as a result of its poor aqueous solubility. Consequently, limited dissolution may have caused the observed non-linear absorption. A similar non-linear pharmacokinetic absorption pattern due to poor aqueous solubility resulting in limited dissolution was observed for the oral anticancer drugs etoposide and the platinum complex JM216 [30,31]. An alternative explanation is that saturation of active transport mechanisms is responsible for the observed non-linear absorption pharmacokinetics of oral paclitaxel. A similar pattern of absorption with saturation of active transport mechanisms has been observed for riboflavin, ascorbic acid and amino-beta-lactam antibiotics [32]. Because neither *in vitro* nor *in vivo* studies have shown the presence of active inward transport mechanisms of paclitaxel and because it is very unlikely that both active inward and outward transport mechanisms for the same drug exist, we hypothesize that the maximum in absorption of oral paclitaxel is caused by its poor aqueous solubility in

the gastro-intestinal tract and not by saturation of putative active transport mechanisms.

Increasing the dose from 15 to 30 mg/kg and splitting the dose of CsA into 2x 15 mg/kg to achieve higher and more sustained levels of the inhibitor did not result in a further increase in the systemic exposure to paclitaxel. Apparently, P-gp inhibition by CsA was maximal at a single dose of CsA of 15 mg/kg. It remains unclear whether CsA was adequate to inhibit P-gp completely. Incomplete P-gp inhibition by CsA may necessitate the use of more potent modulators, such as certain nonimmuno-suppressive analogues of cyclosporin [33]. Incomplete distribution of CsA over the mucosa wall may also contribute to the possible incomplete inhibition of P-gp by CsA.

Increases in paclitaxel dose and thus increases in the amount of ethanol administered did not result in equivalent increases in blood ethanol levels. Maximal ethanol concentrations were significantly correlated with maximal paclitaxel concentrations and, hence, ethanol appears to follow the non-linear absorption profile of orally administered paclitaxel. A maximum in ethanol absorption has not been observed before and is of interest for further investigation.

Cremophor EL levels were undetectable at all oral paclitaxel dose levels. Apparently, Cremophor EL is not absorbed following oral administration of the paclitaxel i.v. formulation (Paxene®). This is important because systemic exposure to Cremophor EL can induce severe hypersensitivity reactions [3-6]. No hypersensitivity reactions were observed in patients who did not receive premedication prior to oral paclitaxel administration (n=8). Evidently, paclitaxel (Paxene®) can be administered orally without premedication directed to prevent hypersensitivity reactions. Furthermore, Cremophor EL is responsible for the non-linear pharmacokinetic behavior of i.v. paclitaxel [34-38]. It increases the affinity of paclitaxel to plasma components which results in a more than proportional increase in plasma paclitaxel levels with increasing doses. However, these higher total drug levels in plasma do not result in higher drug levels in tissues. This pseudo-non-linearity [38] of i.v. paclitaxel has two important implications for the pharmacology of oral paclitaxel. First of all, it will result in a significant underestimation of the true bioavailability of oral paclitaxel. In this study, the bioavailability of oral paclitaxel at a dose of 60 mg/m^2 was determined at 31%. In a dose-finding study performed by Huizing et al. [27], a mean AUC of i.v. paclitaxel at a dose of 100 mg/m^2 of 5.8 μM.h was reported. Re-calculation of the bioavailability of 60 mg/m^2 oral paclitaxel applying the dose-adjusted AUC found by Huizing et al., results in an oral bioavailability of 47% [16]. Second, the pseudo-non-linearity of i.v. paclitaxel implies that after oral administration, when Cremophor EL is not systemically present, plasma levels of paclitaxel represent a higher fraction of free drug, which will result in enhancement of the availability of paclitaxel for the (tumor) tissues [38]. Therefore, interpretation of differences between paclitaxel plasma levels after oral and i.v.

administration, without and with Cremophor EL in the systemic circulation, respectively, should be done with great caution.

In summary, the MTD of oral paclitaxel was 300 mg/m^2. However, because the pharmacokinetic data of oral paclitaxel, in particular at the highest doses applied, revealed non-linear pharmacokinetics with only a moderate further increase of the AUC with doses up to 300 mg/m^2, the oral paclitaxel dose of 180 mg/m^2 in combination with 15 mg/kg oral CsA is considered most appropriate for further investigation. The safety of the oral combination at this dose level was good. Additional studies will focus on multiple dose regimens and combinations with other P-gp inhibitors.

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CHAPTER 2.3

Pharmacokinetics of oral cyclosporin A when co-administered to enhance the oral absorption of paclitaxel

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Submitted

Abstract

Aims: To evaluate the pharmacokinetics of oral cyclosporin A (CsA) when co-administered to enhance the oral absorption of paclitaxel.
Methods: Patients received oral paclitaxel in doses of 60 to 360 mg/m^2 in combination with a dose of oral CsA of 15 mg/kg.
Results: Dose-escalation of paclitaxel from 60 to 300 mg/m^2 resulted in a significant decrease in the area under the concentration-time curve (AUC) of CsA from 24.4 ± 9.9 to 17.6 ± 2.8 mg.h/L (p=0.034) (n=28).
Conclusions: Increases in the paclitaxel dose resulted in a decrease in the AUC of CsA. This observation may be explained by the increase in the co-solvent Cremophor EL of paclitaxel causing reduced absorption of CsA.

Introduction

The oral bioavailability of the anticancer agent paclitaxel is very low, due to several factors one being a high affinity of the drug for the multidrug efflux pump P-glycoprotein (P-gp) abundantly present in the gastro-intestinal tract [1]. Recently we demonstrated the feasibility of oral administration of paclitaxel in cancer patients by co-administration of cyclosporin A (CsA), an efficacious inhibitor of P-gp. Co-administration of oral CsA resulted in a significant increase in the oral bioavailability of paclitaxel from less than 10% without CsA up to approximately 50% in combination with CsA [2,3]. Based on these first promising results we have investigated dose-escalation of paclitaxel in order to further increase the systemic exposure to oral paclitaxel [4]. Here we present the pharmacokinetic data of CsA of the latter study.

Patients and Methods

Study Design
Patients with a histologic proof of cancer for whom no standard therapy of proven benefit existed were eligible. Inclusion and exclusion criteria have been described in detail elsewhere [4]. In brief, patients had to have acceptable bone marrow, liver and renal function. Concomitant use of known P-gp inhibitors and chronic use of H2-receptor antagonists or proton pump inhibitors was not allowed. Patients received oral paclitaxel in doses of 60 to 300 mg/m^2 in combination with an oral CsA dose of 15 mg/kg. For oral paclitaxel administration the i.v. formulation was

used (Paxene®, Baker Norton Pharmaceuticals, Miami, FL). CsA (Neoral®, Novartis, Basel, Switzerland) was administered as an oral solution or as capsules, 10 and 30 minutes prior to paclitaxel intake, respectively. The capsules were preferred due to the bitter taste of the solution. Standard paclitaxel premedication was given to prevent hypersensitivity reactions and consisted of dexamethasone 20 mg orally 12 and 6 hours prior to, and clemastine 2 mg i.v. and cimetidine 300 mg i.v. 30 minutes prior to paclitaxel administration. Three patients (300 and 360 mg/m^2 dose levels) did not receive premedication because plasma levels of Cremophor EL, the co-solvent suspect of causing the hypersensitivity reactions [5], were undetectable after oral administration of paclitaxel. To prevent nausea and vomiting following oral intake of paclitaxel, which occurred more often at the higher paclitaxel dose levels, five patients (300 and 360 mg/m^2 dose levels) received oral granisetron (Kytril®) prior to CsA and paclitaxel administration. To further prevent nausea and vomiting, three patients (300 and 360 mg/m^2 dose levels) received a light breakfast at least 2 hours prior to oral paclitaxel administration. All other patients received CsA and paclitaxel after an overnight fast.

Pharmacokinetics

Blood samples for pharmacokinetic analysis of CsA were collected in heparinized tubes, pre-dose, 15, 30, 45, 60, 75, 90, 105 minutes and 2, 2.5, 3.5, 4.5, 7.5, 10.5, 24.5, 30.5 and 48.5 hours after ingestion of CsA. For CsA analysis whole blood samples were stored at 4ºC and analyzed within one week using a specific fluorescence polarization immuno assay (FPIA) (TDxFLx cyclosporin monoclonal whole blood assay, Abbott Laboratories, Amstelveen, The Netherlands) [6].

Non-compartmental pharmacokinetic methods were applied to process the results [7]. The area under the CsA concentration-time curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity using the terminal rate constant k. The terminal half-life (t1/2) was calculated as ln2/k. The maximal plasma concentration (Cmax) and the time to maximal plasma concentration (Tmax) were observed measured values. Statistical analysis of the CsA data was performed using the Pearson correlation coefficient. The a priori level of significance was p=0.05.

Results

Pharmacokinetics

Pharmacokinetic parameters of CsA (n=28) are outlined in Table 1. Dose-escalation of paclitaxel from 60 to 360 mg/m^2 resulted in a significant decrease in the Cmax and AUC of CsA (Cmax: p=0.002, r=-0.563 and AUC: p=0.034, r=-0.402)

(Figure 1). Cmax and AUC values of CsA in combination with paclitaxel 60 mg/m^2 were 3.10 ± 0.88 mg/L and 24.4 ± 9.9 mg.h/L, respectively and in combination with paclitaxel 300 mg/m^2 1.84 ± 0.31 mg/L and 17.6 ± 2.8 mg.h/L, respectively. At the paclitaxel dose level of 300 mg/m^2 administration of a light breakfast, premedication (dexamethasone, cimetidine and clemastine) or granisetron did not result in differences in the pharmacokinetics of CsA (individual data not shown).

Table 1. *Non-compartmental pharmacokinetics of CsA when co-administered with paclitaxel (pac). Data are presented as means ± SD.*

Pac dose (mg/m^2)	CsA dose (mg/kg)	No. Patients	AUC (mg.h/L)	Cmax (mg/L)	Tmax (h)	t1/2 (h)
Pac 60	15	7	24.4 ± 9.9	3.10 ± 0.88	3.2 ± 0.8	7.9 ± 2.5
Pac 120	15	2	30.4 ± 19.5	2.13 ± 0.53	3.3 ± 2.0	21.9 ± 10.7
Pac 180	15	6	22.2 ± 7.7	2.19 ± 0.58	2.6 ± 1.3	17.9 ± 5.1
Pac 210	15	3	16.4 ± 2.5	1.74 ± 0.25	1.6 ± 0.3	17.4 ± 1.2
Pac 250	15	3	13.4 ± 8.7	1.15 ± 0.38	3.0 ± 1.5	17.3 ± 3.4
Pac 300	15	6	17.6 ± 2.8	1.84 ± 0.31	2.0 ± 1.4	14.5 ± 1.6
Pac 360	15	1	17.3	2.70	0.9	16.3

Discussion

The pharmacokinetics of CsA were evaluated when co-administered with different doses (60-360 mg/m^2) of oral paclitaxel.

The hypothesis of CsA co-administration to enhance absorption of orally administered paclitaxel is based on inhibition of intestinal P-gp by CsA. In addition, inhibition of paclitaxel metabolism by CsA may also be important. Both CsA and paclitaxel are metabolized by cytochrome P450 (CYP) 3A4 [8,9]. Paclitaxel is along with CYP 3A4 metabolized by CYP 2C8 [9]. In our proof of concept study we observed altered paclitaxel metabolism following CsA co-administration with a relative decrease in formation of the CYP 3A4 mediated metabolite 3'p-hydroxypaclitaxel [3]. Following the latter theory, increases in paclitaxel dose could result in relatively more competitive inhibition of CsA metabolism and thus in higher levels of CsA. However, we found that increases in paclitaxel dose resulted in significant decreases in Cmax and AUC values of CsA. Apparently, in this study the potential effect of paclitaxel to competitively inhibit CsA metabolism is absent or

negligible. In our previously published manuscript about the paclitaxel pharmacokinetics in these patients [4], it was clearly shown that orally administered paclitaxel shows non-linear absorption pharmacokinetics with a decrease in oral bioavailability with an increase of dose. At the highest dose level of oral paclitaxel (300 mg/m^2), analysis of feces revealed data implying that the incomplete absorption of orally administered paclitaxel may be due to the co-solvent Cremophor EL [10]. We have subsequently shown in mice that increment of the amount of Cremophor EL with a constant paclitaxel dose causes a substantial reduction in the amount absorbed of orally administered paclitaxel [11]. A comparable phenomenon has been observed for vitamin K1, which showed an increase in the oral bioavailability when the conventional Cremophor EL-solubilized formulation was replaced by a mixed-micellar formulation [12,13]. Parallel with paclitaxel and vitamin K1, absorption of CsA might also be limited by the co-solvent Cremophor EL of the paclitaxel formulation. The decrease in CsA Cmax and AUC values with higher doses of paclitaxel may thus be due to the increase in the amount of co-administered Cremophor EL. One way to test this hypothesis is to evaluate new formulations of paclitaxel without the co-solvent Cremophor EL.

In conclusion, increases in the paclitaxel dose co-administered with a constant CsA dose resulted in a significant decrease in the Cmax and AUC values of CsA. This observation may be explained by the increase in the (paclitaxel) co-solvent Cremophor EL with higher paclitaxel dosages causing reduced absorption of CsA.

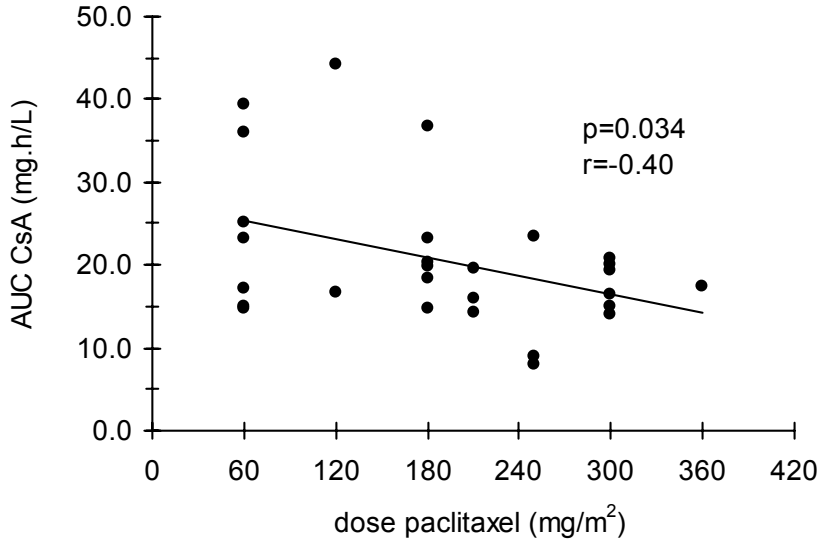

Figure 1. *AUC values of oral CsA versus dose of oral paclitaxel.*

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CHAPTER 2.4

Co-administration of GF120918 significantly increases the systemic exposure to oral paclitaxel in cancer patients

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Abstract

Purpose: Oral bioavailability of paclitaxel is very low, which is due to efficient transport of the drug by the intestinal drug efflux pump P-glycoprotein (P-gp). We have recently demonstrated that the oral bioavailability of paclitaxel can be increased at least 7-fold by co-administration of the P-gp blocker cyclosporin A (CsA). Now we tested the potent alternative orally applicable non-immunosuppressive P-gp blocker GF120918.
Patients and methods: Six patients received one course of oral paclitaxel of 120 mg/m^2 in combination with 1000 mg oral GF120918 (GG918, GW0918). Patients received intravenous (i.v.) paclitaxel 175 mg/m^2 as a 3-hour infusion during subsequent courses.
Results: The mean area under the plasma concentration-time curve (AUC) of paclitaxel after oral drug administration in combination with GF120918 was 3.27 ± 1.67 μM.h. In our previously performed study of 120 mg/m^2 oral paclitaxel in combination with CsA the mean AUC of paclitaxel was 2.55 ± 2.29 μM.h. After i.v. administration of paclitaxel the mean AUC was 15.92 ± 2.46 μM.h. The oral combination of paclitaxel with GF120918 was well tolerated.
Conclusion: The increase in systemic exposure to paclitaxel in combination with GF120918 is of the same magnitude as in combination with CsA. GF120918 is a good and safe alternative for CsA and may enable chronic oral therapy with paclitaxel.

Introduction

Paclitaxel is a potent anticancer drug with proven activity against a number of human solid tumors and has become standard treatment as single agent or in combination chemotherapy for the management of advanced breast, ovarian and non-small cell lung cancer [1,2]. The intravenous (i.v.) administration of paclitaxel is, however, inconvenient for patients and associated with significant and unpredictable side-effects. Severe hypersensitivity reactions have been observed after i.v. infusion of paclitaxel and it is now well established that the pharmaceutical vehicle Cremophor EL contributes largely to this effect [3,4].
Oral administration of paclitaxel is very attractive, because it is convenient and practical for patients and it may circumvent systemic exposure to the toxic vehicle Cremophor EL. Furthermore, oral administration may enable development of chronic treatment schedules resulting in sustained plasma concentrations above a pharmacological relevant threshold level. For paclitaxel a strong positive

relationship has been reported between duration of the paclitaxel plasma concentration above 0.05 μM or 0.1 μM and myelosuppression [5-7]. In addition, Huizing et al. [8] have found, in a retrospective phase I/II study in patients with advanced non-small cell lung cancer, a significant survival benefit in patients who had a exposure duration of paclitaxel above 0.1 μM of more than 15 hours compared with patients who had a shorter duration of exposure above this cut off level. However, these data need confirmation in a prospective study. In view of increasing costs of anticancer therapy, oral treatment of paclitaxel is attractive, as oral administration eliminates the need for hospitalization, physician and nursing assistance and infusion equipment.

Up to now, oral administration of paclitaxel has not appeared feasible because of the low oral bioavailability (<10%) of the drug. Preclinical studies in mice have shown that the low oral bioavailability is due to efficient transport of the drug by the multidrug efflux pump P-glycoprotein (P-gp) abundantly present in the gastro-intestinal tract [9,10]. This became clear when we investigated the oral uptake of paclitaxel in mdr1a knock-out mice, which lack functional P-gp activity in the gut [11]. This mouse model revealed significant bioavailability of orally administered paclitaxel. In wild-type mice good bioavailability of orally administered paclitaxel was achieved when the drug was combined with cyclosporin A (CsA) or the cyclosporin analogue SDZ PSC 833, both efficacious blockers of P-gp [12,13]. Based on our preclinical experiments we recently demonstrated the feasibility of oral administration of paclitaxel in cancer patients by concomitant administration of CsA. Co-administration of CsA resulted in a significant increase of at least 7-fold in the oral bioavailability of paclitaxel and plasma concentrations increased from negligible to therapeutic levels [14,15].

In this study we tested the P-gp blocker GF120918 in combination with oral paclitaxel. GF120918, an acridone carboxamide derivative, is a potent inhibitor of P-gp. In *in vitro* models of P-gp inhibition, GF120918 is active at concentrations around 20 nM, which is about 100 fold more potent than CsA [16]. Importantly, clinical studies of GF120918 with doses up to 1000 mg bid have shown no significant toxicities or side-effects of the drug [17,18]; it may therefore be a better candidate for clinical use, especially for repeated administration, than the immunosuppressive drug CsA.

Based on the promising results of our preclinical studies with oral paclitaxel plus GF120918, revealing an approximately 7-fold increase in the systemic exposure in wild-type mice [19], we initiated this clinical study of orally administered paclitaxel in combination with oral GF120918.

Patients and Methods

Patient Population

Patients with a histologically confirmed cancer refractory to current therapies were eligible for the study. Previous radiotherapy or chemotherapy other than taxoid therapy was allowed, provided that the last treatment was at least four weeks prior to study entry and any resulting toxicities were resolved. Eligibility criteria included acceptable bone marrow function (white blood cells > 3.0 x 10^9/L; platelets > 100 x 10^9/L), liver function (serum bilirubin $\leq$ 25 µmol/L; serum albumin $\geq$ 25 g/L), kidney function (serum creatinine $\leq$ 160 µmol/L or clearance $\geq$ 50 mL/min) and a World Health Organization (WHO) performance status $\leq$ 2. Patients were not eligible if they suffered from uncontrolled infectious disease, neurologic disease, bowel obstruction or symptomatic brain metastases. Other exclusion criteria were concomitant use of known P-gp inhibitors and chronic use of H2-receptor antagonists or proton pump inhibitors. The study protocol was approved by the Medical Ethics Committee of the Institute and all patients gave written informed consent.

Study Design

Six patients received at one occasion paclitaxel orally 120 mg/m^2 in combination with a single oral dose of GF120918 of 1000 mg. At another occasion patients received intravenous (i.v.) paclitaxel administered as a 3-hour infusion at a dose of 175 mg/m^2. The oral and i.v. course were randomized. If it was considered to be in their best interest patients continued on a 3-weekly schedule of i.v. paclitaxel. An oral paclitaxel dose of 120 mg/m^2 was selected for safety reasons because preclinical data of oral paclitaxel revealed that co-administration of a P-gp inhibitor and an oral paclitaxel dose can result in higher systemic exposure than after i.v. administration of the same dose [12]. An oral GF120918 dose of 1000 mg was selected because this dose was well tolerated and was expected to produce significant local P-gp blockade [17,18]. The i.v. formulation of paclitaxel (Taxol®, paclitaxel 6 mg/ml, dissolved in Cremophor EL and ethanol 1:1 v/v, Bristol-Myers Squibb, Syracuse, NY, USA) was used for both i.v. and oral administration of paclitaxel. GF120918 (GG918, GW0918, Glaxo Wellcome, Research Triangle Park, NC, USA) (100 mg tablets) was ingested one hour prior to oral paclitaxel administration. As the absorption of GF120918 is improved after intake of a meal, the drug was ingested 30 minutes after a standard light breakfast. Patients fasted for two hours following oral paclitaxel intake. To prevent nausea and vomiting patients received oral granisetron 1 mg approximately 1 hour prior to oral paclitaxel administration. At all i.v. occasions, patients were premedicated with

dexamethasone 20 mg orally 12 and 6 hours prior to, clemastine 2 mg i.v. 30 minutes prior to and cimetidine 300 mg i.v. shortly prior to paclitaxel administration.

Patient Evaluation

Pretreatment evaluation included a complete medical history and complete physical examination. Before each course, an interim history including concomitant medications taken, toxicities and performance status were registered and a physical examination was performed. Hematology was checked twice weekly after course 1 and 2 and weekly after subsequent courses. Blood chemistries including liver and renal function, serum electrolytes, total protein and albumin and glucose levels, were checked weekly. All toxicities observed were graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTC) [20]. Dose limiting toxicities (DLT) were defined as grade 4 granulocytopenia of a duration of > 5 days, grade 4 thrombocytopenia of any duration or any grade 3/4 non-hematological toxicity except untreated nausea and vomiting. Tumor measurements were performed every other cycle, but initially after the first 2 i.v. courses. Responses were evaluated according to the WHO criteria [21].

Sample Collection and Analysis

Blood samples for pharmacokinetic analyses were collected during course 1 and course 2. For plasma paclitaxel and metabolite concentrations, blood samples of 5 ml each were collected at 0, 15, 30, 45, 60, 75, 90 minutes and 2, 3, 4, 7, 10, 24, 30 and 48 hours after oral intake of paclitaxel. During i.v. administration of paclitaxel a previously established limited sampling model was applied using 2 plasma concentration-time points at 1 and 8 hours after the end of the 3-hour infusion [22]. Blood samples were centrifuged, plasma was separated and samples were immediately stored at -20ºC until analysis. Paclitaxel and metabolite concentrations were determined using a validated high performance liquid chromatography (HPLC) assay [23].

For GF120918 concentrations, blood samples of 7 ml each were collected on ice at 0, 30, 60, 90 minutes and 2, 3, 4, 5, 8, 11, 25, 31 and 49 hours after GF120918 intake. Blood samples were centrifuged at 4ºC, plasma was separated and samples were immediately stored at -20ºC until analysis. GF120918 concentrations were determined using a validated HPLC assay [24].

Pharmacokinetic Analysis

Non-compartmental pharmacokinetic methods were applied to process the results [25]. For orally administered paclitaxel, the maximal drug concentration (Cmax) and time to maximal drug concentration (Tmax) were obtained directly from the

experimental data. The area under the plasma paclitaxel concentration-time curve was calculated by the trapezoidal rule up to the last measured concentration-time point (AUCt) and extrapolated to infinity using the terminal rate constant k (AUC). The time above the threshold concentrations of 0.05 μM and 0.1 μM (T>0.05 μM, T>0.1 μM) was determined using linear interpolation. For i.v. administered paclitaxel the parameters AUC and T>0.1 μM were determined using our previously established limited sampling model [22]. Bioavailability of oral paclitaxel was calculated as the ratio of the AUC after oral and after i.v. administration with a correction for the difference in dose. Statistical analysis of the data was performed using the nonparametric Mann-Whitney U-test. The a priori level of significance was p=0.05.

Results

Patients and Treatment

A total of six patients (3 males/3 females) was enrolled in the study. At study entry the median age of the patients was 58 years (range 49 to 65) with a median performance status of 1 (range 0-1). Primary tumor types included breast (1), non-small cell lung cancer (NSCLC) (2) and adenocarcinoma of unknown primary site (3). Three patients received oral paclitaxel in combination with GF120918 during the first course and i.v. paclitaxel during course 2. The other three patients received i.v. paclitaxel during course 1 and oral paclitaxel in combination with GF120918 during course 2. During all subsequent courses patients received i.v. administered paclitaxel.

The oral combination of paclitaxel and GF120918 was very well tolerated. No significant side-effects were seen after one course of oral paclitaxel in combination with GF120918. Hematological toxicities after oral administration of paclitaxel consisted of anemia grade 1 (3 pts) and 2 (1 pt), which was often pre-existing, leukocytopenia grade 1 (1 pt) and 3 (1 pt) and granulocytopenia grade 2 (1 pt). Non-hematological toxicities after oral intake consisted of nausea grade 1 (1 pt) and 2 (1 pt), vomiting grade 2 (1 pt), arthralgia/myalgia grade 1 (2 pts), stomatitis grade 1 (1 pt), skin reactions grade 1 (1 pt), alopecia grade 1 (1 pt) and fatigue grade 2 (1 pt). Toxicities clearly associated with GF120918 administration were not observed. During subsequent treatment with i.v. paclitaxel a toxicity pattern common to paclitaxel developed with anemia, leukocytopenia, granulocytopenia, arthralgia/myalgia, nausea, vomiting, stomatitis, skin reactions, neurotoxicity and fatigue as main toxicities. In this study no tumor responses were observed.

Pharmacokinetics

Table 1 summarizes the main pharmacokinetic parameters of oral and i.v. administered paclitaxel. The mean AUC value of paclitaxel in patients who received oral paclitaxel in combination with GF120918 was 3.27 ± 1.67 μM.h. There was no statistically significant difference in the paclitaxel AUC values between the patients who started with oral paclitaxel and GF120918 and those who received the drugs during the second course. The mean plasma concentration-time curve of oral paclitaxel in combination with GF120918 is shown in Figure 1. After i.v. administration, the mean AUC value of paclitaxel was 15.92 ± 2.46 μM.h which is in good agreement with earlier data [5,8]. The oral bioavailability of paclitaxel, calculated as the AUC after oral administration (120 mg/m^2) divided by the AUC after i.v. administration (175 mg/m^2) with a correction for the difference in dose, was 30 ± 15%. However, because of the pronounced non-linear pharmacokinetics of i.v. paclitaxel [6,26], this calculation results in an underestimation of the true bioavailability. In a dose-finding study performed by Huizing et al. [8], a mean AUC of i.v. paclitaxel at a dose of 125 mg/m^2 of 6.8 μM.h was reported. Re-calculation of the bioavailability of 120 mg/m^2 orally administered paclitaxel applying the dose-adjusted AUC found by Huizing et al. [8] provides a value of 50% for the oral bioavailability of paclitaxel in combination with GF120918. The mean AUC and AUCt values of GF120918 were 13747 ± 9733 ng.h/mL and 9428 ± 5431 ng.h/mL, respectively. AUCt values have been calculated because of the high per cent of the AUC extrapolated in two patients, i.e. 56% and 54% of the area under the curve. The mean maximum concentration of GF120918 was 434 ± 267 ng/mL, which was reached at 7.7 ± 2.5 hours after intake.

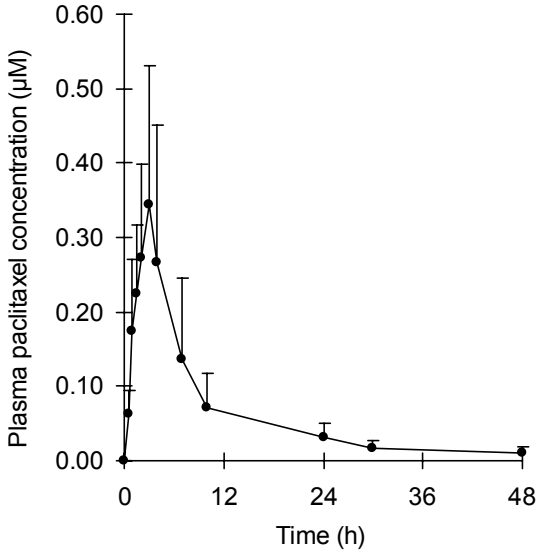

Figure 1. *Plasma concentration-time curve of oral paclitaxel at a dose of 120 mg/m^2 in combination with 1000 mg oral GF120918 (n=6). Data are represented as means ± SD.*

Table 1. *Pharmacokinetics of oral paclitaxel (120 mg/m^2) in combination with GF120918 (1000 mg) and i.v. paclitaxel (175 mg/m^2 administered as a 3-hour infusion).*

	oral paclitaxel							i.v. paclitaxel	
Patient	Course	AUC (μM.h)	Cmax (μM)	Tmax (h)	T>0.1 μM (h)	T>0.05 μM (h)	F (%)	AUC (μM.h)	T>0.1 μM (h)
1	2	4.56	0.48	3.0	8.8	15.1	36	18.59	22.3
2	1	4.36	0.33	3.0	15.3	25.0	49	13.01	12.1
3	1	5.23	0.62	3.0	9.6	23.9	41	18.59	20.9
4	2	1.11	0.17	1.9	2.6	3.9	11	14.90	13.8
5	2	1.92	0.20	3.1	4.2	6.1	17	16.87	16.6
6	1	2.41	0.33	3.0	4.3	6.7	26	13.54	13.9
Mean		3.27	0.36	2.8	7.5	13.5	30	15.92	16.6
SD		1.67	0.17	0.5	4.7	9.3	15	2.46	4.2

In Table 2 a comparison is made between the plasma pharmacokinetic parameters of paclitaxel after oral administration in combination with GF120918 and those of oral paclitaxel at the same dose but in combination with CsA. The latter data were taken from a study that has been performed previously at our Institute [27]. The mean paclitaxel AUC value in patients who received oral paclitaxel combined with GF120918 was 3.27 ± 1.67 μM.h and 2.55 ± 2.29 μM.h in patients who received oral paclitaxel in combination with CsA (not statistically significant).
For the paclitaxel metabolites 6a-hydroxypaclitaxel, 3'p-hydroxypaclitaxel and 6a,3'p-dihydroxypaclitaxel mean AUCt values after oral drug administration in combination with GF120918 (n=5) were 0.40 ± 0.36, 0.36 ± 0.39 and 0.24 ± 0.34 μM.h, respectively. Metabolite data of one patient could not be determined due to (unknown) interfering compounds in the analytical assay. After oral paclitaxel combined with CsA (n=3) these values were 1.69 ± 2.71, 0.48 ± 0.50 and 0.88 ± 1.48 μM.h, respectively (these metabolite data have not been published before). The AUCt ratio for the metabolites 6a-hydroxypaclitaxel and 3'p-hydroxypaclitaxel was 1.1 (0.40/0.36) after oral drug administration with GF120918, whereas this ratio was 3.5 (1.69/0.48) when paclitaxel was combined with CsA. AUCt values have been calculated because extrapolation of the AUC could not be performed properly due to erratic profiles and the limited time that the metabolites could be detected.

Table 2. *Pharmacokinetics of oral paclitaxel (120 mg/m^2) in combination with 1000 mg GF120918 and in combination with 15 mg/kg cyclosporin A [27].*

	GF120918 n=6	Cyclosporin A (CsA) n=3
AUC (μM.h)	3.27 ± 1.67	2.55 ± 2.29
Cmax (μM)	0.36 ± 0.17	0.31 ± 0.13
Tmax (h)	2.8 ± 0.5	3.7 ± 0.7
T>0.1 μM (h)	7.5 ± 4.7	7.9 ± 8.0
T>0.05 μM (h)	13.5 ± 9.3	13.0 ± 12.7

Discussion

We have recently demonstrated that the poor oral bioavailability of paclitaxel can be increased at least 7-fold by co-administration of the P-gp blocker CsA [14,15]. In this study we tested the potent alternative non-immunosuppressive P-gp blocker GF120918 in combination with oral paclitaxel.

The mean AUC value of paclitaxel achieved after oral administration in combination with GF120918 was 3.27 ± 1.67 μM.h, which is comparable to the AUC value achieved after oral paclitaxel administration in combination with CsA, i.e. 2.55 ± 2.29 μM.h [27]. Therefore, GF120918 is a good alternative for CsA in enhancing the oral bioavailability of paclitaxel. In both studies toxicities clearly related to the single dose administration of GF120918 or CsA were not observed. As the feasibility of oral paclitaxel administration will result in repeated dosing of either one of the P-gp blockers, the non-immunosuppressive agent GF120918 may be a better candidate for clinical use than the immunosuppressive drug CsA.

Preclinical studies of GF120918 with P-gp knock-out mice [19], which lack functional activity of P-gp, have shown similar pharmacokinetics of oral paclitaxel with or without co-administration of GF120918 and therefore indicate that the increase in systemic exposure to paclitaxel following GF120918 co-administration is solely due to blockade of P-gp. Our preclinical studies of CsA with wild-type mice [13], however, have shown higher AUC values of oral paclitaxel compared to those in P-gp knock-out mice without CsA [11], indicating interference of CsA in uptake and elimination pathways of orally administered paclitaxel other than mediated by P-gp. For CsA, an important factor that may contribute to the increase in systemic exposure to oral paclitaxel is inhibition of paclitaxel metabolism; both paclitaxel and CsA are substrates for the cytochrome P450 (CYP) 3A4 isozymes [28,29] (Fig. 2).

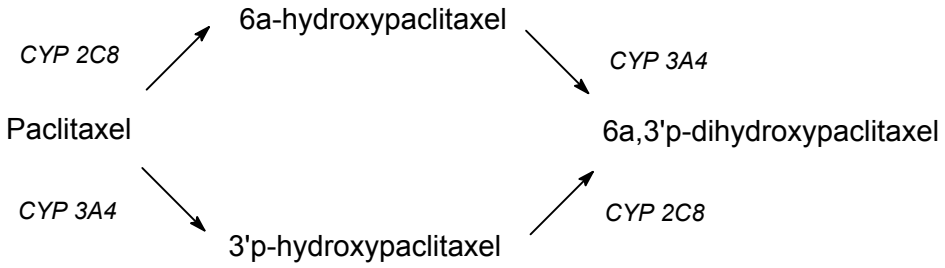

Figure 2. *Major metabolic pathways of paclitaxel.*

In our previous study of oral paclitaxel in combination with CsA [15] we found that after oral drug administration (60 mg/m^2) in combination with CsA the relative contribution of formation of the metabolite 3'p-hydroxypaclitaxel was substantially

lower than after i.v. administration, indicating inhibition of CYP 3A4 mediated paclitaxel metabolism by CsA. In the current study of oral paclitaxel in combination with GF120918 the AUCt ratio of 6a-hydroxypaclitaxel/3'p-hydroxypaclitaxel was 1.1, whereas this ratio was 3.5 when paclitaxel was combined with CsA, revealing a relative lower contribution of 3'p-hydroxypaclitaxel following CsA co-administration. These data also suggest inhibition of the CYP 3A4 mediated metabolic pathway of paclitaxel by CsA. Interpretation of the metabolite data should, however, be done with caution because of the small number of patients enrolled in each study and the very large interpatient variability in the metabolite data of paclitaxel. Furthermore, it is important to realize that inhibition of the CYP 3A4 mediated pathway will not necessarily result in prolonged exposure of active parent compound because drug not handled by CYP 3A4 might be handled by the CYP 2C8 pathway, which is, in general, the predominant metabolic pathway of paclitaxel.

In a control group of patients treated with oral paclitaxel (60 mg/m^2) but without co-administration of a P-gp blocker, a study which has been performed previously at our Institute, bioavailability of single agent oral paclitaxel was determined at 4% [14,15]. In the current study, the oral bioavailability of paclitaxel (120 mg/m^2) in combination with GF120918 is determined at 30%. However, because i.v. paclitaxel shows pronounced non-linear pharmacokinetics [6,26] these oral bioavailabilities, calculated using the AUC of i.v. paclitaxel at a dose of 175 mg/m^2, are underestimated. Using the pharmacokinetic data of i.v. paclitaxel at dose levels of 100 mg/m^2 and 125 mg/m^2 [8] at which less non-linearity is encountered, the apparent bioavailabilities are 6% for oral paclitaxel administered as a single agent [15] and 50% for orally administered paclitaxel in combination with GF120918.

An important pharmacokinetic parameter of paclitaxel is the time-period of exposure above a certain threshold concentration. Earlier data indicate a strong positive relationship between duration of the paclitaxel plasma concentration above 0.05 or 0.1 μM and pharmacological activity [5-8]. The feasibility of oral paclitaxel administration may enable the development of more chronic treatment schedules with sustained plasma concentrations above these pharmacological relevant threshold levels. However, it is important to discuss whether for orally administered paclitaxel these same threshold concentrations of 0.05 and 0.1 μM are relevant and should be pursued. Our previous studies of oral paclitaxel have shown that following oral administration of the drug the co-solvent Cremophor EL is not absorbed [14,15,27]. This is important, first of all, because systemic exposure to Cremophor EL can induce severe hypersensitivity reactions requiring extensive premedication [3,4]. Consequently, paclitaxel can be administered orally without premedication, which has been done in the current study and without complications. On the other hand, however, several studies demonstrated that

Cremophor EL is a potent modulator of multidrug resistance *in vitro*, and it has been hypothesized that this compound contributes to the clinical activity of paclitaxel [30,31]. However, the extremely low volume of distribution of Cremophor EL [32], the undetectable levels in (mouse) tissues [26], and the results in *in vivo* tumor-bearing models [33] suggest that this compound does not play a role in reversing P-gp mediated resistance to paclitaxel *in vivo*. Absence of systemic Cremophor EL after oral paclitaxel administration is also important because this compound is responsible for the non-linear pharmacokinetic behavior of i.v. paclitaxel [6,26]. Cremophor EL increases the affinity of paclitaxel to plasma components which results in a more than proportional increase in plasma paclitaxel levels with increasing doses. However, studies in mice show that these higher total drug levels in plasma do not result in higher drug levels in tissues [34]. This pseudo-non-linearity of i.v. paclitaxel [35] has two important implications for the pharmacology of oral paclitaxel. First, as mentioned in the discussion above, it will result in a significant underestimation of the true bioavailability of oral paclitaxel. Second, the pseudo-non-linearity of i.v. paclitaxel implies that after oral administration, when Cremophor EL is not systemically present, plasma levels of paclitaxel represent a higher fraction of free drug, which will result in enhancement of the availability of paclitaxel for the (tumor) tissues [35]. Consequently, the optimal value of the threshold level may be lower for orally administered paclitaxel compared to i.v. paclitaxel; this needs further confirmation. Thus, comparison of paclitaxel plasma levels after oral and i.v. administration, without and with Cremophor EL in the systemic circulation, respectively, should be done with caution.

In summary, the P-gp inhibitor GF120918 is a good alternative for CsA administration in enhancing the oral bioavailability of paclitaxel. Importantly, GF120918 has no known immunosuppressive activity such as CsA and may therefore be a better candidate for clinical use, especially for repeated administration, than CsA.

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CHAPTER 2.5

Metabolism and excretion of paclitaxel after oral administration in combination with cyclosporin A and after intravenous administration

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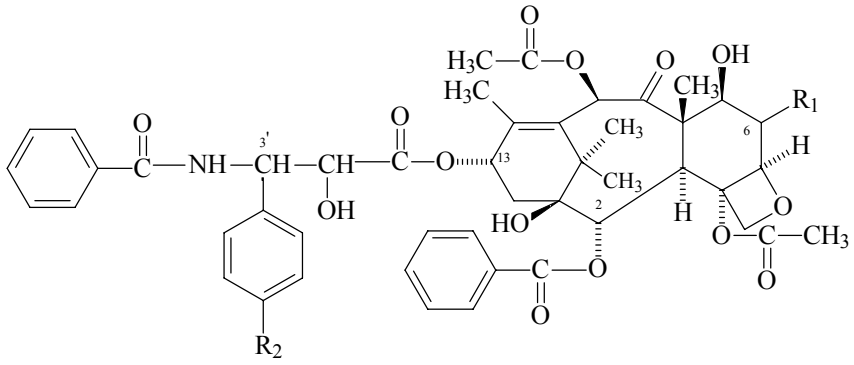
Abstract

The objective of this study was to compare the quantitative excretion of paclitaxel and metabolites after intravenous and oral drug administration.
Four patients received 300 mg/m^2 paclitaxel orally 30 minutes after 15 mg/kg oral cyclosporin A, co-administered to enhance the uptake of paclitaxel. Three weeks later these and three other patients received 175 mg/m^2 paclitaxel by intravenous infusion. Blood samples, urine and feces were collected up to 48 to 96 hours after administration and analyzed for paclitaxel and metabolites.
The area under the plasma concentration-time curve (AUC) of paclitaxel after intravenous administration (175 mg/m^2) was 16.2 ± 1.7 μM.h and after oral administration (300 mg/m^2) 3.8 ± 1.5 μM.h. Following intravenous infusion of paclitaxel total fecal excretion was 56 ± 25%, with the metabolite 6a-hydroxypaclitaxel being the main excretory product (37 ± 18%). After oral administration of paclitaxel total fecal excretion was 76 ± 21% in which paclitaxel accounted for 61 ± 14%.
In conclusion, after intravenous administration of paclitaxel, excretion occurs mainly in the feces with the metabolites as the major excretory products. Orally administered paclitaxel is also mainly excreted in feces but with the parent drug in highest amounts. We assume that this high amount of parent drug is due to incomplete absorption of orally administered paclitaxel from the gastro-intestinal tract.

Introduction

Paclitaxel is a potent anticancer drug with proven activity against a number of human solid tumors and has become standard treatment as single agent or in combination chemotherapy for the management of advanced breast, ovarian and non-small cell lung cancer [1,2]. The drug is currently administered intravenously (i.v.) at different dosages and time schedules and optimization of the clinical application is pursued. Elimination studies in man have shown that the primary route of elimination of i.v. administered paclitaxel occurs via hepatic metabolism and biliary excretion, whereas renal excretion is minimal [3,4]. In man, three major metabolic products of paclitaxel have been detected, i.e. 6a-hydroxypaclitaxel, 3'p-hydroxypaclitaxel and 6a,3'p-dihydroxypaclitaxel (Figure 1) [3,5,6]. The metabolite 6a-hydroxypaclitaxel is in general the principal metabolite. *In vitro* cytotoxicity studies have shown that all three metabolites are substantially less active than paclitaxel [5-7]. Biotransformation of paclitaxel is catalyzed by two cytochrome

P450 (CYP) isoenzymes. The formation of 6a-hydroxypaclitaxel is catalyzed by CYP 2C8, whereas the metabolite 3'p-hydroxypaclitaxel is formed by CYP 3A4 [8-10]. The dihydroxylated metabolite 6a,3'p-dihydroxypaclitaxel results from stepwise hydroxylations by CYPs 2C8 and 3A4 (Figure 2) [8,9].



Structure	R1	R2
Paclitaxel	H	H
6a-hydroxypaclitaxel	OH	H
3'p-hydroxypaclitaxel	H	OH
6a,3'p-dihydroxypaclitaxel	OH	OH

Figure 1. *Molecular structures of paclitaxel, 6a-hydroxypaclitaxel, 3'p-hydroxypaclitaxel and 6a,3'p-dihydroxypaclitaxel.*

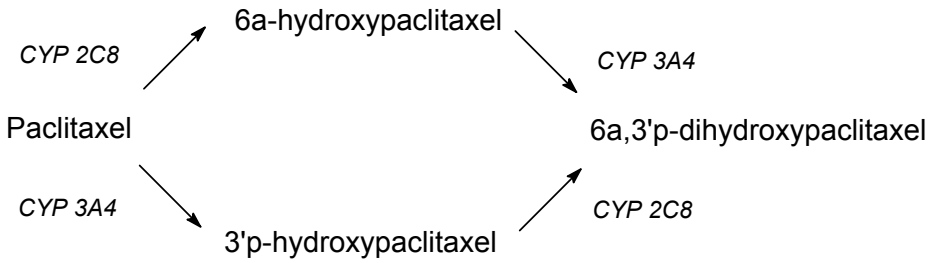


Figure 2. *Major metabolic pathways of paclitaxel.*

Recently, we reported on the oral administration of paclitaxel. Development of treatment of paclitaxel by the oral route has been limited due to its low oral bioavailability (below 10%). Preclinical studies at our Institute have shown that the low oral bioavailability of paclitaxel is, at least in part, due to paclitaxel's affinity for the multidrug efflux pump P-glycoprotein (P-gp), which is abundantly present in the

gastro-intestinal tract. In mdr1a P-gp knock-out mice, which lack functional P-gp activity in the gut, bioavailability of orally administered paclitaxel was increased up to 35% [11]. Because uptake of orally administered paclitaxel was complete, as was shown by the negligible amount of paclitaxel excreted in feces, it can be concluded that first pass metabolism is an important factor in the low oral bioavailability of paclitaxel as well. Additional studies in wild-type mice revealed a pronounced increase in the oral bioavailability of paclitaxel when the drug was combined with cyclosporin A (CsA), an efficacious blocker of P-gp and substrate/inhibitor for the CYP 3A4 metabolic enzymes [12]. Based on our preclinical studies we recently initiated a clinical proof of concept study of orally administered paclitaxel (60 mg/m^2) in combination with oral CsA (15 mg/kg). Co-administration of CsA resulted in a pronounced increase in the systemic exposure of orally administered paclitaxel and oral bioavailability of the drug increased from 6% for paclitaxel administered as a single agent up to 47% when the drug was combined with CsA [13,14]. The increase in systemic exposure by CsA was most likely caused by inhibition of P-gp and in addition, by inhibition of paclitaxel metabolism, as we observed altered paclitaxel metabolism following CsA administration [14]. Furthermore, CsA may have other unknown effects that may influence paclitaxel absorption.

In order to further increase the systemic exposure to orally administered paclitaxel we investigated dose-escalation of oral paclitaxel in combination with CsA [15]. At the maximum tolerated dose of 300 mg/m^2 oral paclitaxel, we studied the quantitative excretion of the drug and compared this with the quantitative excretion of i.v. administered paclitaxel.

Patients and Methods

Patient Population

Patients with a histologically confirmed cancer refractory to current therapies were eligible for the study. Previous radiotherapy or chemotherapy other than taxoid therapy was allowed, provided that the last treatment was at least four weeks prior to study entry and any resulting toxicities were resolved. Eligibility criteria included acceptable bone marrow function (white blood cells > 3.0 x 10^9/L; platelets > 100 x 10^9/L), liver function (serum bilirubin $\leq$ 25 µmol/L; serum albumin $\geq$ 25 g/L), kidney function (serum creatinine $\leq$ 160 µmol/L or clearance $\geq$ 50 mL/min), and a World Health Organization performance status $\leq$ 2. Patients were not eligible if they suffered from uncontrolled infectious disease, neurologic disease, bowel obstruction or symptomatic brain metastases. Other exclusion criteria were

concomitant use of known P-gp inhibitors and chronic use of H2-receptor antagonists or proton pump inhibitors. The study protocol was approved by the Medical Ethics Committee of the Institute and all patients gave written informed consent.

Dosage and Administration

Patients received 300 mg/m^2 paclitaxel orally 30 minutes after the oral administration of 15 mg/kg CsA. Three weeks later these patients received 175 mg/m^2 paclitaxel by a 3-hour i.v. infusion. Patients continued on a 3-weekly schedule of i.v. paclitaxel, if this was considered in their best interest. The i.v. formulation of paclitaxel (Paxene®; paclitaxel 6 mg/mL, dissolved in Cremophor EL and ethanol 1:1 w/v, Baker Norton Pharmaceuticals, Miami, FL, USA) was used for both i.v. and oral administration of paclitaxel. CsA was administered as capsules (Neoral®; Novartis, Basel, Switzerland). To prevent hypersensitivity reactions patients were premedicated with dexamethasone 20 mg orally 12 and 6 hours prior to, clemastine 2 mg i.v. and cimetidine 300 mg i.v. 30 minutes prior to both i.v. and oral paclitaxel administration. To prevent nausea and vomiting patients received 1 mg oral granisetron (Kytril®) prior to oral paclitaxel administration. In addition, two patients received a light breakfast at least 2 hours prior to oral drug administration. Intake of food was not allowed until 2 hours following oral administration of paclitaxel.

Sample Collection

Blood samples for pharmacokinetic analyses were collected during course 1 and 2. Following oral administration samples were obtained pre-dosing, at 15, 30, 45, 60, 75 and 90 minutes and 2, 3, 4, 7, 10, 24, 30 and 48 hours after paclitaxel ingestion. For i.v. administered paclitaxel, a previously established limited sampling model using 2 timed blood samples drawn at 1 and 8 hours post-infusion was used [16]. Blood samples were collected in heparinized tubes. For the analysis of paclitaxel and metabolites blood samples were centrifuged, plasma was separated and immediately stored at -20ºC until analysis. For CsA analysis, 1 mL of whole blood was transferred and stored at 4ºC until analysis. Urine was collected from 0-24 h and 24-48 h after paclitaxel administration. Samples were stabilized with a mixture of 5% Cremophor EL/ethanol 1:1 v/v to prevent paclitaxel precipitation and these samples were stored at -20ºC. The stools were collected in separate portions up to 4 days after dosing. The fecal samples were homogenized in 10 parts of water with a maximum of 2000 mL and aliquots of the suspension were stored at -20ºC.

Sample and Pharmacokinetic Analysis

Paclitaxel and metabolite concentrations in plasma, urine and feces were determined using validated high performance liquid chromatography (HPLC) assays [17,18,11]. All assays used 2'-methylpaclitaxel as the internal standard. Pretreatment of the plasma samples involved solid phase extraction (SPE) on Cyano Bond Elut columns. The concentrations of the plasma metabolic products 6a-hydroxypaclitaxel, 3'p-hydroxypaclitaxel and 6a,3'p-dihydroxypaclitaxel were determined using the paclitaxel standard curve with a correction of 1.14 for the metabolite 6a,3'p-dihydroxypaclitaxel [17]. Pretreatment of urine samples involved liquid-liquid extraction (LLE) with *n*-butylchloride [18]. Fecal samples were pretreated by LLE with diethyl ether followed by automated SPE using Cyano Bond Elut columns. Analysis of the fecal samples was analogous to the assay used by Sparreboom et al. [11] with minor modifications to make the assay more suitable for human feces. Further details and validation of the assay will be published elsewhere. The lower limit of quantitation for paclitaxel and metabolites was 10 ng/mL for plasma, 25 ng/mL for urine and 250 ng/mL for feces. CsA whole blood concentrations were analyzed using a specific fluorescence polarization immuno assay (FPIA, TDxFLx, Abbott Laboratories, Amstelveen, the Netherlands) [19]. The concentration of Cremophor EL in feces was measured after oral intake of paclitaxel using a validated HPLC assay [20] with minor modifications [21].

Non-compartmental pharmacokinetic methods were applied to process the results [22]. The maximal drug concentration (Cmax) and time to maximal drug concentration (Tmax) were obtained directly from the experimental data. The area under the concentration-time curve (AUC) was calculated by the trapezoidal rule up to the last measured time point with extrapolation to infinity using the terminal rate constant k. The time above the threshold concentration of 0.1 μM (T>0.1 μM) was determined using linear interpolation. The excretion of paclitaxel, metabolites and Cremophor EL in feces and urine was calculated relative to the administered dose. Renal clearance of paclitaxel (Cl_r) was calculated by dividing the amount of drug excreted in the urine by the plasma AUC. A statistical analysis of the data was performed using the Pearson correlation coefficient. The a priori level of significance was p=0.05.

Results

Patients and Treatment

Seven patients (four males and three females) were enrolled in the study. At study entry, the median age was 55 years (range 35-78) and the median performance score was 1 (range 0-2). Primary tumor types included breast (1), esophagus (1),

thymoma (1), gall bladder carcinoma (1) and adenocarcinoma of unknown primary site (3). All patients had received prior surgical therapy, radiotherapy and/or chemotherapy. Four patients received both oral and i.v. administered paclitaxel, three other patients received only i.v. administered paclitaxel.

Pharmacokinetics

Cumulative excretion profiles of paclitaxel and metabolites after i.v. and oral administration of paclitaxel are depicted in Figure 3. After both i.v. and oral administration, excretion of paclitaxel and metabolites occurred mainly in the feces, i.e. 56% (n=7) and 76% (n=4), respectively (Tables 1-3). In most of the patients (i.v. n=5 and oral n=4) more than 75% of the total fecal excretion was recovered within 2 days following administration. After i.v. administration, the main compound recovered in the feces was the metabolite 6a-hydroxypaclitaxel accounting for 37% of the administered dose. After oral administration, paclitaxel was mainly excreted as unchanged drug accounting for 61% of the administered dose. The amount of Cremophor EL recovered in feces after oral intake of paclitaxel was 32% of the administered Cremophor EL dose. The total fraction of Cremophor EL excreted in feces for each patient was significantly correlated with the total fraction of paclitaxel excreted in feces ($p=0.037$, $r=0.963$). Urinary excretion of paclitaxel after both i.v. and oral administration was minimal, i.e. 9% (n=6) and 1% (n=4) of the administered dose, respectively (Tables 1-3). Renal clearance (Cl_r) of paclitaxel was 1.1 ± 0.4 L/h/m^2 after i.v. administration and 1.1 ± 0.6 L/h/m^2 after oral drug intake. More than 80% of the total urinary excretion of paclitaxel after i.v. and oral administration occurred within 1 day. In urine samples no metabolites of paclitaxel could be detected.

Using our limited sampling model the calculated plasma AUC after i.v. administration of 175 mg/m^2 given by a 3-hour infusion was 16.2 ± 1.7 μM.h. After oral administration of 300 mg/m^2 paclitaxel in combination with 15 mg/kg CsA, the plasma AUC was 3.8 ± 1.5 μM.h (Table 4). For i.v. administered paclitaxel we could not determine pharmacokinetic parameters of the metabolites due to the fact that only two timed blood samples were drawn. For orally administered paclitaxel, plasma AUC(t) values of the metabolites 6a-hydroxypaclitaxel, 3'p-hydroxy-paclitaxel and 6a,3'p-dihydroxypaclitaxel were 1.5 ± 1.5, 1.0 ± 0.8 and 0.8 ± 0.8 μM.h, respectively. AUC(t) values have been calculated because extrapolation of the AUC could not be performed properly due to the limited detection time of the metabolites. Mean CsA whole blood pharmacokinetic parameters were: Cmax=1.9 ± 0.4 mg/L, AUC=18.8 ± 2.7 mg.h/L and Tmax=2.4 ± 1.5 h (n=4).

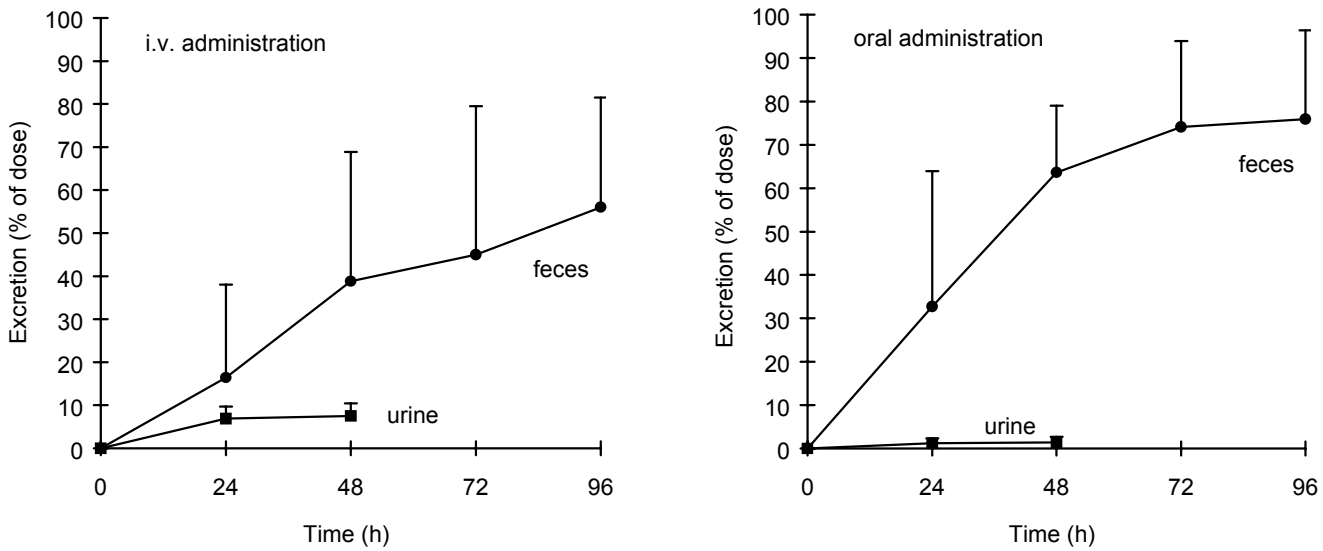


Figure 3. *Cumulative urinary and fecal excretion of paclitaxel and metabolites after i.v. administration (175 mg/m^2 as a 3-h infusion) and oral administration (300 mg/m^2) of paclitaxel.*

Table 1. *Urinary and fecal excretion of paclitaxel and metabolites (6a-HP, 6a-hydroxypaclitaxel; 3'p-HP, 3'p-hydroxypaclitaxel; 6a,3'p-DHP, 6a,3'p-dihydroxypaclitaxel) after intravenous administration of paclitaxel (175 mg/m^2) (n=7).*

Matrix	Urine	Feces	Feces	Feces	Feces
Patient	Paclitaxel (% of dose)	Paclitaxel (% of dose)	6a-HP (% of dose)	3'p-HP (% of dose)	6a,3'p-DHP (% of dose)
1	11.0	5.8	22.7	6.0	4.8
2	n.d.	8.7	33.7	5.4	4.7
3	9.0	11.8	41.6	4.2	5.3
4	13.5	3.4	9.5	1.3	1.8
5	7.5	13.6	62.8	4.4	6.2
6	6.4	13.5	51.3	8.4	12.1
7	3.4	8.4	36.5	3.5	2.2
Mean ± SD	8.5 ± 3.5	9.3 ± 3.9	36.9 ± 17.6	4.7 ± 2.2	5.3 ± 3.4

n.d. not determined due to loss of urine.

Table 2. *Urinary and fecal excretion of paclitaxel and metabolites and Cremophor EL after oral administration of paclitaxel (300 mg/m^2) (n=4).*

Matrix	Urine	Feces	Feces	Feces	Feces	Feces
Patient	Paclitaxel (% of dose)	Paclitaxel (% of dose)	6a-HP (% of dose)	3'p-HP (% of dose)	6a,3'p-DHP (% of dose)	Cremophor EL (% of dose)
1	3.4	54.1	12.7	3.7	3.5	31.0
2	0.4	68.0	5.5	1.0	0.7	33.8
3	0.8	76.0	18.9	3.2	4.3	39.8
4	1.1	45.6	5.0	0.6	1.0	23.1
Mean ± SD	1.4 ± 1.3	60.9 ± 13.6	10.5 ± 6.6	2.1 ± 1.6	2.4 ± 1.8	31.9 ± 6.9

Table 3. *Urinary and fecal excretion values of paclitaxel and metabolites following oral (P.O.) and intravenous (I.V.) administration of paclitaxel (mean ± SD).*

		P.O. 300 mg/m^2 (n=4)	I.V. 175 mg/m^2 (n=7)
Urine (% of dose)	Paclitaxel	1.4 ± 1.3	8.5 ± 3.5
Feces (% of dose)	Paclitaxel	60.9 ± 13.6	9.3 ± 3.9
	6a-HP	10.5 ± 6.6	36.9 ± 17.6
	3'p-HP	2.1 ± 1.6	4.7 ± 2.2
	6a,3'p-DHP	2.4 ± 1.8	5.3 ± 3.4
	Total	76.0 ± 20.6	56.2 ± 25.1

Table 4. *Plasma pharmacokinetic parameters of paclitaxel and metabolites after intravenous (175 mg/m^2) and oral administration (300 mg/m^2) (mean ± SD).*

		Paclitaxel	6a-HP	3'p-HP	6a,3'p-DHP
I.V.	AUC (μM.h)	16.2 ± 1.7	n.d.	n.d.	n.d.
(n=6)[a]	T> 0.1 μM (h)	22.3 ± 2.8	n.d.	n.d.	n.d.
P.O.	AUC (μM.h)	3.8 ± 1.5	1.5 ± 1.5[b]	1.0 ± 0.8[b]	0.8 ± 0.8[b]
(n=4)	T> 0.1 μM (h)	9.3 ± 4.7	n.d.	n.d.	n.d.
	Cmax (μM)	0.36 ± 0.17	0.15 ± 0.12	0.09 ± 0.05	0.08 ± 0.07
	Tmax (h)	3.8 ± 0.5	5.2 ± 2.0	6.0 ± 2.0	7.0 ± 0.1

n.d. not determined; [a]one patient was not evaluable because one sample of the limited sampling model was not taken; [b]AUC(t) values.

Discussion

Following i.v. administration of paclitaxel the major excretion route of paclitaxel and metabolites was feces, i.e. 56% of the administered dose. The major compounds detected in feces were the metabolites, i.e. 47% of the administered dose, of which 6a-hydroxypaclitaxel accounted for 37%. Extensive excretion of metabolites in feces supports the hypothesis that paclitaxel metabolism, especially biotransformation to 6a-hydroxypaclitaxel, followed by biliary excretion comprises an important elimination route of i.v. administered paclitaxel. Our results are in good agreement with those obtained by Walle et al. [4] who treated patients with radiolabelled paclitaxel (Taxol®) and extracted 59% of the administered radioactivity from feces of which 5% consisted of paclitaxel and 26% of the metabolite 6a-hydroxypaclitaxel. In that study total radioactivity recovered in feces

amounted up to 71%. Our preclinical studies in mice treated with i.v. administered paclitaxel also showed that a substantial fraction of the administered dose (26%) was excreted in feces as metabolites, however, the excretion of unchanged paclitaxel of 51% in feces was substantially higher than in humans [23]. Thus, although paclitaxel metabolism in mice qualitatively resembles that in humans, the drug is less extensively metabolized in mice than in humans.

Following oral paclitaxel administration in combination with the P-gp inhibitor CsA the major excretion route of paclitaxel and metabolites was also with feces, i.e. 76% of the administered dose. The major compound recovered in feces was paclitaxel, accounting for 61% of the administered dose. In our preclinical studies with oral paclitaxel in both wild-type and mdr1a P-gp knock-out mice we observed that the fecal excretion of paclitaxel decreased from 87% in wild-type mice to 2% in the mdr1a P-gp knock-out mice [11]. This large decrease in fecal excretion of paclitaxel suggests almost complete (re)uptake of the drug from the gastro-intestinal tract in P-gp knock-out mice. Thus, according to our preclinical studies we expected only a small fraction of the paclitaxel dose excreted in the feces instead of the observed 61%. We assume that the large amount of paclitaxel recovered in feces in our study is largely due to excretion of unabsorbed drug, which is supported by the lower plasma AUC values of orally administered paclitaxel (300 mg/m^2) compared to i.v. administered paclitaxel (175 mg/m^2), i.e. 3.8 and 16.2 μM.h, respectively. The plasma pharmacokinetic data of oral paclitaxel in the dose-escalation study [15], of which this excretion study was part, revealed significant increases in the paclitaxel AUC values when the dose was escalated, however, the increases in systemic exposure were disproportional with the increases in dose, suggesting incomplete absorption of the drug, which was more pronounced at the higher dose levels. In that study, we suggested that the incomplete absorption of orally administered paclitaxel was most likely caused by the poor aqueous solubility of paclitaxel in the gastro-intestinal tract. A second potential explanation we proposed was incomplete blockade of intestinal P-gp by CsA. Incomplete P-gp inhibition by CsA would necessitate the use of more potent P-gp modulators such as PSC 833 [24,25]. In this study, we propose a third possibility, i.e. entrapment of paclitaxel by Cremophor EL in the gastro-intestinal tract, which will hamper its release and may therefore lead to incomplete absorption. This hypothesis is supported by the large amounts of Cremophor EL that we detected in feces after oral intake of paclitaxel, i.e. 32% of the administered Cremophor EL dose, which are in contrast to the undetectable Cremophor EL levels in feces of the mdr1a P-gp knock-out mice after oral paclitaxel administration (unpublished data). Moreover, the total fraction of Cremophor EL excreted in feces for each patient was significantly correlated with the total fraction of paclitaxel excreted in feces

(p=0.037, r=0.963). Further research is warranted to get a better picture of the incomplete absorption of paclitaxel after oral ingestion. We are currently investigating the absorption of oral paclitaxel administered in a formulation without Cremophor EL.

Urinary excretion of paclitaxel was low after both i.v. and oral administration and accounted for 9% and 1% of the administered dose, respectively. Clearly, urinary excretion contributes minimally to the excretion of paclitaxel, as was shown in previous studies [3,4]. The lower urinary excretion fraction of paclitaxel after oral drug administration compared to i.v. administration can be explained by the incomplete absorption of orally administered paclitaxel. In addition, CsA may inhibit urinary paclitaxel excretion and may therefore contribute to the lower amount of the dose recovered in urine following oral administration. Lum et al. [26] found a 40% decrease in renal clearance of i.v. administered etoposide in combination with CsA compared to etoposide alone, which was presumed to be caused by inhibition of P-gp mediated drug transport in the kidneys. However, in this study, renal clearance of paclitaxel after i.v. and oral administration was comparable, suggesting that either P-gp is not a major factor in urinary excretion of paclitaxel or that P-gp in the renal tubule is not inhibited by CsA given at the current dose level.

The total urinary and fecal excretion of paclitaxel and the three metabolites 6a-hydroxypaclitaxel, 3'p-hydroxypaclitaxel and 6a,3'p-dihydroxypaclitaxel amounts to 65% and 77% of the administered i.v. and oral paclitaxel dose, respectively. One patient in this study (patient 4) suffered from obstipation, which resulted in incomplete feces collection following both i.v. and orally administered paclitaxel. If this patient is omitted, total recovery in the remaining patients becomes 71% and 86% of the administered i.v. and oral dose, respectively. Thus, in our study the majority of parent drug and metabolites after both i.v. and oral paclitaxel administration was recovered. The remaining unrecovered fraction of the administered dose may be lost due to incomplete urine and feces collection and/or metabolism to yet unidentified metabolites. Monsarrat et al. [3] detected five metabolites in human bile and Huizing et al. [27] found 11 putative metabolites of paclitaxel in human plasma.

In conclusion, paclitaxel given by i.v. infusion is mainly excreted in the feces with the hydroxylated metabolites as the major excretory products. Orally administered paclitaxel is also mainly excreted in the feces, but with the parent drug in highest amounts. We assume that the high amount of parent drug recovered in feces after oral administration is due to incomplete absorption from the gastro-intestinal tract, which may be due to paclitaxel's poor aqueous solubility, incomplete P-gp inhibition by CsA, entrapment of paclitaxel by Cremophor EL and/or other, yet unknown, factors.

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CHAPTER 2.6

The co-solvent Cremophor EL limits absorption of orally administered paclitaxel in cancer patients

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Submitted

Abstract

Purpose: To investigate the effect of the co-solvents Cremophor EL and polysorbate 80 on the absorption of orally administered paclitaxel.
Patients and methods: Six patients received in a randomized setting, one week apart oral paclitaxel 60 mg/m^2 dissolved in polysorbate 80 or Cremophor EL. For three patients the amount of Cremophor EL was 5 mL/m^2, for the other three 15 ml/m^2. Prior to paclitaxel administration patients received 15 mg/kg oral cyclosporin A to enhance the oral absorption of the drug.
Results: Paclitaxel formulated in polysorbate 80 resulted in a significant increase in the maximal concentration (Cmax) and area under the concentration-time curve (AUC) of paclitaxel in comparison with the Cremophor EL formulations (p=0.046 for both parameters). Compared to the 5 mL/m^2 Cremophor EL formulation, Cmax and AUC values were 1.5-fold higher; at the level of 15 mL/m^2 these differences were 3.9 and 3.2-fold respectively. Fecal data revealed a decrease in excretion of unchanged paclitaxel for the polysorbate 80 formulation compared to the Cremophor EL formulations. The amount of paclitaxel excreted in feces was significantly correlated with the amount of Cremophor EL excreted in feces (p=0.019).
Conclusion: The results show that the co-solvent Cremophor EL is an important factor limiting the absorption of orally administered paclitaxel from the intestinal lumen. They highlight the need for designing a better drug formulation in order to increase the usefulness of the oral route of paclitaxel.

Introduction

Paclitaxel is an important anticancer agent widely applied in the treatment of breast, ovarian and lung cancer and AIDS-related Kaposi's sarcoma [1,2]. The drug is marketed as an intravenous (i.v.) formulation consisting of 6 mg/mL paclitaxel dissolved in Cremophor EL:ethanol 1:1 v/v. Many different dosages and time schedules have been tested and further optimization of the clinical application is currently pursued. Recently, we reported about the oral route for administering paclitaxel to patients using the i.v. formulation as a drinking solution diluted with water [3,4]. This work was based on preclinical studies highlighting the important role of P-glycoprotein (P-gp) in the oral bioavailability of paclitaxel [5,6]. P-gp in the gut builds a barrier to many substrate xenotoxins and drugs, including paclitaxel. In patients, administration of 60 mg/m^2 paclitaxel with 15 mg/kg CsA, a competitive inhibitor of both P-gp and cytochrome P450 3A4, significantly increased the oral

bioavailability of paclitaxel by at least 7-fold and plasma concentrations rose from negligible to potentially therapeutic levels [3,4]. To further enhance the systemic exposure to paclitaxel we performed a dose-escalation study of oral paclitaxel in combination with CsA [7]. Although dose-escalation of oral paclitaxel from 60 to 300 mg/m^2 resulted in a significantly higher systemic exposure to paclitaxel, this increase was moderate and not proportional with dose. Similar results have been obtained by Rowinsky and co-workers [8]. A mass balance study, which was performed in patients receiving the highest dose level (300 mg/m^2), revealed that a high fraction of the dose was recovered in the feces as unchanged drug suggesting incomplete absorption [9]. Moreover, high amounts of the co-solvent Cremophor EL were also recovered in feces and the fractions of the dose of Cremophor EL and paclitaxel excreted in feces were significantly correlated [9]. We therefore hypothesized that Cremophor EL limits the absorption of paclitaxel by entrapment of the drug in the gastro-intestinal tract.

This hypothesis was recently substantiated in preclinical models using mdr1ab P-gp knock-out mice [10]. Cremophor EL given at dosages relevant to cancer patients resulted in considerably decreased paclitaxel plasma levels and substantially increased fecal excretion of unchanged paclitaxel. Based on these preclinical results, we initiated this clinical study in which each patient received 60 mg/m^2 of oral paclitaxel (in combination with 15 mg/kg of CsA) formulated in Cremophor EL 5 mL/m^2 or 15 mL/m^2 at one occasion and in polysorbate 80 at the other with pharmacokinetic monitoring at both occasions.

Patients and Methods

Patient Population

Patients with a histologically confirmed cancer refractory to current therapies were eligible for the study. Previous radiotherapy or chemotherapy was allowed, provided that the last treatment was at least four weeks prior to study entry and any resulting toxicities were resolved. Eligibility criteria included acceptable bone marrow (white blood cells > 3.0 x 10^9/L; platelets > 100 x 10^9/L), liver function (serum bilirubin $\leq$ 25 µmol/L; serum albumin $\geq$ 25 g/L), renal function (serum creatinine $\leq$ 160 µmol/L or clearance $\geq$ 50 mL/min) and a World Health Organization (WHO) performance status $\leq$ 2. Patients were not eligible if they suffered from uncontrolled infectious disease, neurologic disease, bowel obstruction or symptomatic brain metastases. Other exclusion criteria were concomitant use of known P-gp inhibitors and chronic use of H2-receptor antagonists or proton pump inhibitors. The study protocol was approved

by the Medical Ethics Committee of the Institute and all patients gave written informed consent.

Study Design

Six patients received at two occasions, which were one week apart and randomized, oral paclitaxel at a dose of 60 mg/m^2 formulated in Cremophor EL/ethanol and the same oral paclitaxel dose formulated in polysorbate 80/ethanol. Two cohorts of each three patients were made, one of which received Cremophor EL 5 mL/m^2 in the oral paclitaxel formulation and the other Cremophor EL 15 mL/m^2. The polysorbate 80 formulation was the same between the two cohorts. Prior to oral paclitaxel intake patients received 15 mg/kg oral CsA.

For the oral paclitaxel formulation with Cremophor EL (5 and 15 mL/m^2), the standard i.v. formulation of paclitaxel was used (Taxol®; 6 mg/mL paclitaxel in Cremophor EL:ethanol 1:1 v/v). Three of the six patients received additional Cremophor EL (BASF, Brussels, Belgium) of 10 mL/m^2 to this formulation. The polysorbate 80 formulation was made similar to the i.v. Cremophor EL formulation of paclitaxel with replacement of Cremophor EL by polysorbate 80 (6 mg/mL paclitaxel in polysorbate 80:ethanol 1:1 v/v). To all formulations 25 mL of water was added to decrease viscosity. Paclitaxel was retrieved from Hauser, Inc (Boulder, USA), polysorbate 80 from Kolb (Hedingen, Switzerland). CsA was administered as capsules (Neoral® Novartis, Basel, Switzerland; base: corn oil, polyoxyl 40 hydrogenated castor oil) 30 minutes prior to oral intake of paclitaxel.

An oral paclitaxel dose of 60 mg/m^2 was chosen for safety reasons. As we expected that the oral bioavailability of paclitaxel formulated in polysorbate 80 would approach the bioavailability of orally administered docetaxel, i.e. 90% [11], a dose of 60 mg/m^2 oral paclitaxel administered within a time period of 2 weeks was considered to be therapeutic and safe.

To prevent nausea and vomiting patients received 1 mg oral granisetron (Kytril®) approximately 2 hours prior to oral paclitaxel administration. In addition, patients received a light standard breakfast (2 crackers and a cup of tea) at least 2 hours prior to oral drug administration. Intake of food was not allowed until 2 hours following intake of paclitaxel.

Two weeks after the second oral course of paclitaxel, patients received i.v. paclitaxel (Taxol®) administered as a 3-hour infusion at a dose of 175 mg/m^2. If it was considered to be in their best interest patients continued on a 3-weekly schedule of i.v. paclitaxel. At the i.v. occasions, patients were premedicated to prevent hypersensitivity reactions with dexamethasone 20 mg orally 12 and 6 hours prior to, clemastine 2 mg i.v. 30 minutes prior to and cimetidine 300 mg i.v. shortly prior to paclitaxel administration. Oral doses were given without this premedication regimen

as previous studies of oral paclitaxel have revealed that the co-solvent Cremophor EL, suspect of causing the hypersensitivity reactions [12], was not absorbed following oral administration of paclitaxel [3,4,7].

Sample Collection

Blood samples for pharmacokinetic analyses were collected during the two oral courses. Samples were obtained in heparinized tubes pre-dosing, at 15, 30, 45, 60, 75 and 90 minutes and 2, 3, 4, 7, 10, 24, and 30 hours after paclitaxel ingestion. For the analysis of paclitaxel, blood samples were centrifuged, plasma was separated and immediately stored at -20ºC until analysis. For CsA analysis, 1 mL of whole blood was transferred, stored at 4ºC and analyzed within one week after treatment. Urine was collected from 0-24 h and 24-30 h after paclitaxel administration. Samples were stabilized with a mixture of 5% Cremophor EL/ethanol 1:1 v/v to prevent paclitaxel precipitation and these samples were stored at -20ºC until analysis. The stools were collected in separate portions up to 6 days after dosing. The stools collected up to 30 hours after dosing were immediately stored at -20ºC, the stools collected from 30 hours up to 6 days after dosing were stored at the patients home and were frozen at day 6 at -20ºC. After defrosting, the fecal samples were homogenized in 10 parts of water, with a maximum of 2000 mL, and aliquots of the suspension were stored at -20ºC until analysis.

Sample Analysis

Paclitaxel and metabolite concentrations in plasma, urine and feces were determined using validated high performance liquid chromatography (HPLC) assays [13-15]. All assays used 2'-methylpaclitaxel as the internal standard. Pretreatment of the plasma samples involved solid phase extraction (SPE) on Cyano Bond Elut columns. Pretreatment of urine samples involved liquid-liquid extraction (LLE) with *n*-butylchloride. Fecal samples were pretreated by LLE with diethyl ether followed by automated SPE using Cyano Bond Elut columns. The lower limit of quantitation for paclitaxel and metabolites was 10 ng/mL for plasma, 25 ng/mL for urine and 250 ng/mL for feces homogenates. CsA whole blood concentrations were analyzed using a specific fluorescence polarization immunoassay (FPIA, Abbott Laboratories, Amstelveen, The Netherlands) [16]. The concentration of Cremophor EL in feces was measured using a validated HPLC assay [17] with minor modifications and corrections for the presence of liberated free ricinoleic acid [18].

Pharmacokinetic Analysis

Non-compartmental pharmacokinetic methods were applied to process the results [19]. The maximal drug concentration (Cmax) and time to maximal drug concentration (Tmax) were obtained directly from the experimental data. The area under the concentration-time curve (AUC) was calculated by the trapezoidal rule up to the last measured time point (AUCt) with extrapolation to infinity using the terminal rate constant k. The excretion of paclitaxel, metabolites and Cremophor EL in feces and urine was calculated relative to the administered dose. Statistical analysis of the data was performed using the nonparametric Wilcoxon matched-pairs signed-rank test. The a priori level of significance was p=0.05.

Results

Formulation in polysorbate 80 resulted in significantly higher Cmax and AUC values of paclitaxel in comparison with the Cremophor EL formulations (n=6; p=0.046 for both parameters) (Figures 1-2, Table 1). Compared to the 5 ml/m^2 Cremophor EL formulation, Cmax and AUC values were 1.5-fold higher, whereas these differences were 3.9 and 3.2-fold, respectively with 15 ml/m^2 Cremophor EL. Tmax values of paclitaxel were significantly lower with the polysorbate 80 formulation compared to the Cremophor EL formulations (n=6; p=0.046).

Formulation of paclitaxel in polysorbate 80 also resulted in significantly higher Cmax and AUC values of CsA when compared to the Cremophor EL formulations (n=6; p=0.028 for both parameters) (Figure 3, Table 2). Compared to 5 ml/m^2 Cremophor EL, Cmax and AUC values of CsA were 1.4 and 1.3-fold higher, whereas these differences were 1.5 and 1.4-fold, respectively with 15 mL/m^2 Cremophor EL. Tmax values of CsA were not significantly different between the two paclitaxel formulations.

Excretion of unchanged paclitaxel in feces was lower in case of paclitaxel being formulated in polysorbate 80 compared to Cremophor EL (Figure 4, Table 3). However, these differences did not reach statistical significance (n=6; p=0.115). Importantly, in two patients receiving the Cremophor EL formulation, feces collection was incomplete, resulting in relative low amounts of paclitaxel excreted in feces. Excretion of the metabolite 6a-hydroxypaclitaxel was significantly higher with the polysorbate 80 formulation (n=6; p=0.046). Excretion of the metabolites 3'p-hydroxypaclitaxel and 6a,3'p-dihydroxypaclitaxel was not different between the two formulations. The amount of Cremophor EL excreted in feces was 10.3 ± 4.9% of the administered dose for the 5 mL/m^2 group and 20.9 ± 16.0% of the administered dose for the 15 mL/m^2 group. The total fraction of Cremophor EL excreted in feces

was significantly correlated with the amount of paclitaxel excreted in feces (p=0.019, r=0.886) (n=6).

Excretion of orally administered paclitaxel in urine was minimal, as observed previously [7,9]. Urinary excretion of paclitaxel was 2.4 ± 1.1% of the administered dose for the polysorbate 80 formulation (n=6), 2.1 ± 0.7% of the administered dose for the 5 mL/m^2 Cremophor EL formulation (n=3) and 2.6 ± 0.7% of the administered dose for the 15 mL/m^2 Cremophor EL formulation (n=2). Of one patient urine collection was incomplete due to loss of urine during collection.

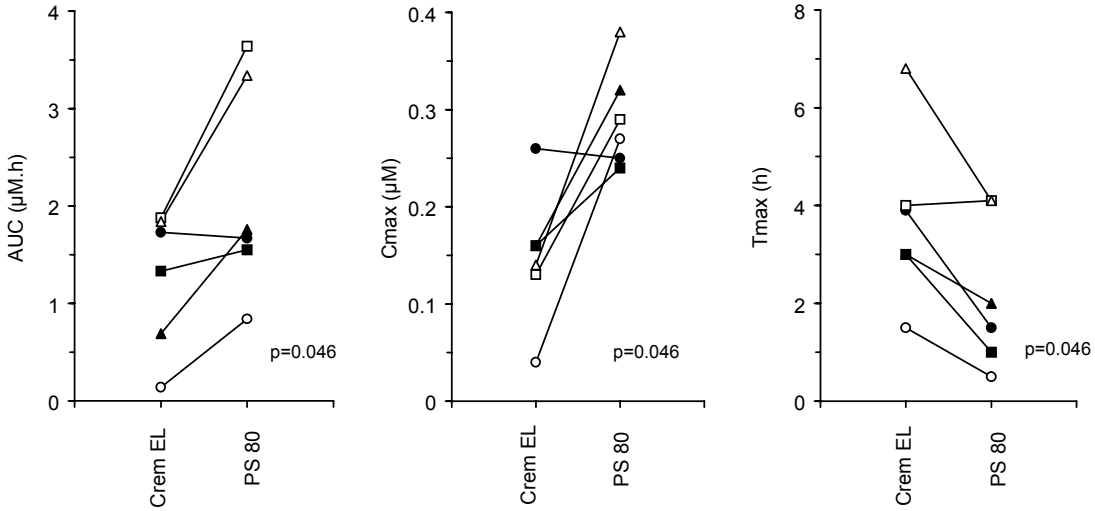

Figure 1. *Pharmacokinetic parameters of paclitaxel formulated in Cremophor EL (Crem EL) 5 mL/m^2 (closed symbols) or 15 mL/m^2 (open symbols) and polysorbate 80 (PS 80).*

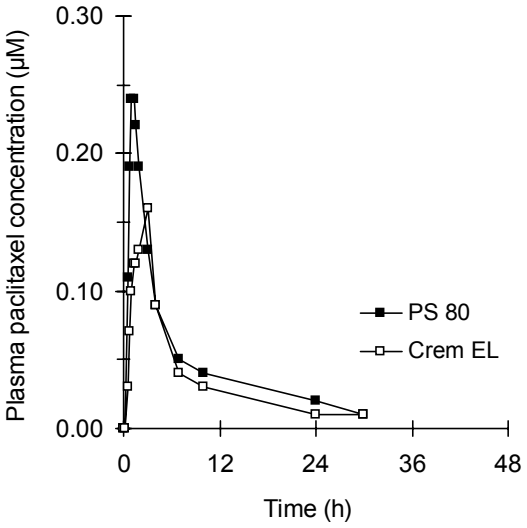

Figure 2. *Individual paclitaxel plasma concentration-time curves of a patient receiving oral paclitaxel formulated in Cremophor EL (Crem EL) 5 mL/m^2 and polysorbate 80 (PS 80).*

Table 1. *Pharmacokinetic parameters of oral paclitaxel (60 mg/m^2) formulated in polysorbate 80 (PS 80) and Cremophor EL (Crem EL) 5 mL/m^2 (cohort 1) or 15 mL/m^2 (cohort 2). Data are presented as means ± SD.*

Cohort	AUC (µM.h)		Cmax (µM)		Tmax (h)	
	Crem EL	PS 80	Crem EL	PS 80	Crem EL	PS 80
1	1.25	1.66	0.19	0.27	3.3	1.5
	(0.52)	(0.11)	(0.06)	(0.04)	(0.5)	(0.5)
2	1.29	2.61	0.10	0.31	4.1	2.9
	(0.99)	(1.54)	(0.06)	(0.06)	(2.7)	(2.1)

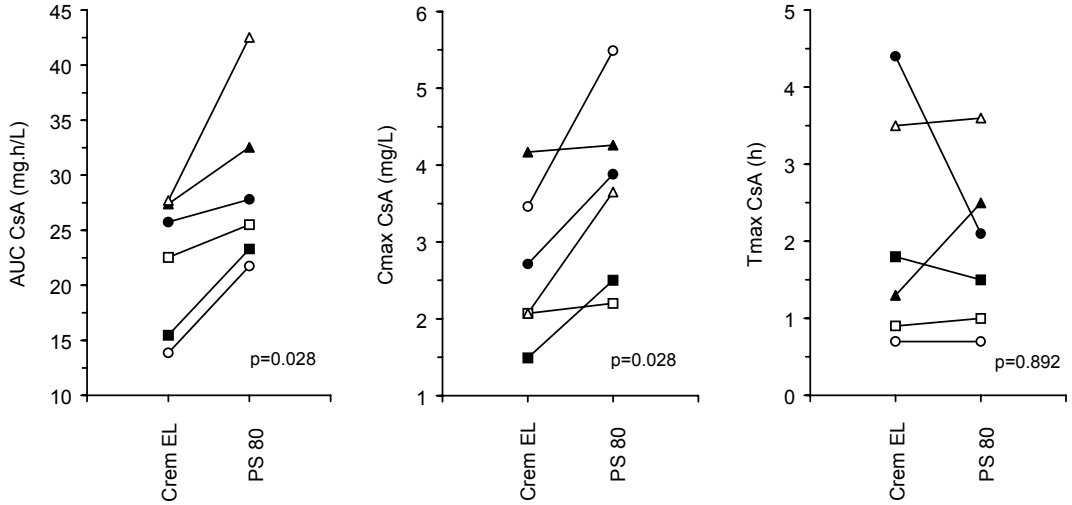

Figure 3. *Pharmacokinetics of cyclosporin A when co-administered to oral paclitaxel formulated in Cremophor EL (Crem EL) 5 mL/m^2 (closed symbols) or 15 mL/m^2 (open symbols) and polysorbate 80 (PS 80).*

Table 2. *Pharmacokinetic parameters of oral cyclosporin A (15 mg/kg) co-administered to oral paclitaxel (60 mg/m^2) formulated in polysorbate 80 (PS 80) and Cremophor EL (Crem EL) 5 mL/m^2 (cohort 1) or 15 mL/m^2 (cohort 2). Data are presented as means ± SD.*

Cohort	AUC (mg.h/L)		Cmax (mg/L)		Tmax (h)	
	Crem EL	PS 80	Crem EL	PS 80	Crem EL	PS 80
1	22.9	27.9	2.79	3.54	2.5	2.0
	(6.5)	(4.6)	(1.34)	(0.93)	(1.7)	(0.5)
2	21.4	29.9	2.53	3.78	1.7	1.8
	(7.0)	(11.1)	(0.80)	(1.65)	(1.6)	(1.6)

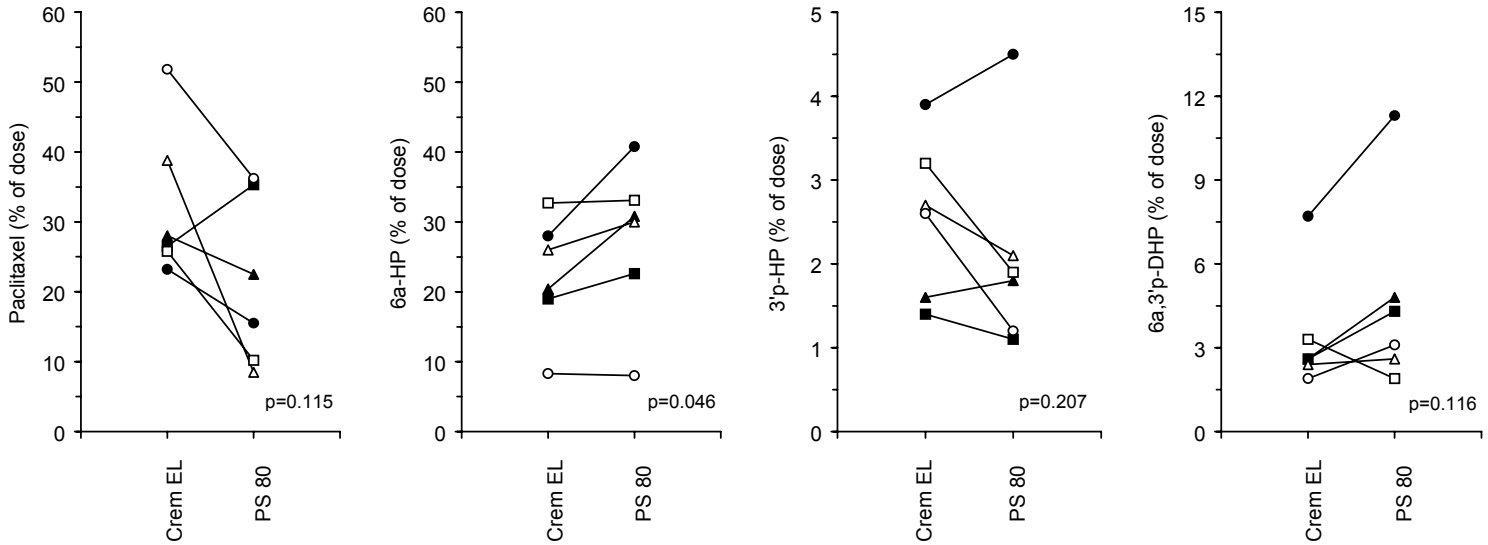

Figure 4. *Fecal excretion of paclitaxel and the metabolites 6a-hydroxypaclitaxel (6a-HP), 3'p-hydroxypaclitaxel (3'p-HP) and 6a,3'p-dihydroxypaclitaxel (6a,3'p-DHP) after oral paclitaxel administration in Cremophor EL (Crem EL) 5 mL/m^2 (closed symbols) or 15 mL/m^2 (open symbols) and polysorbate 80 (PS 80).*

Table 3. *Fecal excretion of paclitaxel and the metabolites 6a-hydroxypaclitaxel (6a-HP), 3'p-hydroxypaclitaxel (3'p-HP) and 6a,3'p-dihydroxypaclitaxel (6a,3'p-DHP) after oral paclitaxel administration (60 mg/m^2) formulated in polysorbate 80 (PS 80) and Cremophor EL (Crem EL) 5 mL/m^2 (cohort 1) or 15 mL/m^2 (cohort 2). Data are presented as means ± SD.*

Cohort	Paclitaxel (% of dose)		6a-HP (% of dose)		3'p-HP (% of dose)		6a,3'p-DHP (% of dose)		Total recovery (% of dose)	
	Crem EL	PS 80	Crem EL	PS 80	Crem EL	PS 80	Crem EL	PS 80	Crem EL	PS 80
1	25.9	24.4	22.5	31.4	2.3	2.5	4.3	6.8	55.0	65.1
	(2.5)	(10.0)	(4.8)	(9.1)	(1.4)	(1.8)	(2.9)	(3.9)	(7.0)	(6.2)
2	38.8	18.3	22.3	23.7	2.8	1.7	2.5	2.5	66.4	46.2
	(13.0)	(15.5)	(12.6)	(13.7)	(0.3)	(0.5)	(0.7)	(0.7)	(3.0)	(2.8)

Discussion

The results of this study show that the presence of Cremophor EL in the i.v. formulation of paclitaxel used orally reduces the absorption of paclitaxel from the gut. Formulation of paclitaxel in polysorbate 80 resulted in a significant increase in the Cmax and AUC values of paclitaxel. The excretion of unchanged paclitaxel in feces was substantially lower for the polysorbate 80 formulation and indicates an improved oral uptake. At the same time, excretion of the major paclitaxel metabolite 6a-hydroxypaclitaxel was significantly increased, which is also indicative of increased absorption of the drug. The relationship between reduction in the absorption of paclitaxel and Cremophor EL was evident. The amount of paclitaxel excreted in feces was significantly correlated with the amount of Cremophor EL excreted in feces. Furthermore, differences in the amount of paclitaxel excreted in feces between the two formulations were more pronounced for cohort 2 (15 mL/m^2 Cremophor EL) than cohort 1 (5 mL/m^2 Cremophor EL). Differences between the two cohorts were also seen in the paclitaxel plasma pharmacokinetic data. This implies that the effect of Cremophor EL in limiting the absorption of orally administered paclitaxel increases with dose. This is supported by the results of our previous study of oral paclitaxel at a dose of 300 mg/m^2 (corresponding with 25 mL/m^2 Cremophor EL), revealing a very high recovery of paclitaxel in feces of 61% of the administered dose [9]. Interestingly, Cremophor EL of the paclitaxel formulation also reduced the absorption of CsA. Significantly higher Cmax and AUC values of CsA were observed when paclitaxel was formulated in polysorbate 80 rather than in Cremophor EL. This result is in line with those obtained in our previously performed dose-escalation study of oral paclitaxel given together with a constant dose of CsA [7]. Apparently, absorption of orally administered paclitaxel and CsA are influenced by Cremophor EL by the same mechanism.

The results of this clinical study are in good agreement with our preclinical data [10]. In mdr1ab P-gp knock-out mice, receiving 10 mg/kg paclitaxel in the standard formulation, only about 7% of the dose was excreted in feces as unchanged drug, suggesting almost complete absorption from the gastro-intestinal tract. However, when the dose of Cremophor EL was increased by 7-fold, fecal excretion of unchanged drug increased to 35% of the dose. Moreover, the plasma Cmax and AUC values of paclitaxel were 4- and 1.6-fold lower, respectively. For reasons of availability our preclinical and clinical studies with oral paclitaxel performed thus far have used the standard i.v. formulation of paclitaxel. Cremophor EL, a mixture of polyoxyethylated triglycerides, is an essential compound in this formulation used to solubilize paclitaxel in aqueous dilutions by formation of micelles, which include the drug molecules within their hydrophobic core. After oral paclitaxel administration,

Cremophor EL was assumed to be degraded in the gastro-intestinal tract as paclitaxel and Cremophor EL levels recovered in feces of mdr1a P-gp knock-out mice were very low [5,10]. However, in our clinical study of oral paclitaxel 300 mg/m^2, a substantial fraction of the dose of Cremophor EL, i.e. 32%, was recovered in feces together with 61% of the dose of paclitaxel, indicative for incomplete degradation of Cremophor EL and poor uptake of paclitaxel [9]. By use of an *in vitro* assay we have shown that micelles are being formed in the intestines of mice at Cremophor EL concentrations of 0.33% w/v and higher [10]. With the addition of extra Cremophor EL to mdr1ab P-gp knock-out mice, the levels of Cremophor EL in the intestinal contents were approximately 10-fold higher. It could then be concluded that the mechanism of interaction between paclitaxel and Cremophor EL rests on the property of Cremophor EL to form micelles, which entrap paclitaxel thus reducing the availability of paclitaxel for uptake [10]. In line with this hypothesis it is likely that the oral bioavailability of CsA is similarly affected.

The selection of polysorbate 80 as vehicle used to replace Cremophor EL was based on the following considerations 1) the very good oral bioavailability of docetaxel, a taxane drug formulated in polysorbate 80/ethanol [11] and 2) the rapid degradation of polysorbate 80 by esterases in plasma [20]. Initially, we planned to formulate paclitaxel in polysorbate 80 similar to docetaxel (Taxotere®) with 20 mg drug per 0.5 mL polysorbate 80 and 1.5 mL ethanol 13% g/g. However, no clear solution of paclitaxel could be made. Therefore, it was decided to formulate paclitaxel in polysorbate 80 similar to the i.v. Cremophor EL formulation with 6 mg drug per 0.5 mL polysorbate 80 and 0.5 mL absolute ethanol. This formulation was clear and appeared feasible. Paclitaxel formulated in polysorbate 80 resulted in a mean paclitaxel excretion in feces of 21%, which suggests incomplete uptake of the drug from the gut. We previously determined that after i.v. administration of paclitaxel (175 mg/^{2}) only 9% of the drug was recovered as unchanged paclitaxel in feces [9]. The incomplete uptake of oral paclitaxel formulated in polysorbate 80 may be caused by the relative high amount of polysorbate 80, which may have, to a certain extent, similar capabilities as Cremophor EL of forming micelles, especially at higher concentrations. We are currently investigating a new formulation of oral paclitaxel with a different solvent, which may shed more light on this issue.

In conclusion, the results show that the co-solvent Cremophor EL is an important factor limiting the absorption of orally administered paclitaxel from the intestinal lumen, in particular at the higher dose levels. Development of a better, non-Cremophor EL based drug formulation is needed in order to increase the usefulness of the oral route of paclitaxel.

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CHAPTER 3.1

A phase I and pharmacokinetic study of bi-daily dosing of oral paclitaxel in combination with cyclosporin A

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Abstract

Purpose: to investigate dose-escalation of bi-daily (bid) oral paclitaxel in combination with cyclosporin A in order to improve and prolong the systemic exposure to paclitaxel and to explore the maximum tolerated dose (MTD) and dose limiting toxicity (DLT) of this combination.
Patients and methods: A total of 15 patients received during course 1 two doses of oral paclitaxel (2x 60, 2x 90, 2x 120 or 2x 160 mg/m^2) 7 hours apart in combination with 15 mg/kg of cyclosporin A, co-administered to enhance the absorption of paclitaxel. During subsequent courses patients received 3-weekly intravenous paclitaxel at a dose of 175 mg/m^2 as a 3-h infusion.
Results: Toxicities observed following bid dosing of oral paclitaxel were generally mild and consisted of toxicities common to paclitaxel administration and mild gastro-intestinal toxicities such as nausea, vomiting and diarrhea, which occurred more often at the higher dose levels. Dose-escalation of bid oral paclitaxel from 2x 60 to 2x 160 mg/m^2 did not result in a significant increase in the area under the plasma concentration-time curve (AUC) of paclitaxel. The AUC after doses of 2x 60, 90, 120 and 160 mg/m^2 were 3.77 ± 2.70, 4.57 ± 2.43, 3.62 ± 1.58 and 8.58 ± 7.87 μM.h, respectively. The AUC achieved after intravenous administration of paclitaxel 175 mg/m^2 was 17.95 ± 3.94 μM.h.
In conclusion, dose-increment of paclitaxel did not result on average in a significant additional increase in the AUC values of the drug. Dose-escalation of the bid dosing regimen was therefore not continued up to DLT. As bid dosing appeared to result in higher AUC values compared with single dose administration (data which we have published previously), we recommend bid dosing of oral paclitaxel for future studies. Although pharmacokinetic data are difficult to interpret, due to the limited number of patients at each dose level and the large interpatient variability, we recommend the dose level of 2x 90 mg/m^2 for further investigation as this dose level showed the highest systemic exposure to paclitaxel combined with good safety.

Introduction

Paclitaxel is an important anticancer agent widely applied for the treatment of breast, ovarian, and lung cancer and AIDS-related Kaposi's sarcoma [1,2]. The cellular target for paclitaxel has been identified as the tubulin/microtubule system that plays a significant role in mitosis, intracellular transport, cell motility and maintenance of cell shape. Paclitaxel promotes the assembly of stable

microtubules and inhibits their depolymerization, resulting in the arrest of cells in the G_2-M phase of the cell cycle [3-5].

Paclitaxel is currently administered intravenously (i.v.) at different dosages and time schedules and optimization of the clinical application is under investigation. Preclinical data from a variety of human cancer cell lines reveal that the cytotoxicity of paclitaxel is schedule dependent. In studies in which investigators evaluated both concentration and exposure duration, prolongation of drug exposure seemed more important for the activity of paclitaxel than an increase in concentration [6-10]. Furthermore, paclitaxel, like some other drugs to which resistance is conferred by the multidrug resistance (mdr) phenotype, was more effective *in vitro* when applied to mdr cells for a longer duration [11]. In clinical studies, prolongation of the infusion duration was associated with an increase in severity of bone marrow suppression. Myelosuppression appeared to be related with the duration of plasma paclitaxel concentrations above either a threshold concentration of 0.05 μM [12,13] or 0.1 μM [14]. This increased toxicity with longer infusion duration suggests that tumor-cell cytotoxicity may also increase as exposure duration increases. Huizing et al. [15] found, in the presence of carboplatin, a positive correlation between the duration of paclitaxel exposure above 0.1 μM and the median survival time in non-small cell lung cancer patients, indicating that prolongation of paclitaxel exposure may improve the response rate and overall survival.

Oral administration of paclitaxel is investigated because the oral route of administration is more practical and convenient to patients and may enable chronic continuous dosing of paclitaxel. However, paclitaxel shows very low oral bioavailability, which has limited treatment of the drug by the oral route.

Preclinical studies in mice have shown that the low oral bioavailability is due to efficient transport of the drug by the multidrug efflux pump P-glycoprotein (P-gp) abundantly present in the gastro-intestinal tract [16]. Efficient oral uptake of paclitaxel has recently been made possible in mice [17] and men [18,19] by co-administration of oral cyclosporin A (CsA), an inhibitor of P-gp and cytochrome P450 (CYP) 3A4-mediated drug metabolism. In men, co-administration of CsA resulted in a significant increase of at least 7-fold in the systemic exposure of paclitaxel and plasma concentrations increased from negligible to therapeutic levels [18,19].

The first promising clinical results at low paclitaxel dosages in a proof of principle study [18,19] and those obtained in a phase I dose-escalating study using a once daily dosing schedule [20] encouraged us to explore a twice daily dosing schedule in an attempt to further increase and prolong the systemic exposure to orally administered paclitaxel.

Patients and Methods

Patient Population

Patients with a histological proof of cancer for whom no standard therapy of proven benefit existed were eligible for the study. Previous radiotherapy or chemotherapy other than taxoid therapy was allowed, provided that the last treatment was at least four weeks prior to study entry and any resulting toxicities were resolved. Patients had to have acceptable bone marrow (white blood cells > 3.0 x 10^9/L; platelets > 100 x 10^9/L), liver function (serum bilirubin $\leq$ 25 µmol/L; serum albumin $\geq$ 25 g/L), renal function (serum creatinine $\leq$ 160 µmol/L or clearance $\geq$ 50 mL/min), and a World Health Organization (WHO) performance status $\leq$ 2. Patients were not eligible if they suffered from uncontrolled infectious disease, neurologic disease, bowel obstruction or symptomatic brain metastases. Other exclusion criteria were concomitant use of known P-gp inhibitors and chronic use of H2-receptor antagonists or proton pump inhibitors. The study protocol was approved by the medical ethics committee of The Netherlands Cancer Institute and all patients had to give written informed consent.

Study Design

Patients received oral paclitaxel at doses of 2x 60, 2x 90, 2x 120 or 2x 160 mg/m^2 during course 1 and i.v. paclitaxel administered as a 3-hour infusion at a dose of 175 mg/m^2 during course 2. If it was considered to be in their best interest patients continued on a 3-weekly schedule of i.v. paclitaxel. Per dose level of oral paclitaxel 3 eligible patients were entered; 3 additional patients (total of 6) were treated at a dose level if 1 of the first 3 patients exhibited dose limiting toxicity (DLT). DLTs were defined as grade 4 granulocytopenia of a duration of > 5 days, grade 4 thrombocytopenia of any duration or any grade 3/4 non-hematological toxicity except untreated nausea and vomiting. The maximum tolerated dose (MTD) was defined as the highest dose level producing DLTs in < 2 of 6 patients. The i.v. formulation of paclitaxel (Paxene®, paclitaxel 6 mg/ml, dissolved in Cremophor EL and ethanol 1:1 w/v, Baker Norton Pharmaceuticals, Miami, FL, USA) was used for both i.v. and oral administration of paclitaxel. Oral paclitaxel was administered in two doses 7 hours apart and 30 minutes prior to each paclitaxel dose patients received 15 mg/kg CsA (Neoral®, Novartis, Basel, Switzerland). The first oral paclitaxel dose was administered after an overnight fast and patients remained fasted until 2 hours following administration. For the second oral dose patients were refused food and drinks 1.5 hours prior to paclitaxel administration and up to 1 hour after administration. To prevent nausea and vomiting following administration of CsA and oral paclitaxel, 1 patient at the dose level 2x 90 mg/m^2 and all patients at

dose levels 2x 120 and 2x 160 mg/m^2 received 1 mg oral granisetron (Kytril®) 1 hour prior to CsA administration. In addition, 1 patient at dose level 2x 160 mg/m^2 received a light breakfast at least 2 hours prior to the first oral paclitaxel dose. Prior to i.v. administration of paclitaxel, patients received standard i.v. premedication to prevent hypersensitivity reactions, consisting of dexamethasone 20 mg orally 12 and 6 hours prior to, and clemastine 2 mg i.v. and cimetidine 300 mg i.v. 30 minutes prior to paclitaxel administration. Premedication was not administered prior to oral administration of paclitaxel.

Patient Evaluation

Pre-treatment evaluation included a complete medical history and complete physical examination. Before each course, an interim history including concomitant medications taken, toxicities and performance status was recorded and a physical examination was performed. Hematology was checked twice weekly after course 1 and 2 and weekly after subsequent courses. Blood chemistries including liver and renal function, serum electrolytes, total protein and albumin and glucose levels, were checked weekly. All toxicities observed were graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTC) [21]. Tumor measurements were performed every other cycle, but initially after the first two i.v. courses. Responses were evaluated according to the WHO criteria [22].

Sample Collection and Analysis

After oral drug administration of paclitaxel and CsA, blood samples and urine were collected for pharmacokinetic analysis. Blood samples were obtained in heparinized tubes, pre-dose, 30 minutes and 1, 2, 3, 4, 6, 7, 7.5, 8, 9, 10, 11, 13, 24 and 48 hours after ingestion of the two oral doses. For CsA whole blood concentrations, an aliquot of the blood sample was stored at 4ºC and analyzed within one week using a specific fluorescence polarization immuno assay (FPIA, Abbott TDxFLx, Amstelveen the Netherlands) [23]. For paclitaxel plasma concentrations, the remainder of the blood samples was centrifuged and plasma samples were stored at -20ºC until analysis. Paclitaxel plasma concentrations were determined using a validated high-performance liquid chromatography (HPLC) assay [24]. In addition to measuring CsA and paclitaxel levels after oral drug administration, ethanol and Cremophor EL concentrations were measured. The plasma samples obtained for paclitaxel analysis were used for analysis of ethanol and Cremophor EL. Plasma ethanol levels were measured for all patients at the dose levels of 2x 90, 120 and 160 mg/m^2 at 30 minutes and 1 hour following each oral dose of paclitaxel and analyzed by gas chromatography. Plasma concentrations of Cremophor EL were measured for 2 patients (dose level 2x 160

mg/m^2) at six time points up to 13 hours after the first oral dose of paclitaxel using a validated HPLC assay [25] with minor modifications as described elsewhere [26]. Urine was collected in 24-hour aliquots for 48 hours. Urine samples were stabilized with a mixture of 5% Cremophor EL/ethanol 1:1 v/v and stored at -20ºC until analysis. Paclitaxel concentrations in urine were determined using a validated HPLC assay [27]. During i.v. administration of paclitaxel blood samples for paclitaxel analysis were obtained according to a previously established limited sampling model using two concentration-time points at 1 and 8 hours after the end of paclitaxel infusion [28]. Blood samples were collected in heparinized tubes, centrifuged and plasma samples were stored at -20ºC until analysis. Paclitaxel plasma concentrations were determined using a validated HPLC assay [24].

Pharmacokinetic Analysis

Non-compartmental pharmacokinetic methods were applied to process the results [29]. For orally administered paclitaxel, the maximal drug concentration (Cmax) and time to maximal drug concentration (Tmax) were obtained directly from the experimental data. The area under the plasma paclitaxel concentration-time curve (AUC) was estimated by the trapezoidal rule up to the last measured concentration-time point (AUCt) and extrapolated to infinity using the terminal rate constant k. The terminal half-life (t1/2) was calculated as ln2/k. The time above the previously defined threshold concentrations of 0.05 μM and 0.1 μM (T>0.05 μM, T>0.1 μM) was determined using linear interpolation. For i.v. administered paclitaxel the parameters AUC and T>0.1 μM were determined using our previously established limited sampling model [28]. The percentage of the administered dose recovered in the urine (U_{excr}) was calculated as the amount excreted in the urine divided by the actual administered dose times 100%. Statistical analysis of the data was performed using the nonparametric Jonckheere-Terpstra-test [30] and the Mann-Whitney U-test. The a priori level of significance was p=0.05.

Results

Patients and Treatment

A total of 15 patients (3 males and 12 females) was enrolled in the study. At study entry, the median age of the patients was 57 years (range 34-75 years) and the median WHO performance status was 0 (range 0-1). Primary tumor types included breast (3), ovarian (3), non-small cell lung cancer (3), adenocarcinomas of unknown primary site (3), colon (2) and pancreas (1) tumors. All patients, except 2, had received prior surgical therapy, radiotherapy and/or chemotherapy. Toxicities

observed following bid dosing of oral paclitaxel and after the first i.v. course of paclitaxel are presented in Tables 1 and 2. After oral paclitaxel administration, hematological toxicities observed included anemia, which was often pre-existing, and leukocytopenia/granulocytopenia. The main non-hematological toxicities were alopecia, arthralgia/myalgia, fatigue, neurotoxicity, mucositis, diarrhea, nausea and vomiting. Other, incidental toxicities were gastric pain (1 patient), skin reactions (1 patient), flushes (2 patients) and mild and reversible hypotension (1 patient) (not listed in Table 2). Toxicities observed were generally mild (grade 1-2); 1 patient experienced granulocytopenia grade 3 (dose level 2x 160 mg/m^2). Toxicities clearly related to CsA administration were nausea and vomiting, which were observed in 3 patients. These toxicities arose prior to paclitaxel intake. During the first course of i.v. paclitaxel, a similar profile of hematological and non-hematological toxicities was observed as after oral intake of the drug. Toxicities observed were generally mild; 1 patient developed leukocytopenia grade 3 and another patient experienced granulocytopenia grade 4. In this study 1 partial response, which was documented after the third course (1 oral and 2 i.v.), was observed in a patient with ovarian cancer (dose level 2x 120 mg/m^2).

Pharmacokinetics

Three patients were considered not evaluable for pharmacokinetic analysis. In 1 patient the oral course was interrupted due to respiratory problems (not drug-related) and 2 other patients vomited within 2 hours of intake of oral paclitaxel. Therefore, 12 patients, 3 at each dose level, were considered eligible for pharmacokinetic analysis. Pharmacokinetic parameters of bid dosing of oral paclitaxel are presented in Table 3. Dose-increment of oral paclitaxel from 2x 60 to 2x 160 mg/m^2 did not result in a significant increase in the AUC of paclitaxel nor in a significant increase in time above the threshold concentrations of 0.05 μM and 0.1 μM (Jonckheere-Terpstra-test). An individual plasma concentration-time curve of bid dosing of 2x 90 mg/m^2 oral paclitaxel is depicted in Figure 1. CsA whole blood pharmacokinetic parameters are shown in Table 4. Cremophor EL plasma levels after oral administration of paclitaxel were measured in 2 patients (dose level 2x 160 mg/m^2) and were at all investigated time points lower than the limit of quantitation of the assay (< 0.01% v/v). Maximal blood ethanol concentrations were reached within 1 hour of oral intake of either dose of paclitaxel. Paclitaxel doses of 2x 90, 120 and 160 mg/m^2 (corresponding to 2x 7.5, 10 and 13.3 ml/m^2 ethanol) resulted in mean maximal ethanol concentrations of 0.07 ± 0.05, 0.21 ± 0.04 and 0.29 ± 0.12‰, respectively, after the first dose. After the second dose mean maximal ethanol concentrations were comparable to those after the first dose.

Table 1. *Hematological toxicities observed following bid dosing of oral paclitaxel and after the first i.v. course of paclitaxel.*

	Oral paclitaxel 2x 60 mg/m^2	Oral paclitaxel 2x 90 mg/m^2	Oral paclitaxel 2x 120 mg/m^2	Oral paclitaxel 2x 160 mg/m^2	Oral paclitaxel all dose levels	i.v. paclitaxel 175 mg/m^2 (3-h inf)
No. of patients	4	3	3	5	15	11
Anemia						
Grade 1	1	0	1	3	5	5
Grade 2	2	2	1	0	5	1
Grade 3	0	0	0	0	0	1
Leukocytopenia						
Grade 1	0	0	0	0	0	2
Grade 2	0	1	0	2	3	1
Grade 3	0	0	0	0	0	1
Granulocytopenia						
Grade 1	0	0	0	0	0	2
Grade 2	0	1	0	1	2	2
Grade 3	0	0	0	1	1	0
Grade 4	0	0	0	0	0	1

Table 2. *Non-hematological toxicities observed following bid dosing of oral paclitaxel and after the first i.v. course of paclitaxel.*

	Oral paclitaxel 2x 60 mg/m^2	Oral paclitaxel 2x 90 mg/m^2	Oral paclitaxel 2x 120 mg/m^2	Oral paclitaxel 2x 160 mg/m^2	Oral paclitaxel all dose levels	i.v. paclitaxel 175 mg/m^2 (3-h inf)
No. of patients	4	3	3	5	15	11
Alopecia						
Grade 1	2	1	2	0	5	0
Grade 2	0	1	1	2	4	3
Arthralgia/myalgia						
Grade 1	1	1	2	2	6	3
Grade 2	1	0	0	0	1	2
Fatigue						
Grade 1	2	0	1	1	4	3
Grade 2	0	1	1	0	2	1
Neurotoxicity						
Grade 1	1	1	0	2	4	3
Mucositis						
Grade 1	2	1	1	0	4	4
Diarrhea						
Grade 1	0	1	1	3	5	1
Nausea						
Grade 1	0	1	1	1	3	1
Vomiting						
Grade 1	2	1	0	1	4	1

Table 3. *Pharmacokinetic parameters of bid dosing of oral paclitaxel (data listed as mean ± (SD)).*

Pac dose (mg/m^2)	CsA dose (mg/kg)	No. of patients	Tmax (h)	Cmax (μM)	AUC (μM.h)	T> 0.1 μM (h)	T> 0.05 μM (h)	U_{excr} (% dose)
2x 60	2x 15	3	3.4 (0.6)	0.21 (0.10)	3.77 (2.70)	11.4 (10.9)	26.9 (11.1)	1.7 (1.1)
			3.4 (0.6)	0.21 (0.08)				
2x 90	2x 15	3	3.4 (0.6)	0.23 (0.16)	4.57 (2.43)	12.1 (8.8)	21.8 (10.3)	2.2 (1.0)
			3.2 (1.0)	0.32 (0.16)				
2x 120	2x 15	3	3.0 (1.0)	0.20 (0.09)	3.62 (1.58)	8.7 (7.7)	17.1 (7.9)	1.2 (0.7)
			0.9 (1.5)	0.25 (0.17)				
2x 160	2x 15	3	3.5 (2.5)	0.44 (0.37)	8.58 (7.87)	19.1 (18.6)	28.6 (23.1)	1.0 (1.0)
			2.3 (1.6)	0.49 (0.41)				

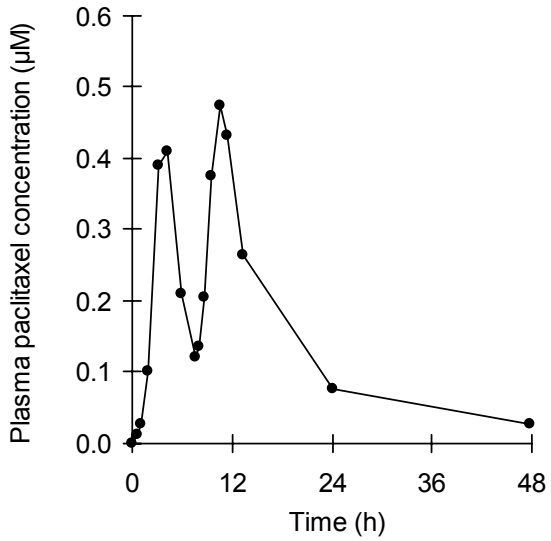

Figure 1. *Typical individual paclitaxel plasma concentration-time curve of bi-daily dosing of 2x 90 mg/m^2 oral paclitaxel.*

Table 4. *Pharmacokinetic parameters of bid dosing of CsA (data listed as mean ± (SD)).*

Pac dose (mg/m^2)	CsA dose (mg/kg)	No. of patients	Tmax (h)	Cmax (mg/L)	AUC (mg.h/L)
2x 60	2x 15	3	2.1 (1.3)	2.75 (0.34)	55.43 (29.73)
			5.2 (2.0)	2.81 (1.10)	
2x 90	2x 15	3	2.1 (1.5)	3.01 (0.69)	62.75 (23.53)
			3.5 (2.2)	3.65 (1.42)	
2x 120	2x 15	3	1.4 (0.4)	2.60 (0.62)	42.40 (12.49)
			2.6 (1.9)	2.22 (0.87)	
2x 160	2x 15	3	1.1 (0.0)	2.98 (1.58)	50.37 (37.03)
			0.7 (2.5)	2.90 (2.25)	

The pharmacokinetic data of i.v. paclitaxel (175 mg/m^2 as a 3-hour infusion) were in good agreement with earlier observations [14,15]. The mean plasma AUC and T>0.1 µM values were 17.95 ± 3.94 µM.h and 17.1 ± 6.7 h, respectively (n=11).
In Table 5 a comparison is made between the pharmacokinetic data of bid dosing of oral paclitaxel (2x 60, 2x 90 and 2x 120 mg/m^2) with those of single dose administration of oral paclitaxel (120, 180 and 250 mg/m^2) [20]. At all dose levels fractionated administration of oral paclitaxel resulted in consistently higher values of AUC and T>0.1 µM. Differences were, however, not statistically significant (Mann-Whitney U-test).

Table 5. *Pharmacokinetic parameters of bid dosing of oral paclitaxel compared with single dose administration of the drug [20] (data listed as mean ± (SD)).*

Pac dose (mg/m^2)	AUC (μM.h)	T> 0.1 μM (h)	T> 0.05 μM (h)
2x 60	3.77 (2.70)[a]	11.4 (10.9)[a]	26.9 (11.1)[a]
120	2.55 (2.29)	7.9 (8.0)	13.0 (12.7)
2x 90	4.57 (2.43)[b]	12.1 (8.8)[b]	21.8 (10.3)[b]
180	3.33 (2.39)	7.9 (6.7)	14.6 (12.3)
2x 120	3.62 (1.58)[c]	8.7 (7.7)[c]	17.1 (7.9)[c]
250	3.27 (2.94)	7.0 (9.3)	13.6 (11.1)

[a] not statistically significant compared to 120 mg/m^2,
[b] not statistically significant compared to 180 mg/m^2,
[c] not statistically significant compared to 250 mg/m^2.

Discussion

Dose-escalation of bid dosing of oral paclitaxel plus CsA was performed starting at 2x 60 mg/m^2 up to 2x 160 mg/m^2. Pharmacokinetic analysis revealed that dose-increment of oral paclitaxel from 2x 60 mg/m^2 to 2x 90 mg/m^2 or higher doses did not result in a significant additional increase in the systemic exposure to paclitaxel nor in the time above the threshold concentrations of 0.05 and 0.1 μM. Apparently, the absorption of orally administered paclitaxel is limited. Saturation of absorption after oral paclitaxel administration was also observed in the dose-escalation study of single dose oral paclitaxel [20]. It was then hypothesized that limited dissolution, due to release of paclitaxel from its pharmaceutical formulation and precipitation as a result of its poor aqueous solubility, could cause the apparent saturation in absorption of orally administered paclitaxel. At the highest dose level of 2x 160 mg/m^2, Cmax and AUC values appear to be higher than those at the lower paclitaxel dose levels. However, at this dose level the extremely high Cmax (0.80 and 0.90 μM) and AUC (16.79 μM.h) values of 1 patient contribute largely to the high mean values. It remains unclear why this patient absorbed oral paclitaxel this well. Saturation of drug absorption has also been observed for other anticancer agents, including methotrexate, etoposide and leucovorin [31-33]. This has led to hyperfractionated approaches, whereby the drug has been administered multiple

times a day rather than as one large daily dose, to achieve a greater overall daily systemic exposure. Comparison of the pharmacokinetic data of bid dosing of oral paclitaxel with those of single dose administration of the drug [20] showed that fractionated administration of oral paclitaxel resulted in consistently higher values of the systemic exposure (AUC) of paclitaxel and the duration of systemic exposure (T>0.1 μM and T>0.05 μM) to the drug. However, due to the large interpatient variability and the small number of patients enrolled at each dose level, these differences were not statistically significant. Nevertheless, we suggest that for oral paclitaxel, administration of a multiple dose regimen is a realistic option to further increase and prolong the systemic exposure to paclitaxel.

An important pharmacokinetic parameter of paclitaxel is the time period of exposure above a certain threshold concentration. Earlier data indicate a strong positive relationship between duration of the paclitaxel plasma concentration above 0.05 or 0.1 μM and pharmacological activity [12-15]. The feasibility of oral paclitaxel administration may enable the development of more chronic treatment schedules with the aim of achieving sustained plasma concentrations above these pharmacological relevant threshold levels. However, it is unclear whether for orally administered paclitaxel the threshold concentrations of 0.05 and 0.1 μM are relevant and should be pursued. The plasma Cremophor EL concentrations are a key factor in this discussion. After oral administration of paclitaxel, plasma Cremophor EL plasma levels were undetectable, which was also seen in our previous studies of orally administered paclitaxel [18-20]. Thus, after oral administration of the paclitaxel i.v. formulation (Paxene®) the co-solvent Cremophor EL is not absorbed. This is important, first because systemic exposure to Cremophor EL can induce severe hypersensitivity reactions requiring extensive premedication [34,35]. In the current study patients did not receive premedication prior to oral administration of paclitaxel. Potential hypersensitivity reactions observed following orally administered paclitaxel were very mild (grade 1) and consisted of flushes (2 patients), skin reactions (1 patient) and mild and reversible hypotension (1 patient), which did not require additional measures. Evidently, paclitaxel (Paxene®) can be administered orally without premedication. Furthermore, Cremophor EL is responsible for the non-linear pharmacokinetic behavior of i.v. paclitaxel [12,36]. It entraps paclitaxel in the plasma compartment, which results in a more than proportional increase in plasma paclitaxel levels with increasing doses. However, studies in mice show that these higher total drug levels in plasma do not result in higher drug levels in tissues [37]. This pseudo-non-linearity of i.v. paclitaxel [26] has two important implications for the pharmacology of oral paclitaxel. First, oral bioavailability of paclitaxel, calculated by comparing plasma AUC values after oral and i.v. administration, will be underestimated as the

affinity of paclitaxel for the plasma compartment is increased after i.v. administration due to the presence of systemic Cremophor EL. Secondly, the pseudo-non-linearity of i.v. paclitaxel implies that after oral administration, when Cremophor EL is not present, plasma levels of paclitaxel represent a higher fraction of free drug, which will result in enhancement of the availability of paclitaxel for the (tumor) tissues [26]. Consequently, the optimal value of the threshold level may be lower for orally administered paclitaxel than i.v. paclitaxel; this needs further confirmation. Thus, pharmacokinetics of i.v. paclitaxel and oral paclitaxel, with and without Cremophor EL in the systemic circulation, respectively, are substantially different and therefore comparison of pharmacokinetic parameters of i.v. and oral paclitaxel should be done with caution.

In this study we have used CsA to increase the systemic exposure to oral paclitaxel. CsA is an efficacious inhibitor of P-gp and has been one of the first agents applied to modulate P-gp [38]. In addition to CsA, more potent inhibitors of P-gp have been developed, such as the CsA analogue SDZ PSC 833 [39] or the acridone carboxamide derivative GF120918 [40]. Importantly, these newly developed modulators of P-gp have no known immunosuppressive activity such as CsA and may therefore be better candidates for clinical use, especially for repeated administration than CsA. CsA is, however, commercially available and an advantage in its use to increase the systemic exposure to oral paclitaxel is its potential to inhibit metabolism of paclitaxel. Metabolism of paclitaxel is catalyzed by two cytochrome P450 (CYP) isoenzymes; CYP 2C8 catalyses the degradation to the 6a-hydroxypaclitaxel metabolite and CYP 3A4 results in formation of the 3'p-hydroxypaclitaxel metabolite [41,42]. Both metabolites are substantially less active than the parent drug [42]. CsA itself is also metabolized by CYP 3A4 [43]. In our previous study of single dose oral paclitaxel in combination with CsA [26] we found that after oral paclitaxel administration in combination with CsA the relative contribution of formation of the metabolite 3'p-hydroxypaclitaxel was substantially lower than after i.v. administration of the drug, indicating inhibition of CYP 3A4-mediated paclitaxel metabolism by CsA.

Because pharmacokinetic analysis revealed limited absorption of orally administered paclitaxel, we did not continue dose-escalation of bid dosing oral paclitaxel up to DLT. Toxicities observed following oral paclitaxel administration in combination with CsA were mild (CTC grade 1-2) at all investigated dose levels. At the dose level 2x 160 mg/m^2 diarrhea occurred more often (3 of 5 patients) than at other dose levels and at this dose level 1 patient continued to experience acute nausea and vomiting despite granisetron administration. Therefore, we considered the lower dose levels more suitable for future studies.

In conclusion, dose-escalation of bid dosing of oral paclitaxel was not continued up to DLT, as the pharmacokinetic data revealed no significant additional increase in the systemic exposure to paclitaxel with increment of the administered dose. Because fractionated administration of oral paclitaxel resulted in consistently higher values of the paclitaxel pharmacokinetic parameters than single dose administration, we will continue with additional clinical studies focused on multiple dose regimens of oral paclitaxel. Although pharmacokinetic data are difficult to interpret, due to the limited number of patients at each dose level and the large interpatient variability, we recommend the dose level of 2x 90 mg/m^2 for further investigation as this dose level showed the highest systemic exposure to paclitaxel combined with good safety.

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CHAPTER 3.2

The effect of different doses of cyclosporin A on the systemic exposure of orally administered paclitaxel

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Abstract

The objective of this study was to define the minimally effective dose of cyclosporin A (CsA) that would result in a maximal increase of the systemic exposure to oral paclitaxel.
Six evaluable patients participated in this randomized cross-over study in which they received at two occasions two doses of 90 mg/m^2 oral paclitaxel 7 hours apart in combination with 10 or 5 mg/kg CsA.
Dose-reduction of CsA from 10 to 5 mg/kg resulted in a statistically significant decrease in the area under the plasma concentration-time curve (AUC) and time above the threshold concentration of 0.1 μM (T>0.1 μM) of oral paclitaxel. The mean (± SD) AUC and T>0.1 μM values of oral paclitaxel with CsA 10 mg/kg were 4.29 ± 0.88 μM.h and 12.0 ± 2.1 h, respectively. With CsA 5 mg/kg these values were 2.75 ± 0.63 μM.h and 7.0 ± 2.1 h, respectively (p=0.028 for both parameters).
In conclusion, dose-reduction of CsA from 10 to 5 mg/kg resulted in a significant decrease in the AUC and T>0.1 μM values of oral paclitaxel. Because CsA 10 mg/kg resulted in similar paclitaxel AUC and T>0.1 μM values compared to CsA 15 mg/kg (data which we have published previously), the minimally effective dose of CsA is determined at 10 mg/kg.

Introduction

Paclitaxel is an important anticancer agent widely applied for the treatment of breast, ovarian, and lung cancer and AIDS-related Kaposi's sarcoma [1,2]. The drug is currently administered intravenously (i.v.) at different dosages and time schedules and optimization of the clinical application is still pursued. Recently, we reported about the feasibility of oral administration of paclitaxel [3-7]. Oral administration is investigated because it is convenient to patients, it reduces administration costs and facilitates the use of more chronic treatment regimens. The latter is important as there are strong indications in both preclinical [8-12] and clinical studies [13-16] that the pharmacological activity of paclitaxel is related to duration of exposure to the drug.
Oral administration of paclitaxel appeared feasible with co-administration of cyclosporin A (CsA) [6,7], an efficacious inhibitor of the gastro-intestinal drug efflux pump P-glycoprotein (P-gp), which was shown in our preclinical studies to cause the low oral bioavailability of paclitaxel [3]. In our clinical proof of principle study, co-administration of 15 mg/kg CsA resulted in an approximately 7-fold increase in the systemic exposure of 60 mg/m^2 orally administered paclitaxel [6,7]. These first

promising results with low paclitaxel dosages encouraged us to investigate dose-increment of CsA and dose-escalation of paclitaxel in order to further increase the systemic exposure. Dose-increment of CsA from 15 to 30 mg/kg did not result in an additional increase in the systemic exposure of paclitaxel and hence CsA 15 mg/kg was used in further studies [17]. Dose-escalation of paclitaxel from 60 up to 300 mg/m^2 resulted in a significant increase in systemic exposure of paclitaxel, however these increases were moderate and not proportional with the increases in dose, indicating limited absorption for orally administered paclitaxel [17]. In order to further increase the systemic exposure we consecutively investigated dose-escalation of a twice daily dose regimen of oral paclitaxel [18]. Compared to single dose administration, hyperfractionated administration resulted in consistently higher values of the systemic exposure of paclitaxel. As observed for single dose paclitaxel, twice daily dose administration also revealed limited absorption of the drug. Based on these observations the oral paclitaxel dose level of 2x 90 mg/m^2 in combination with CsA 15 mg/kg was recommended for further studies [18].
In this study we investigated dose-reduction of CsA from 10 to 5 mg/kg co-administered to 2x 90 mg/m^2 oral paclitaxel, in order to define the minimally effective dose of CsA that would result in a maximal increase in the systemic exposure to paclitaxel. CsA is an immunosuppressive drug widely used in transplantation to prevent rejection of the transplanted organ [19]. It felt important that the immunosuppressive effect is minimized. In addition, a dose-related nephrotoxicity of CsA should be considered [20]. It was our aim to determine the minimally effective dose of CsA resulting in a maximal increase in systemic exposure to paclitaxel.

Patients and Methods

Patient Population

Patients with a histologic proof of cancer for whom no standard therapy of proven benefit existed were eligible for the study. Previous radiotherapy or chemotherapy other than taxoid therapy was allowed, provided that the last treatment was at least four weeks prior to study entry and any resulting toxicities were resolved. Patients had to have acceptable bone marrow function (white blood cells > 3.0 x 10^9/L; platelets > 100 x 10^9/L), liver function (serum bilirubin $\leq$ 25 µmol/L; serum albumin $\geq$ 25 g/L), renal function (serum creatinine $\leq$ 160 µmol/L or clearance $\geq$ 50 mL/min), and a World Health Organization (WHO) performance status $\leq$ 2. Patients were not eligible if they suffered from uncontrolled infectious disease, neurologic disease, bowel obstruction or symptomatic brain metastases. Other exclusion criteria were

concomitant use of known P-gp inhibitors and chronic use of H2-receptor antagonists or proton pump inhibitors. The study protocol was approved by the Medical Ethics Committee of the Institute and all patients had to give written informed consent.

Study Design

The study had a randomized cross-over trial design. Patients received, at one occasion, two doses of 90 mg/m^2 paclitaxel orally 7 hours apart in combination with 10 or 5 mg/kg CsA. Three weeks later, these patients received the two doses of 90 mg/m^2 oral paclitaxel in combination with the alternate CsA dose. If it was considered to be in their best interest patients continued on a 3-weekly schedule of i.v. paclitaxel 175 mg/m^2 administered as a 3-hour infusion.

The i.v. formulation of paclitaxel (Paxene®, paclitaxel 6 mg/ml, dissolved in Cremophor EL and ethanol 1:1 w/v, Baker Norton Pharmaceuticals, Miami, FL, USA) was used for both i.v. and oral administration of paclitaxel. CsA (Neoral®, Novartis, Basel, Switzerland) was administered 30 minutes prior to each oral paclitaxel dose. The first oral paclitaxel dose was administered at least two hours after a standard light breakfast (2 crackers and a cup of tea) and patients remained fasted until 2 hours following administration. For the second oral paclitaxel dose, patients were refrained from food and drinks 1.5 hours prior to paclitaxel administration and up to 1 hour after administration. To prevent nausea and vomiting following administration of CsA and oral paclitaxel, patients received oral granisetron 1 mg (Kytril®) 2 hours prior to the first CsA dose and 1 hour prior to the second CsA dose. Prior to i.v. administration of paclitaxel patients received standard i.v. premedication to prevent hypersensitivity reactions, i.e. dexamethasone 20 mg orally 12 and 6 hours prior to, and clemastine 2 mg i.v. and cimetidine 300 mg i.v. 30 minutes prior to paclitaxel administration. Oral paclitaxel doses were given without this premedication regimen as previous studies of oral paclitaxel have shown that the co-solvent Cremophor EL, suspect of causing the hypersensitivity reactions [21], was not absorbed following oral intake of the drug [6,7,17,18].

Patient Evaluation

Pre-treatment evaluation included a complete medical history and complete physical examination. Before each course, an interim history including concomitant medications taken, toxicities and performance status were registered and a physical examination was performed. Hematology was checked twice weekly after course 1, 2 and 3 and weekly after subsequent courses. Blood chemistries including liver and renal function, serum electrolytes, total protein and albumin and

glucose levels, were checked weekly. All toxicities observed were graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTC) [22]. Tumor measurements were performed every other cycle, but initially after the first 2 i.v. courses. Responses were evaluated according to the WHO criteria [23].

Sample Collection and Analysis

After oral paclitaxel administration blood samples and urine were collected for pharmacokinetic analysis. Blood samples were obtained in heparinized tubes, pre-dose, 30 minutes and 1, 2, 3, 4, 6, 7, 7.5, 8, 9, 10, 11, 13, 24 and 48 hours after ingestion of the two oral doses. For CsA whole blood concentrations, an aliquot of the blood sample was stored at 4°C and analyzed within one week using a specific fluorescence polarization immuno assay (FPIA, Abbott TDxFLx, Amstelveen the Netherlands) [24]. For plasma paclitaxel and metabolite concentrations, the remaining of the blood samples was centrifuged and plasma samples were stored at -20°C until analysis. Paclitaxel and metabolite concentrations were determined using a validated high-performance liquid chromatography (HPLC) assay [25]. In addition to measuring CsA and paclitaxel levels after oral drug administration, Cremophor EL concentrations were measured in one patient during both oral courses. The plasma samples obtained for paclitaxel analysis were used for analysis of Cremophor EL. Plasma Cremophor EL concentrations were measured at 6 time points up to 13 hours after the first oral dose of paclitaxel using a validated HPLC assay [26] with minor modifications as described elsewhere [27]. Urine was collected in 24-hour aliquots for 48 hours. Urine samples were stabilized with a mixture of 5% Cremophor EL/ethanol 1:1 v/v and stored at -20°C until analysis. Paclitaxel concentrations in urine were determined using a validated HPLC assay [28].

During i.v. administration of paclitaxel blood samples for paclitaxel analysis were obtained according to a previously established limited sampling model using 2 concentration-time points at 1 and 8 hours after the end of paclitaxel infusion [29]. Blood samples were collected in heparinized tubes, centrifuged and plasma samples were stored at -20°C until analysis. Paclitaxel concentrations were determined using a validated HPLC assay [25].

Pharmacokinetic Analysis

Non-compartmental pharmacokinetic methods were applied to process the results [30]. For orally administered paclitaxel, the maximal drug concentration (Cmax) and time to maximal drug concentration (Tmax) were obtained directly from the experimental data. The area under the plasma paclitaxel concentration-time curve (AUC) was estimated by the trapezoidal rule up to the last measured concentration-

time point (AUCt) and extrapolated to infinity using the terminal rate constant k. The terminal half-life (t1/2) was calculated as ln2/k. The time above the previously defined threshold concentrations of 0.05 μM and 0.1 μM (T>0.05 μM, T>0.1 μM) was determined using linear interpolation. For i.v. administered paclitaxel the parameters AUC and T>0.1 μM were determined using our previously established limited sampling model [29]. The percentage of the administered dose recovered in the urine (U_{excr}) was calculated as the amount excreted in urine divided by the actual administered dose times 100%. Statistical analysis of the data was performed using the nonparametric Wilcoxon matched-pairs signed-rank test. The a priori level of significance was p=0.05.

Results

Patients and Treatment

A total of 8 patients (5 males and 3 females) was enrolled in the study. At study entry, the median age of the patients was 47 years (range 36 to 69) and the median WHO performance status was 0 (range 0 to 1). Primary tumor types included non-small cell lung cancer (2), breast (1), stomach (2), cervix (1) and uterus (1) tumors and adenocarcinoma of unknown primary site (1). All patients, except one, had received prior surgical therapy, radiotherapy and/or chemotherapy.

The oral combination of paclitaxel and CsA was in general well tolerated. Hematological toxicities after oral intake of paclitaxel plus CsA (in total 16 courses) consisted of anemia (12), leukocytopenia (4) and granulocytopenia (3), which were generally mild (CTC grade 1-2), except for two patients who experienced leukocytopenia grade 3. No pronounced differences could be observed in hematological toxicities between the two CsA dose levels. Non-hematological toxicities after oral administration of paclitaxel plus CsA were nausea (7), vomiting (2), diarrhea (5), mucositis (1), arthralgia/myalgia (5), alopecia (7) and fatigue (2). Non-hematological toxicities did not exceed grade 1 in severity, except for one patient who experienced alopecia grade 2. Again, no pronounced differences between the two CsA levels were observed. However, the limited number of observations does not allow a definite comparison of toxicities. After the first course of i.v. paclitaxel (in total 8 courses) the pattern of hematological and non-hematological toxicities was as expected for i.v. paclitaxel and consisted anemia (3), leukocytopenia (4), granulocytopenia (4), arthralgia/myalgia (7), alopecia (3), neurotoxicity (2) and mucositis (1).

In this study two partial responses were observed, one in a patient with a cervix tumor after six courses of paclitaxel (two oral and four i.v.) and one in a patient with

adenocarcinoma of unknown primary site after five courses of paclitaxel (two oral and three i.v.).

Pharmacokinetics

A total of six patients was evaluable for pharmacokinetic analysis. One patient was considered not evaluable because of vomiting within 1 hour after intake of oral paclitaxel. For another patient, plasma samples of one oral course were lost due to power failure of the freezer.

In Table 1 the mean (± SD) values of the plasma pharmacokinetic parameters of orally administered paclitaxel in combination with 5 and 10 mg/kg CsA are presented. In addition, in this table the data of 2x 90 mg/m^2 oral paclitaxel in combination with 15 mg/kg CsA, data from our previously performed dose-escalation study of twice daily dosing of oral paclitaxel, are given [18]. Dose-reduction of CsA from 10 to 5 mg/kg resulted in a significant decrease in the AUC, T>0.1 μM and T>0.05 μM values of orally administered paclitaxel (p=0.028 for all three parameters). Compared to CsA 15 mg/kg (in a different cohort of patients) [18], co-administration of 10 mg/kg CsA resulted in comparable paclitaxel AUC, T>0.1 μM and T>0.05 μM values. Figure 1 shows the paclitaxel concentration-time curves of a patient receiving oral paclitaxel 2x 90 mg/m^2 in combination with 5 and 10 mg/kg CsA on two occasions. Plasma Cremophor EL levels were lower than the lower limit of quantitation of the assay (< 0.01% v/v) at all investigated time-points.

Table 1. *Pharmacokinetic parameters of paclitaxel after oral administration at a dose of 2x 90 mg/m^2 in combination with CsA 5, 10 and 15 mg/kg (mean ± SD). The data of CsA 15 mg/kg are retrieved from a previously performed study [18].*

Paclitaxel data	CsA 5 mg/kg (n=6)	CsA 10 mg/kg (n=6)	CsA 15 mg/kg (n=3)
AUC (μM.h)	2.75 ± 0.63[a]	4.29 ± 0.88	4.57 ± 2.43
T> 0.1 μM (h)	7.0 ± 2.1[a]	12.0 ± 2.1	12.1 ± 8.8
T> 0.05 μM (h)	14.5 ± 4.9[a]	23.7 ± 4.4	21.8 ± 10.3
t1/2 (h)	10.5 ± 5.6	13.6 ± 7.5	11.4 ± 2.2
$Cmax_1$ (μM)	0.16 ± 0.05	0.21 ± 0.05	0.23 ± 0.16
$Cmax_2$ (μM)	0.28 ± 0.08	0.35 ± 0.11	0.32 ± 0.16
$Tmax_1$ (h)	2.5 ± 1.4	2.3 ± 1.0	3.4 ± 0.6
$Tmax_2$ (h)	2.7 ± 0.9	2.5 ± 0.5	3.2 ± 1.0

[a]p-values < 0.05 compared to CsA 10 mg/kg (randomized cross-over trial design).

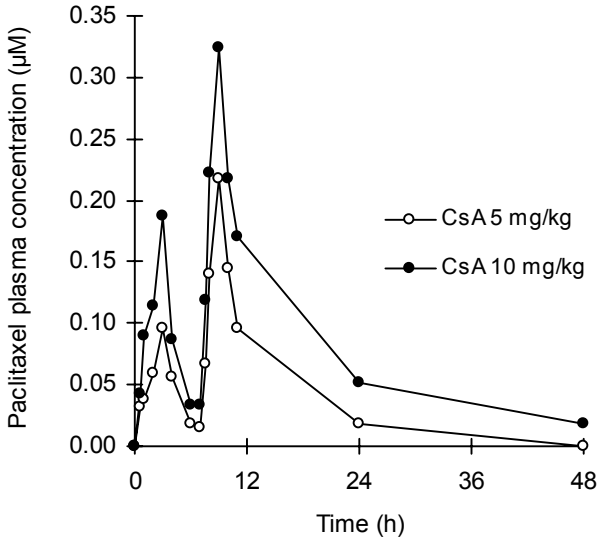

Figure 1. *Paclitaxel plasma concentration-time curves obtained in one patient dosed with oral paclitaxel 2x 90 mg/m^2 in combination with 5 and 10 mg/kg CsA.*

Table 2 presents the AUCt values of the paclitaxel metabolites 6a-hydroxypaclitaxel, 3'p-hydroxypaclitaxel and 6a,3'p-dihydroxypaclitaxel after oral administration of paclitaxel in combination with CsA 5, 10 and 15 mg/kg. The latter data are again derived from our previously performed dose-escalation study of twice daily dosing of oral paclitaxel [18]. In this study of CsA 5 and 10 mg/kg, metabolite data of two patients could not be determined due to interference of (unknown) compounds in the analytical assay. AUCt values have been calculated because extrapolation of the AUC could not be performed properly due to erratic profiles and the limited time that these metabolites could be detected. Higher doses of CsA appeared to result in a relative decrease in the formation of the 3'p-hydroxypaclitaxel metabolite. The AUCt ratio for the metabolites 6a-hydroxypaclitaxel and 3'p-hydroxypaclitaxel was 1.0 (0.49/0.48) after CsA 5 mg/kg, 4.0 (2.63/0.66) after CsA 10 mg/kg and 6.9 (6.19/0.90) after CsA 15 mg/kg.

CsA whole blood pharmacokinetic parameters of 5, 10 and 15 mg/kg co-administered to 2x 90 mg/m^2 oral paclitaxel are shown in Table 3. As done for paclitaxel, we added in this table CsA data from our previous study of oral paclitaxel in combination with 15 mg/kg CsA [18]. Dose-increment of CsA from 5 to 10 mg/kg resulted in an approximately 2-fold increase in $Cmax_1$, $Cmax_2$ and AUC values of CsA, whereas $Tmax_1$, $Tmax_2$ and t1/2 revealed rather constant values. Administration of CsA 15 mg/kg revealed further increases in Cmax and AUC values of CsA in proportion with the further increase in dose.

Urinary excretion after orally administered paclitaxel in combination with CsA was minimal, as was shown in our previous clinical studies of oral paclitaxel [17,18].

Different CsA doses did not seem to result in pronounced differences in urinary excretion of the drug. Paclitaxel was excreted as unchanged drug for 2.0 ± 1.2% after CsA 5 mg/kg and for 2.8 ± 0.9% after CsA 10 mg/kg. After CsA 15 mg/kg paclitaxel was excreted in the urine for 2.2 ± 1.0% [18].

The pharmacokinetic data of i.v. paclitaxel (175 mg/m^2 as a 3-h infusion) were in good agreement with earlier observations [13,16]. The mean plasma AUC and T> 0.1 μM values were 15.4 ± 1.8 μM.h and 16.3 ± 0.7 h, respectively (n=4). For two patients i.v. paclitaxel pharmacokinetics could not be determined because infusion duration or blood sampling were not within the range of the limited sampling model [29].

Table 2. *AUCt values of the paclitaxel metabolites 6a-hydroxypaclitaxel (6a-HP), 3'p-hydroxypaclitaxel (3'p-HP) and 6a,3'p-dihydroxypaclitaxel (6a,3'p-DHP) after oral paclitaxel administration of 2x 90 mg/m^2 in combination with CsA 5, 10 and 15 mg/kg (mean ± SD). The data of CsA 15 mg/kg are retrieved from a previously performed study [18].*

AUCt (μM.h)	CsA 5 mg/kg (n=4)	CsA 10 mg/kg (n=4)	CsA 15 mg/kg (n=3)
6a-HP (μM.h)	0.49 ± 0.24	2.63 ± 0.87	6.19 ± 6.79
3'p-HP (μM.h)	0.48 ± 0.51	0.66 ± 0.21	0.90 ± 0.88
6a,3'p-DHP (μM.h)	0.35 ± 0.62	1.73 ± 1.06	4.66 ± 6.75

Table 3. *Pharmacokinetic parameters of CsA administered at doses of 2x 5, 2x 10 and 2x 15 mg/kg combined with 2x 90 mg/m^2 oral paclitaxel (mean ± SD). The data of CsA 15 mg/kg are retrieved from a previously performed study [18].*

	CsA 5 mg/kg (n=6)	CsA 10 mg/kg (n=6)	CsA 15 mg/kg (n=3)
AUC (mg.h/L)	16.64 ± 6.00	37.09 ± 6.77	62.75 ± 23.53
t1/2 (h)	10.3 ± 2.4	10.5 ± 1.1	12.4 ± 2.4
$Cmax_1$ (mg/L)	1.43 ± 0.93	2.44 ± 0.78	3.01 ± 0.69
$Cmax_2$ (mg/L)	1.26 ± 0.52	2.37 ± 0.73	3.65 ± 1.42
$Tmax_1$ (h)	1.9 ± 1.1	1.1 ± 0.2	2.1 ± 1.5
$Tmax_2$ (h)	2.8 ± 0.8	2.7 ± 1.4	3.5 ± 2.2

Discussion

In this randomized cross-over trial we investigated dose-reduction of CsA from 10 to 5 mg/kg co-administered to oral paclitaxel 2x 90 mg/m^2 in order to define the minimally effective dose of CsA that would still result in a maximal increase of the systemic exposure to paclitaxel. Dose-reduction of CsA from 10 to 5 mg/kg resulted in a significant decrease in the AUC, T>0.1 μM and T>0.05 μM values of orally administered paclitaxel at a dose of 2x 90 mg/m^2 in the same group of patients. After CsA 10 mg/kg these parameters were approximately 1.6-1.7 fold higher than after CsA 5 mg/kg. Compared to CsA 15 mg/kg [18], co-administration of 10 mg/kg CsA revealed similar paclitaxel AUC, T>0.1 μM and T>0.05 μM values. Parameters differed in a range of 0.9-1.1-fold between the CsA doses of 10 and 15 mg/kg. Previously we investigated the effect of dose-increment and dose-scheduling of CsA on the systemic exposure of orally administered paclitaxel 60 mg/m^2 in a single dose regimen [17]. Increasing the CsA dose from 15 to 30 mg/kg to achieve higher levels of the inhibitor did not result in an increase in the systemic exposure of paclitaxel [17]. In addition, administration of two doses of 15 mg/kg CsA, 10 minutes prior to and 2 hours after the oral intake of paclitaxel, to achieve more sustained levels of the inhibitor, did also not result in a further increase in the systemic exposure to paclitaxel [17]. Thus, combining the results from dose-increment and dose-scheduling of CsA with those of dose-reduction of CsA, P-gp inhibition by CsA appears to be maximal at CsA 10 mg/kg, which is therefore recommended for further studies of orally administered paclitaxel.

In our proof of principle study of oral paclitaxel with and without CsA [6,7] we suggested that the increase in systemic exposure to orally administered paclitaxel by CsA was most likely caused by inhibition of intestinal P-gp by CsA. In addition, we hypothesized that inhibition of paclitaxel metabolism by CsA may have contributed as we observed altered paclitaxel metabolism following CsA co-administration [7]. Paclitaxel is metabolized by the cytochrome P450 (CYP) isoenzymes 2C8 and 3A4, resulting in the metabolites 6a-hydroxypaclitaxel and 3'p-hydroxypaclitaxel, respectively (Figure 2) [31,32]. Both metabolites are substantially less active than the parent compound [32]. CsA itself is also metabolized by CYP 3A4 [33]. In our proof of principle study of oral paclitaxel [7], we found that after oral paclitaxel administration in combination with CsA, the relative contribution of formation of the metabolite 3'p-hydroxypaclitaxel was substantially lower than after i.v. administration of the drug, indicating inhibition of CYP 3A4 mediated paclitaxel metabolism by CsA. In this study, we were able to compare metabolite levels after three different doses of CsA. Higher doses of CsA resulted in a pronounced increase in the AUCt ratio of the metabolites 6a-hydroxypaclitaxel and 3'p-hydroxypaclitaxel, indicating a relative decrease in the

formation of the 3'p-hydroxypaclitaxel metabolite. These data also suggest inhibition of the CYP 3A4 mediated metabolic pathway of paclitaxel by CsA. Interpretation of the metabolite data should, however, be done with caution because of the small number of patients enrolled at each CsA dose level and the very large interpatient variability in the data. Furthermore, it is important to note that inhibition of the CYP 3A4 mediated pathway will not necessarily result in prolonged exposure of active parent compound as drug not handled by CYP 3A4 may escape through the CYP 2C8 pathway, which is, in general, the predominant metabolic pathway of paclitaxel.

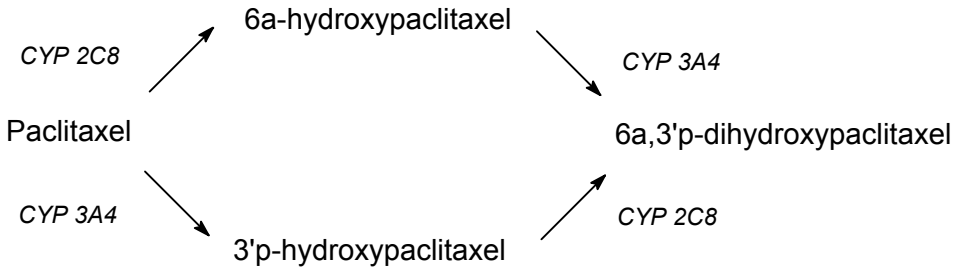

Figure 2. *Major metabolic pathways of paclitaxel.*

As plasma levels of Cremophor EL, the co-solvent suspect of causing the hypersensitivity reactions related to paclitaxel administration [21], were undetectable in our previous studies of oral paclitaxel [6,7,17,18], patients in this study, and previous studies of oral paclitaxel [17,18], received oral paclitaxel without premedication. We have confirmed these previously established data by measuring Cremophor EL levels in one patient at both oral courses, which were at all investigated time-points lower than the lower limit of quantitation. No hypersensitivity reactions were observed and evidently, paclitaxel (Paxene®) can be administered orally without the premedication regimen. Furthermore, absence of systemic Cremophor EL is important, because the co-solvent is responsible for the non-linear pharmacokinetic behavior of i.v. paclitaxel [14,34]. It entraps paclitaxel in the plasma compartment, which results in a more than proportional increase in plasma paclitaxel levels with increasing doses. However, studies in mice show that these higher total drug levels in plasma do not result in higher drug levels in tissues [35]. This pseudo-non-linearity of i.v. paclitaxel [27] implies that after oral paclitaxel administration, when Cremophor EL is not present, plasma levels of paclitaxel represent a higher fraction of free drug, which will result in enhancement of the availability of paclitaxel for the (tumor)tissues [27]. Therefore, pharmacokinetics of i.v. paclitaxel and orally administered paclitaxel, with and without systemic Cremophor EL, are substantially different and comparison of the pharmacokinetic parameters should be done with caution.

In conclusion, dose-reduction of CsA from 10 to 5 mg/kg resulted in a significant decrease in the AUC, T>0.1 μM and T>0.05 μM values of orally administered paclitaxel. However, the 5 mg/kg dose still provided paclitaxel levels that were greater than after paclitaxel alone [6]. CsA 10 mg/kg resulted in paclitaxel AUC, T>0.1 μM and T>0.05 μM values comparable to CsA 15 mg/kg, which was previously shown to reveal maximal inhibition of P-gp. Thus, CsA 10 mg/kg is determined as the minimally effective dose of CsA with a maximal increase in the systemic exposure to paclitaxel and is recommended for further studies of orally administered paclitaxel.

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CHAPTER 4.1

Co-administration of cyclosporin A strongly enhances the oral bioavailability of docetaxel

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Abstract

Purpose: Oral bioavailability of docetaxel is very low, which is, at least in part, due to its affinity for the intestinal drug efflux pump P-glycoprotein (P-gp). In addition, metabolism of docetaxel by cytochrome P450 (CYP) 3A4 in gut and liver may also contribute. The purpose of this study was to enhance the systemic exposure to oral docetaxel upon co-administration of cyclosporin A (CsA), an efficacious inhibitor of P-gp and substrate for CYP 3A4.
Patients and methods: A proof of concept study was carried out in 14 patients with solid tumors. Patients received one course of oral docetaxel 75 mg/m^2 with or without a single oral dose of CsA 15 mg/kg. CsA preceded oral docetaxel by 30 minutes. During subsequent courses patients received intravenous (i.v.) docetaxel 100 mg/m^2.
Results: The mean (± SD) area under the concentration-time curve (AUC) in patients who received oral docetaxel 75 mg/m^2 without CsA was 0.37 ± 0.33 mg.h/L and 2.71 ± 1.81 mg.h/L for the same oral docetaxel dose with CsA. The mean AUC of i.v. docetaxel 100 mg/m^2 was 4.41 ± 2.10 mg.h/L. The absolute bioavailability of oral docetaxel was 8 ± 6 % without and 90 ± 44 % with CsA. The oral combination of docetaxel and CsA was well tolerated.
Conclusion: Co-administration of oral CsA strongly enhanced the oral bioavailability of docetaxel. Interpatient variability in the systemic exposure after oral drug administration was of the same order as after intravenous administration. These data are promising and form the basis for the further development of a clinically useful oral formulation of docetaxel.

Introduction

In past years there has been an increasing interest in the development of oral treatment regimens of cytotoxic drugs. Patient convenience, practicality and pharmacoeconomics are major arguments in favor of oral therapy [1,2]. In addition, the oral route facilitates the use of more chronic treatment regimens, which results in prolonged exposure to the cytotoxic agent. For the taxanes paclitaxel and docetaxel, however, the low oral bioavailability has limited development of treatment by the oral route. The low systemic exposure of the taxanes after oral drug administration is, at least in part, due to their high affinity for the multidrug efflux pump P-glycoprotein (P-gp) [3,4]. P-gp in the mucosa of the gastro-intestinal tract limits the absorption of the orally administered taxanes and mediates their direct excretion into the gut lumen [3]. In addition, first-pass elimination by

cytochrome P450 (CYP) isoenzymes in the liver and/or gut wall may also contribute to the low oral bioavailability of paclitaxel (CYP 2C8 and CYP 3A4) and docetaxel (CYP 3A4) [5-7].

Preclinical experiments performed at the Netherlands Cancer Institute with mdr1a P-gp knock-out mice, which lack functional P-gp activity in the gut, have shown significant bioavailability of orally administered paclitaxel [3] and docetaxel (unpublished data). Additional studies with wild-type mice revealed good bioavailability after oral administration when paclitaxel [8] or docetaxel (unpublished data) was combined with cyclosporin A (CsA), an efficacious blocker of P-gp and substrate for the CYP 3A4 metabolic enzymes. Recently, we performed a clinical proof of concept study of orally administered paclitaxel in combination with oral CsA [9,10]. Co-administration of CsA resulted in a pronounced increase of at least 7-fold in the systemic exposure of paclitaxel. The most plausible explanation for the increase in the systemic exposure is inhibition of P-gp by CsA. In addition, inhibition of paclitaxel metabolism, mediated by CYP 3A4, most likely contributed, as we observed altered paclitaxel metabolism after CsA co-administration [10]. Given our preclinical research and clinical data of oral paclitaxel with CsA, we hypothesized that the systemic exposure of orally administered docetaxel would be increased by co-administration of oral CsA. To investigate this, we initiated a proof of concept study in patients with solid tumors.

Patients and Methods

Patient Population

Patients with a histologically confirmed cancer refractory to current therapies were eligible for the study. Previous radiotherapy or chemotherapy other than taxoid therapy was allowed, provided that the last treatment was at least 4 weeks prior to study entry and any resulting toxicities were resolved. Eligibility criteria included acceptable bone marrow function (WBC count > 3.0 x 10^9/L; platelet count > 100 x 10^9/L), liver function (serum bilirubin level $\leq$ 20 µmol/L; serum albumin level $\geq$ 25 g/L), kidney function (serum creatinine $\leq$ 160 µmol/L or clearance $\geq$ 50 mL/min) and a World Health Organization (WHO) performance status $\leq$ 2. Patients were not eligible if they suffered from uncontrolled infectious disease, neurologic disease, bowel obstruction or symptomatic brain metastases. Other exclusion criteria were concomitant use of known P-gp inhibitors and chronic use of H2-receptor antagonists or proton pump inhibitors. The study protocol was approved by the medical ethics committee of the Institute, and all patients had to give written informed consent.

Study Design

In the first part of the study, a small cohort of four patients received oral docetaxel without CsA at a dose of 75 mg/m^2 during course 1 and intravenous (i.v.) docetaxel at a dose of 100 mg/m^2 administered as a 1-hour infusion during course 2. In the second part of the study, 10 patients received oral docetaxel 75 mg/m^2 plus oral CsA 15 mg/kg at one occasion and i.v. docetaxel at another occasion. In this part of the study the oral course and i.v. course were randomized. If it was considered to be in their best interest patients continued on a 3-weekly schedule of i.v. docetaxel with a maximum of six i.v. courses. An oral docetaxel dose of 75 mg/m^2 (< 100 mg/m^2) was selected for safety reasons because preclinical data on oral paclitaxel revealed that co-administration of a P-gp inhibitor and an oral paclitaxel dose can result in a higher systemic exposure than after i.v. administration of the same dose [11].

Drug Administration

The i.v. formulation of docetaxel (Taxotere®, Rhône-Poulenc Rorer/Aventis, Antony, France) was used for both i.v. and oral administration of the agent. Thirty minutes prior to oral docetaxel administration patients ingested the CsA capsules (Neoral®, Novartis, Basel, Switzerland). Two patients received the oral solution of CsA (Neoral®), which was ingested 10 minutes prior to intake of docetaxel. Oral drugs were taken with 100 mL of tap water after an overnight fast. Patients remained fasted until 2 hours after oral docetaxel administration. Standard docetaxel pretreatment was given with all courses and consisted of oral dexamethasone 8 mg 1 hour before drug administration and 4 mg every 12 hours (four times) after drug administration. Before oral docetaxel administration patients received 1 mg of oral granisetron (Kytril®).

Patient Evaluation

Pretreatment evaluation included a complete medical history and complete physical examination. Before each course, an interim history including concomitant medications taken, toxicities and performance status were registered and a physical examination was performed. Hematology was checked twice weekly after courses 1 and 2 and weekly after subsequent courses. Blood chemistries including liver and renal function, serum electrolytes, total protein and albumin and glucose levels, were checked weekly. All toxicities observed were graded according to National Cancer Institute common toxicity criteria [12]. Dose limiting toxicity was defined as grade 4 granulocytopenia of a duration of >5 days, grade 4 thrombocytopenia of any duration or any grade 3 or 4 nonhematologic toxicity except alopecia and untreated nausea and vomiting. Tumor measurements were

performed every other cycle, but initially after the first two i.v. courses. Responses were evaluated according to the WHO criteria [13].

Analysis

Pharmacokinetic monitoring was performed during course 1 and course 2. For plasma docetaxel and metabolite concentrations, blood samples of 5 ml each were collected in heparinized tubes. After oral administration, samples were obtained before dosing, at 15, 30, 45, 60, 75 and 90 minutes and 2, 3, 4, 7, 10, 24, 30, and 48 hours after docetaxel ingestion. During i.v. administration, samples were obtained before starting, 30 and 45 minutes after starting, at the end of the infusion, and at 5, 10, 20, 30, 60 and 90 minutes and 2, 3, 4, 7, 10, 24, 30, and 48 hours after infusion. Blood samples were centrifuged, plasma was separated and samples were immediately stored at -20ºC until analysis. Docetaxel and metabolite concentrations in plasma were determined using a validated high performance liquid chromatography assay [14]. For CsA whole blood concentrations, blood samples drawn for docetaxel analysis were used. An aliquot of the whole blood sample was stored at 4ºC and analyzed within 1 week using a specific fluorescence polarization immunoassay (TDxFLx, Abbott Laboratories) [15].

Pharmacokinetics

Non-compartmental pharmacokinetic methods were applied to process the results [16]. The area under the concentration-time curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity using the terminal rate constant k. Bioavailability of oral docetaxel was calculated as the ratio of the AUC after oral and after i.v. administration with a correction for the difference in dose. Other parameters to be assessed were the maximal concentration, the time to maximal concentration, the terminal half life, total plasma clearance after i.v. administration, and the apparent volume of distribution at steady state after i.v. administration. The maximal concentration and time to maximal concentration were observed measured values; the other parameters were calculated using noncompartmental methods [16]. Statistical analysis of the data was performed using the nonparametric Mann-Whitney U-test. The a priori level of significance was p=0.05.

Results

Patient Characteristics

A total of 14 patients (men/women 4/10) was enrolled onto the study (Table 1). At study entry, the median age of the patients was 52 years (range 31 to 73 years)

and the median WHO performance status was 1 (range 0 to 2). Primary tumor types included breast cancer (n=8), non-small-cell lung cancer (n=3), small-cell lung cancer (n=1), esophageal cancer (n=1), and stomach cancer (n=1). All patients had received prior surgical therapy, radiotherapy and/or chemotherapy.

Table 1. *Patient characteristics.*

No. of patients	14
Male/Female	4/10
Age, years	
Median (range)	52 (31-73)
WHO performance status	
Median (range)	1 (0-2)
Tumor type	
Breast	8
Non-small-cell lung	3
Small-cell lung	1
Esophagus	1
Stomach	1
Prior treatment	
Surgical therapy	1
Chemotherapy	2
Surgical therapy and chemotherapy	2
Radiotherapy and chemotherapy	4
Surgical therapy, radiotherapy and chemotherapy	5

Pharmacokinetics

Individual plasma pharmacokinetic parameters of orally administered docetaxel are presented in Table 2. The pharmacokinetic data of one patient, who received oral docetaxel without CsA, could not be determined due to (unknown) interfering compounds in the analytical assay. The data revealed that co-administration of CsA resulted in a pronounced increase in the mean AUC value of orally administered docetaxel (75 mg/m^2) from 0.37 ± 0.33 mg.h/L (n=3) without CsA up to 2.71 ± 1.81 mg.h/L (n=10) in combination with CsA (Figure 1). The mean oral AUC value in the cohort of 10 patients who started with oral docetaxel plus CsA was not significantly different from the mean oral AUC in the patients who received oral docetaxel plus CsA at the second occasion. The oral bioavailability of docetaxel, calculated as the ratio of the AUC after oral and after i.v. administration with a correction for the difference in dose, was 8 ± 6% without CsA and 90 ± 44% in combination with CsA

(p=0.011). The pharmacokinetic data for i.v. docetaxel were in good agreement with data from previous studies [17] (Table 3). The mean AUC value of i.v. administered docetaxel (100 mg/m^2 as a 1-h infusion) was 4.27 ± 2.26 mg.h/L (n=10). The coefficient of variation of the AUC after oral docetaxel administration in combination with CsA was 67% (n=10), and after i.v. administration 53% (n=10).

The plasma pharmacokinetic parameters of the docetaxel metabolites M1, M2, M3 and M4 (Figure 2) after oral administration of docetaxel with CsA and after i.v. administration are presented in Table 4. After oral administration of docetaxel without CsA, metabolites could not be detected. After oral administration with CsA, the mean AUC ratios of M1+M2 (not separated in the analytical assay), M3 and M4 to docetaxel were 0.31, 0.11 and 0.11, respectively. After i.v. administration, only metabolite M4 could be detected with a mean AUC ratio to docetaxel of 0.01.

Whole blood CsA concentrations were measured in nine patients. The mean maximum CsA concentration was 3.92 ± 0.88 mg/L and was reached at 2.0 ± 0.8 h after intake. The mean AUC value of CsA was 31.0 ± 9.3 mg.h/L.

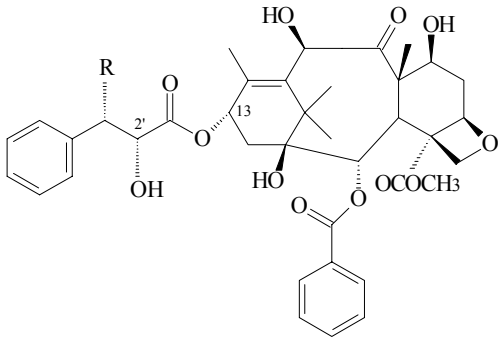
Table 2. *Main pharmacokinetic parameters of docetaxel after oral administration at a dose of 75 mg/m^2 without (patients no. 1-4) and with (patients no. 5-14) CsA.*

Patient No.	Tmax (h)	Cmax (mg/L)	AUC (mg.h/L)	F (%)
1	NA	NA	NA	NA
2	1.5	0.03	0.08	3
3	1.3	0.13	0.30	8
4	3.0	0.30	0.73	14
Mean	1.9	0.15	0.37	8
SD	0.9	0.14	0.33	6
5	0.4	0.55	1.14	72
6	1.9	1.20	2.60	109
7	0.7	0.39	1.40	56
8	1.6	0.57	1.85	52
9	0.8	0.58	1.58	88
10	1.0	1.07	2.43	33
11	2.0	1.13	3.76	111
12	2.1	1.49	7.41	187
13	2.0	0.79	2.45	76
14	2.0	0.81	2.48	112
Mean	1.5	0.86	2.71	90
SD	0.7	0.35	1.81	44

NA, not assessable.

Table 3. *Main pharmacokinetic parameters of docetaxel 100 mg/m^2 after a 1-hour i.v. infusion (mean ± SD).*

PK parameter	Patients No. 5-14	Patients No. 1-14
Cmax (mg/L)	3.15 ± 0.67	3.21 ± 0.77
AUC (mg.h/L)	4.27 ± 2.26	4.41 ± 2.10
CL (L/h/m^2)	28 ± 11	27 ± 10
Vss (L/m^2)	190 ± 240	180 ± 210



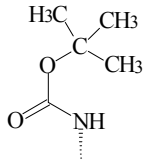
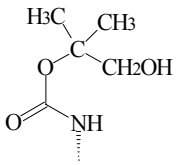
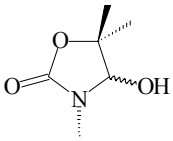
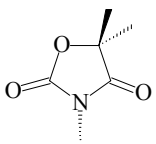
Compound	R
Docetaxel	
M2	
M1+M3	
M4	

Figure 2. *Structural formulas of docetaxel and the metabolites M1, M2, M3 and M4.*

Table 4. *Pharmacokinetic parameters of the metabolites M1, M2, M3 and M4 (mean ± SD).*

	No.	Tmax (hr)	Cmax (mg/L)	AUCt (mg.h/L)	AUCt metabolite/ AUC docetaxel
M1+M2[a]					
Oral docetaxel + CsA	9	1.9 ± 0.8	0.20 ± 0.09	0.84 ± 0.51	0.31 ± 0.11
IV docetaxel		ND	ND	ND	ND
M3					
Oral docetaxel + CsA	2	2.0 ± 1.4	0.05 ± 0.04	0.37 ± 0.46	0.11 ± 0.11
IV docetaxel		ND	ND	ND	ND
M4					
Oral docetaxel + CsA	9	3.6 ± 1.5	0.07 ± 0.03	0.28 ± 0.16	0.11 ± 0.05
IV docetaxel	9	1.2 ± 0.1	0.06 ± 0.07	0.03 ± 0.02	0.01 ± 0.01

No metabolites could be detected after oral administration of docetaxel without CsA;
[a]metabolites M1 and M2 could not be separated in the analytical assay, the sum is reported;
ND, not detectable.

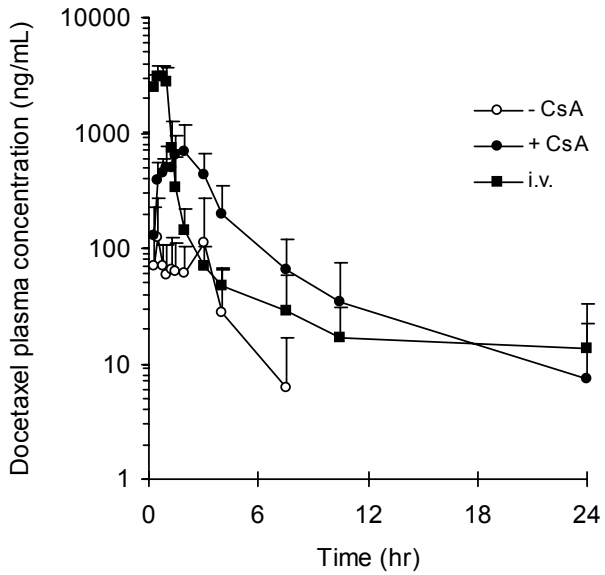


Figure 1. *Plasma concentration-time curves of i.v. administered docetaxel and oral docetaxel with and without CsA represented as means ± SD.*

Toxicities

Docetaxel administered orally was very well tolerated. After oral docetaxel administration in combination with CsA (n=10), the hematologic toxicities observed were anemia (6 patients), which was often pre-existing, leukocytopenia (8 patients) and granulocytopenia (6 patients) (Table 5). Hematologic toxicities were relatively

mild (grade 1 or 2), except for leukocytopenia and granulocytopenia grade 3 in two patients. The main non-hematologic toxicities after oral intake of docetaxel in combination with CsA were fatigue (7 patients), alopecia (4 patients), diarrhea (3 patients), nausea (3 patients), and flu-like feelings (3 patients) (Table 6). Non-hematologic toxicities did not exceed grade 2 in severity. Toxicities clearly associated with CsA administration were not observed. During the first course of i.v. administered docetaxel (n=10), hematologic toxicities observed were anemia (6 patients), which was often pre-existing, leukocytopenia (nine patients) and granulocytopenia (8 patients) (Table 5). Hematologic toxicities were generally grade 3 or less in severity, except for grade 4 granulocytopenia in two patients. One patient developed grade 3 neutropenic fever that required hospitalization and was treated with antibiotics. The principal non-hematologic toxicities after i.v. administered docetaxel (n=10) were fatigue (5 patients), alopecia (5 patients), arthralgia/myalgia (4 patients), flu-like feelings (4 patients), infections (3 patients), skin reactions (3 patients), diarrhea (2 patients), and nausea (2 patients) (Table 6). Nonhematologic toxicities were relatively mild (grade 1 or 2), except for grade 3 diarrhea in one patient.

Table 5. *Hematologic toxicities observed after oral docetaxel administration in combination with CsA and after the first i.v. course of docetaxel.*

	Oral docetaxel 75 mg/m^2 + CsA	IV docetaxel 100 mg/m^2	IV docetaxel 100 mg/m^2
Patients No.	5-14	5-14	1-14
Anemia			
Grade 1	5	5	5
Grade 2	1	1	1
Leukocytopenia			
Grade 1	5	2	2
Grade 2	1	1	1
Grade 3	2	6	9
Grade 4	0	0	0
Granulocytopenia			
Grade 1	4	0	0
Grade 2	0	3	3
Grade 3	2	3	4
Grade 4	0	2	4

No significant toxicities were observed in the four patients who received oral docetaxel without CsA.

Table 6. *Nonhematologic toxicities observed after oral docetaxel administration in combination with CsA and after the first i.v. course of docetaxel.*

	Oral docetaxel 75 mg/m^2 + CsA	IV docetaxel 100 mg/m^2	IV docetaxel 100 mg/m^2
Patients No.	5-14	5-14	1-14
Nausea			
Grade 1/2	3	2	2
Grade 3/4	0	0	1
Vomiting			
Grade 1/2	2	1	2
Grade 3/4	0	0	1
Diarrhea			
Grade 1/2	3	1	3
Grade 3/4	0	1	1
Abdominal pain			
Grade 1/2	2	0	0
Arthralgia/myalgia			
Grade 1/2	1	4	7
Alopecia			
Grade 1/2	4	5	7
Fatigue			
Grade 1/2	7	5	9
Neurosensory toxicities			
Grade 1	2	2	3
Grade 2	0	0	1
Mucositis			
Grade 1/2	0	2	2
Sore throat			
Grade 1/2	1	2	2
Dry mouth			
Grade 1/2	2	2	2
Flu-like feelings			
Grade 1/2	3	4	4
Infection			
Grade 1/2	0	3	3
Skin reactions			
Grade 1/2	1	3	3

No significant toxicities were observed in the four patients who received oral docetaxel without CsA.

Antitumor Activity

One complete response and three partial responses were documented after three or four courses (one oral course and two or three i.v. courses). Evaluation in one patient with esophageal carcinoma revealed a substantial decrease in subcarinal mass (pathologic lymph node) after one oral course of docetaxel. After the third course a partial response was observed. After the seventh course a radiologic complete response was observed. In one patient with breast cancer the CA 15.3 marker decreased by approximately 20% after one oral course of docetaxel. After the third course a partial response of the liver metastases was observed. Two other patients with breast cancer developed a partial response after four courses of docetaxel.

Discussion

The results presented here show that co-administration of oral CsA strongly enhances the systemic exposure to orally administered docetaxel. Docetaxel administered without CsA exhibits poor oral bioavailability of only 8 ± 6 %, whereas oral docetaxel in combination with CsA reaches a bioavailability of 90 ± 44 % (p=0.011). Furthermore, the coefficient of variation in the systemic exposure after oral drug administration was of the same order as after i.v. administration, i.e. 67% and 53%, respectively. Thus, oral administration did not result in a notable increase in the interpatient difference in systemic exposure.

Our preclinical data obtained in wild-type mice and mdr1ab P-gp knock-out mice (unpublished data) combined with these first clinical data indicate that CsA increases the absorption of orally administered docetaxel by effectively blocking P-gp in the gastro-intestinal tract. In addition, inhibition of docetaxel metabolism in the gut wall and/or liver by CsA may also contribute to the increased systemic exposure, as both docetaxel and CsA are substrates for the CYP 3A4 metabolic system [6,7,18,19]. The four major metabolites of docetaxel - M1, M2, M3, and M4 - originate from successive oxidations of the parent compound by CYP 3A4 [6,7]. In *in vitro* cytotoxicity studies, all four metabolites were significantly less potent than docetaxel [20]. Competition for CYP 3A4 by CsA may result in altered plasma levels of docetaxel and metabolites and thereby may result in altered ratios of metabolite to docetaxel. After oral docetaxel administration without CsA no metabolites were detected in plasma. Therefore, the effect of CsA on the metabolism of orally administered docetaxel could not be determined. Oral ingestion of docetaxel in combination with CsA, however, resulted in an increase of the mean AUC ratio of metabolite M4 to docetaxel compared with i.v.

administration, i.e. 0.11 and 0.01, respectively. This relative increase in docetaxel metabolism after oral administration can be explained by the relatively higher initial amount of drug passing through the liver (first-pass effect). Additionally, metabolism of docetaxel in the intestinal wall may also contribute to the higher metabolite levels after oral administration. Increased metabolism after oral drug administration may result in lower levels of the active drug and possibly, reduced efficacy. However, the results show that the achieved gain in increased uptake largely outweighs the possible loss by the increased metabolism.

The oral combination of docetaxel and CsA was very well tolerated. The main side effects were myelosuppression and fatigue, which were mild to moderate. Theoretically, co-administration of a P-gp inhibitor may cause toxicities due to inhibition of the physiologic protective function of P-gp. P-gp inhibition could cause an increase in the docetaxel levels in P-gp protected brain tissue and may therefore enhance the risk of central neurotoxicity [21,22]. However, we did not observe any signs or symptoms of central neurotoxicity in our study or in the animal studies. The single oral dose of CsA 15 mg/kg resulted in peak and trough CsA concentrations that were in the therapeutic range for immunosuppression and may be associated with toxicity, particularly renal toxicity. In this study we did not observe renal toxicity nor any other side effects clearly related to the single administered CsA dose.

After the first i.v. course of docetaxel, a similar pattern of toxicities was observed as after oral drug administration, which order was randomized. However, myelosuppression seemed to occur more often and to be more severe after i.v. administration than after oral drug administration. This may be related to the higher peak concentrations and AUC values of docetaxel after i.v. administration (100 mg/m^2) compared with oral drug administration (75 mg/m^2). Myelosuppression is often observed after i.v. administration of docetaxel 100 mg/m^2 every 3 weeks, and reduction of myelosuppression is one of the reasons for initiation of weekly i.v. schedules of docetaxel at lower doses. In addition, the hypothesis that dose intensification and more frequent exposure of tumor cells to docetaxel may enhance activity of the drug has also contributed to the start of weekly docetaxel schedules. Recent clinical studies have shown that administration of i.v. docetaxel on a weekly schedule decreases the hematologic toxicity profile of the drug while therapeutic activity is maintained [23-25]. The feasibility of oral drug administration may stimulate and facilitate the use of weekly treatment schedules of docetaxel. We are currently investigating weekly oral docetaxel in combination with CsA in a phase II study in patients with advanced breast cancer.

These promising results of a substantial increase in the oral bioavailability of docetaxel due to inhibition of P-gp suggests that this concept may well be applied to other drugs, including non-cytotoxic agents, that have a high affinity for P-gp and

associated poor oral bioavailability, eg, human immunodeficiency virus protease inhibitors [26]. At present, it remains uncertain to what extent inhibition of docetaxel metabolism by CsA contributes. In addition, other CsA induced actions on currently unknown transporters may also contribute to the increase in oral docetaxel bioavailability. Further investigations with more selective P-gp inhibitors are planned to differentiate between inhibition of P-gp and inhibition of drug metabolism.

In summary, co-administration of oral CsA strongly enhanced the oral bioavailability of docetaxel. Furthermore, the interpatient variability was of the same order for orally and intravenously administered docetaxel. The safety of the single oral course was very good. These data are stimulating for the further development of a clinically useful oral formulation of docetaxel. A phase II study in patients with advanced breast cancer aimed at assessment of the antitumor activity of the weekly oral combination of docetaxel and CsA is currently ongoing.

Acknowledgements

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CHAPTER 4.2

Pharmacokinetics of oral cyclosporin A when co-administered to enhance the absorption of orally administered docetaxel

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Abstract

Objective: To evaluate the pharmacokinetics of oral cyclosporin A (CsA) when co-administered to enhance the absorption of orally administered docetaxel.
Methods: Patients (n=9) with histologic proof of solid cancer received oral docetaxel 75 mg/m^2 in combination with oral CsA 15 mg/kg.
Results: The area under the blood concentration-time curve (AUC) of CsA when combined with docetaxel 75 mg/m^2 was 31.0 ± 9.3 mg.h/L (mean ± SD). Compared to literature data of the same dose of CsA, AUC values in our study appear to be substantially higher. In addition, compared to the AUC values of CsA in combination with oral paclitaxel (previously published data) AUC values in this study are approximately 1.5-fold higher.
Conclusions: The higher AUC values of CsA obtained in this study compared to literature data may be explained by competitive inhibition of cytochrome P450 (CYP) 3A4 mediated metabolism of CsA by docetaxel. In addition, the higher levels of CsA with docetaxel compared to paclitaxel co-administration may be explained by the fact that docetaxel is almost exclusively metabolized by CYP 3A4, whereas paclitaxel is predominantly metabolized by CYP 2C8 and to a lesser extent by CYP 3A4.

Introduction

The anticancer agent docetaxel shows very low oral bioavailability which is, at least in part, due to its affinity for the intestinal drug efflux pump P-glycoprotein (P-gp) [1]. In addition, metabolism of docetaxel by cytochrome P450 (CYP) 3A4 in gut and liver may also contribute, as docetaxel is a known substrate for these enzymes [2]. Recently, we demonstrated the feasibility of oral administration of docetaxel in cancer patients by co-administration of cyclosporin A (CsA), an efficacious blocker of P-gp [3] and substrate/inhibitor of CYP 3A4 [4]. Co-administration of CsA resulted in a pronounced increase in the oral bioavailability of docetaxel from 8% without CsA up to 90% in combination with CsA [5]. Here we present the pharmacokinetic data of CsA when used as ‘absorption enhancer’ of docetaxel.

Patients and Methods

Study Design
Patients with histologic proof of solid cancer for whom no standard therapy of proven benefit existed and who had not received prior taxoid therapy were eligible. Inclusion and exclusion criteria have been described in detail elsewhere [5].

Patients received oral docetaxel (i.v. formulation) (Taxotere®, Rhône-Poulenc Rorer/Aventis, Antony, France) at a dose of 75 mg/m^2 in combination with oral CsA 15 mg/kg, a dose which was previously shown to reveal maximal inhibition of P-gp [6]. CsA (Neoral®, Novartis, Basel, Switzerland) was taken either 30 minutes prior to docetaxel intake when ingested as capsules or 10 minutes when taken as an oral solution. Docetaxel pretreatment consisted of oral dexamethasone 8 mg 1 hour prior to and 4 mg every 12 hours (4 times) after drug administration. Prior to the oral drug combination patients received 1 mg oral granisetron (Kytril®). Oral drugs were taken after an overnight fast and patients remained fasted until 2 hours following drug ingestion.

Pharmacokinetics

Blood samples for pharmacokinetic analysis of CsA were collected in heparinized tubes, pre-dose, 15, 30, 45, 60, 75, 90, 105 minutes and 2, 2.5, 3.5, 4.5, 7.5, 10.5, 24.5, 30.5 and 48.5 hours after ingestion of CsA. Whole blood samples were stored at 4ºC and analyzed within one week using a specific fluorescence polarization immuno assay (specific-FPIA) (TDxFLx, Abbott Laboratories, Amstelveen, The Netherlands) [7,8]. Non-compartmental pharmacokinetic methods were applied to process the results [9]. The area under the CsA concentration-time curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity using the terminal rate constant k. The maximal plasma concentration (Cmax) and the time to maximal plasma concentration (Tmax) were observed measured values. Data are presented as means ± SD.

Results

In total nine patients were sampled for CsA pharmacokinetic analysis. These patients were 4 male and 5 female with a median age of 53 years (range 38-65) and median weigth of 73 kg (range 57-90). The mean CsA AUC value in these patients was 31.0 ± 9.3 mg.h/L. The mean CsA Cmax value was 3.92 ± 0.88 mg/L, which was reached at 2.0 ± 0.8 hours after intake (Figure 1). Ingestion of CsA as capsules (n=7) or as the oral solution (n=2) did not appear to show differences in the pharmacokinetics of CsA.

Comparison of these CsA data with those available in the literature shows higher AUC values for this study (Table 1). We have used the CsA (Neoral®) data by Mueller et al. [10], who investigated single dose CsA administration in a dose-range of 200 to 800 mg in healthy volunteers. This dose-range revealed a linear relationship between dose and AUC, with an AUC of CsA of 12.4 mg.h/L for the

dose of 800 mg. By linear extrapolation, the median applied CsA dose in our study of 1100 mg would then result in an AUC of CsA of 17.0 mg.h/L. No pharmacokinetic data are known to us of a single dose of CsA of 15 mg/kg. When given as an immunosuppressant, CsA is generally given (bi-daily) at lower dosages. In addition, we have compared our CsA data with those of CsA (Neoral®) in combination with paclitaxel administration, which we have published previously [6]. From Table 1 it can be seen that docetaxel co-administration appears to result in higher CsA AUC values than paclitaxel co-administration.

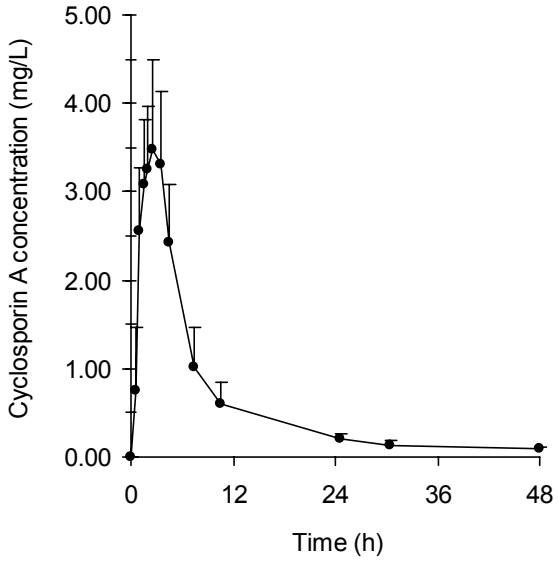

Figure 1. *Plasma concentration-time curve of oral CsA at a dose of 15 mg/kg co-administered with oral docetaxel 75 mg/m^2 (n=9). Data are presented as means ± SD.*

Table 1. *AUC values of CsA when given in combination with oral docetaxel (this study), given as single agent [10] and given in combination with oral paclitaxel [6]. Data are presented as means ± SD.*

CsA dose	In combination with	AUC value (mg.h/L)	Reference
15 mg/kg	Oral docetaxel 75 mg/m^2	31.0 ± 9.3	This study
800 mg	Single agent	12.4 ± 3.1	[10]
15 mg/kg	Oral paclitaxel 60 mg/m^2	24.4 ± 9.9	[6]
15 mg/kg	Oral paclitaxel 300 mg/m^2	17.6 ± 2.8	[6]

Discussion and Conclusions

This is the first study that describes the pharmacokinetics of CsA when co-administered with oral docetaxel.

Compared to the pharmacokinetic data of CsA administered as single agent as presented by Mueller and co-workers [10] AUC values of CsA in our study appear to be considerably higher. These higher AUC values may be explained by competitive inhibition of CYP 3A4 mediated CsA metabolism by docetaxel. Docetaxel may inhibit CsA metabolism in both the liver and the intestinal wall, which has been shown to be a major site of CsA breakdown [11]. Increases in CsA levels due to inhibition of metabolism have also been observed for other drugs such as ketoconazol, erythromycine, amiodaron, allopurinol, verapamil, diltiazem, nicardipine and danazol [12]. In addition, competitive inhibition of intestinal P-gp by docetaxel may also contribute to the high levels of CsA in our study. Lown and co-workers [13] have shown a significant role of intestinal P-gp in the first-pass elimination of CsA. It should be noted, however, that our CsA data have been generated with a different analytical assay than those of Mueller, who used a specific ^{3}H radioimmunoassay (RIA, Sandimmun, Sandoz Ltd). It has been shown that the specific FPIA we used results in 6-25% higher CsA whole blood levels compared to the RIA with the higher deviation for the lower levels [14-16]. For the FPIA, cross reactivity with CsA metabolites has been reported with percentages up to 20% [7]. However, even taking the highest range into account, CsA AUC values in our study still appear to be substantially higher. Furthermore, the potential effect of dexamethasone co-administration in our study as an inducer of CYP 3A4 metabolism [17] strengthens our hypothesis of increased CsA levels by co-administration of docetaxel therapy.

Comparison of the CsA pharmacokinetic data between paclitaxel and docetaxel co-administration suggests higher CsA AUC values when the drug was combined with docetaxel. These higher values may be explained by the fact that metabolism of docetaxel is almost exclusively performed by CYP 3A4 [2], whereas metabolism of paclitaxel is predominantly mediated by CYP 2C8 and to a lesser extent by CYP 3A4 [18]. Consequently, relatively more competitive inhibition of the CYP 3A4 mediated CsA metabolism can be expected from docetaxel than with paclitaxel.

The therapeutic range of CsA as immunosuppressant in kidney and heart transplant patients is 150-250 ng/mL determined as trough levels 12 hours after intake and analyzed with the specific FPIA assay [8]. Higher levels may result in toxicity of the drug of which nephropathy is most prominent [19]. CsA levels in our study at 10.5 hours after intake were far above this range, i.e. 620 ± 250 ng/mL (n=9). Toxicities of CsA were, however, not observed. We measured serum

creatinine levels prior to and after treatment with CsA and docetaxel, which were very similar. The absence of CsA toxicities in this study can most likely be attributed to the single dose administration of the drug, while in the transplantation setting CsA is ingested on a continuous daily basis. When CsA is given repeatedly to enhance absorption of oral docetaxel, accurate monitoring of the renal function is recommended.

In conclusion, our study of CsA in combination with oral docetaxel reveals substantially higher CsA levels compared to those administered as a single agent or in combination with paclitaxel. Most likely this is caused by specific inhibition of CYP 3A4 mediated CsA metabolism in gut and/or liver by docetaxel.

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CHAPTER 4.3

A phase II study with weekly oral docetaxel and cyclosporin A in patients with metastatic breast cancer

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Interim Analysis

Abstract

The oral bioavailability of docetaxel is low and variable due to the high affinity of docetaxel for the drug efflux pump P-glycoprotein (P-gp). Pharmacokinetic results of a phase I study have shown that co-administration of cyclosporin A (CsA), a P-gp blocker, resulted in a pronounced increase in the oral bioavailability of docetaxel from 8 ± 5% without CsA up to 90 ± 44% with CsA. The aim of this phase II study was to asses response and toxicity of this combination in patients with metastatic breast cancer.

Patients and methods: Currently, twenty patients with metastatic breast cancer and bidimensionally measurable disease and who had received prior anthracycline containing chemotherapy were entered in this study. A weekly oral dose of 100 mg docetaxel was given which leads to an area under the plasma concentration versus time curve (AUC) equivalent to an intravenous dose of 40 mg/m^2. One course consisted of a weekly dose of docetaxel for six weeks every 8 weeks. Thirty minutes prior to intake of docetaxel, CsA capsules were taken in a dose of 15 mg/kg. The drugs were taken with 100 ml tap water after an overnight fast. Pharmacokinetics of docetaxel were determined during week 1 and 9.

Results: At interim analysis all twenty patients were evaluable for toxicity. Sixteen patients were evaluable for response. Four patients went off study prematurely because of vomiting (2) and malaise (1) and one patient died after 4 weekly cycles because of neutropenic fever. Median age was 48 years (range 39-60) and median WHO score was 1 (range 0-2). There were 2 complete (12.5%) and 6 partial responses (37.5%) with an overall response rate (ORR) of 50%. Five patients (31%) had stable disease and 3 patients (19%) progressive disease. Most frequently recorded toxicities were: leucopenia CTC grade 3 and 4 (50%) neutropenia grade 3 and 4 (60%), diarrhea grade 2 (35%), stomatitis grade 2 (25%), nail toxicity grade 2 (45%), fatigue grade 2 and 3 (45%) and fluid retention grade 2 (20%). The mean docetaxel AUC in these patients was 2.50 ± 1.43 mg.h/l in week 1 and 2.12 ± 0.48 mg.h/l in week 9 with interpatient variabilities of 57% and 23%, respectively. The intrapatient variability was 35%.

Conclusion: Weekly oral docetaxel in combination with CsA is feasible and shows at interim analysis an ORR of 50%. The non-hematological toxicity profile consists mainly of fatigue, gastro-intestinal toxicity especially manageable diarrhea and nail toxicity. Hematological toxicity seems to be less severe than after intravenous administration. The study will continue and will recruit 25 evaluable patients.

Introduction

Docetaxel, a prototype taxane, is an effective anticancer agent in patients with metastatic breast cancer. The drug has shown good activity both in first line and second line of therapy, including patients previously treated with anthracyclines. Response rates vary between 35 and 58% [1-5]. A recently published phase III study in patients with metastatic breast cancer, previously treated with anthracyclines, has shown that the docetaxel arm is significantly superior to the mitomycin/vinblastin arm with an overall response rate (ORR) of 30%, median time to progression (TTP) of 19 weeks and an overall survival (OS) of 11.4 months [6]. The standard dose is 75-100 mg/m^2 given as a 1-hour infusion every 21 days with dose limiting toxicity (DLT) of myelosuppression. Other common toxicities are fatigue, alopecia, skin and nail toxicity and fluid retention. At the moment weekly schedules of docetaxel are increasingly used. The rationale behind weekly administration of docetaxel is based on the potential to increase dose intensity resulting in more frequent exposure of the tumor cells to the drug. Phase I studies have shown that the toxicity profile of a weekly schedule markedly alters and is different from the registered 3-weekly schedule [7-11]. In the weekly regimen the DLT is fatigue and less hematological toxicity is observed [7-11]. The low rate of myelosuppression makes this schedule a more convenient way to administer docetaxel. Other reported side effects are nail changes, alopecia, and sensory neuropathy. The recommended dose from phase I studies ranged from 35-45 mg/m^2/week [7-11]. The therapeutic activity of a weekly schedule has been maintained and is promising. A recently published phase II study revealed an ORR of 41 % (CI 95%, 24-61%) in 29 evaluable patients and the regimen was generally well tolerated [12]. Another study also showed encouraging efficacy (ORR 48%) in heavily pretreated patients with metastatic breast cancer [13]. An additional benefit is that weekly docetaxel can be combined with other weekly regimens for example with vinorelbine or gemcitabine [14] or in combination with radiotherapy [15]. In the past years there has been an increasing interest in the development of oral treatment regimens of cytotoxic drugs as oral administration is more convenient and practical for patients and facilitates the use of treatment schedules with more frequent dosing [16]. However, the low oral bioavailability of docetaxel, due to the high affinity of docetaxel for the drug efflux pump P-glycoprotein (P-gp) in the gut [17], has limited development of treatment by the oral route. P-gp in the mucosa of the gastro-intestinal tract limits the absorption of orally administered docetaxel. In mdr1 P-gp knock-out mice, which lack functional P-gp activity in the gut, the oral bioavailability of docetaxel was significantly increased compared to wild-type mice [18]. In addition, good oral bioavailability of docetaxel was achieved in wild-type

mice when the drug was combined with the P-gp blocker cyclosporin A (CsA) [18]. Pharmacokinetic results of a phase I study performed at our Institute have shown that co-administration of oral CsA resulted in an increase in the oral bioavailability of docetaxel from 8% ± 5% without CsA to 90% ± 44% with CsA [19].
The aim of this phase II study was to asses activity and toxicity of the combination of oral docetaxel and oral CsA given on a weekly basis in patients with metastatic breast cancer previously treated with anthracyclines. In addition, we evaluated the pharmacokinetics of this combination.

Patients and Methods

Eligibility Criteria
Patients with histologically confirmed metastatic breast cancer were eligible for this study. Patients were required to be at least 18 years of age. All patients had to have received at least one prior anthracycline containing chemotherapy regimen for the treatment of metastatic or (neo) adjuvant disease. Prior taxane therapy was not allowed. At entry patients were required to have bidimensionally measurable disease according to the WHO-criteria [20]. They had to have adequate hematological, renal and hepatic functions (ANC $\geq$ 2.0 x 10^9/L, platelets $\geq$ 100 x 10^9/L, total bilirubin $\leq$ 20 µmol/L, AST (SGOT) and/or ALT (SGPT) $\leq$ 2.5 upper normal limit, unless liver metastases then $\leq$ 5 x upper normal limit, serum creatinine $\leq$ 160 µmol/L). All patients had to have a WHO performance status of $\leq$ grade 2 and an estimated life expectancy of at least 12 weeks. If indicated patients had to practice appropriate contraception. Exclusion criteria were: concomitant use of known P-gp inhibitors and chronic use of H2-receptor antagonists or proton pump inhibitors; known history of cerebral or leptomeningeal metastases or neurologic disease; history of prior malignancy except completely excised in situ carcinoma of the cervix or non-melanoma skin cancer; other serious illness; bowel obstruction or motility disorders that may influence the absorption of drugs; concurrent treatment with other experimental drugs. The study protocol was approved by the Medical Ethics Committee of the Institute and all patients gave written informed consent.

Treatment Plan , Evaluation of Response and Toxicity
All patients were treated with oral docetaxel 100 mg in combination with oral CsA 15 mg/kg weekly for 6 weeks followed by two weeks rest. The i.v formulation of docetaxel (Taxotere®, Aventis Pharma, Antony, France; 10 mg/mL docetaxel in polysorbate 80:ethanol 13% 1:3 v/v) was used for the oral administration of

docetaxel. Thirty minutes prior to intake of oral docetaxel, CsA capsules (Neoral®, Novartis, Basel, Switzerland) were ingested. Oral drugs were taken with 100 ml tap water after an overnight fast and patients remained fasted until 2 hours following oral docetaxel administration. Standard docetaxel pretreatment was given all courses and consisted of oral dexamethason 8 mg 1 hour prior to and 8 mg every 12 hours (2 times) and was changed after the first three patients into 4 mg 1 hour prior to and 4 mg every 12 hours (2 times) to minimize possible corticosteroid related side-effects. Prior to oral docetaxel intake patients received 1 mg oral granisetron (Kytril®) to prevent nausea and/or vomiting.

Pretreatment evaluation included a complete medical history and complete physical examination. Hematology and blood chemistries were checked prior to treatment and subsequently weekly. All toxicities observed were graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) [21]. All patients who received at least one cycle of therapy were considered evaluable for toxicity. Standard clinical measurement and radiologic examination such as chest X- ray or CT scan were used to ensure bidimensionally measurable disease according to the WHO criteria [20] and were checked after every 8 weeks. Patients who completed one course (6 weekly cycles) were considered evaluable for response. Patients were planned to have 3 courses of 6 weekly cycles but in the best interest of the patient therapy could be continued. Therapy stopped in case of progressive disease or unacceptable toxicity. Patients who experienced uncomplicated CTC-grade 4 hematologic toxicity, or grade 3 hematological toxicity complicated with infection or bleeding were treated at a 25% lower dose, following recovery to $\leq$ grade 1.

Patients who did not recover to $\leq$ grade 1 toxicity within two weeks after their planned day of retreatment went off study. For patients who required dose reduction, the dosage was not re-escalated in subsequent cycles. For evaluation of response the study consisted of two parts: A: Fourteen patients evaluable for response were entered: if no responses were observed the study had to be closed. B: The maximum number of patients evaluable entered in the study would be 25 when there were 4 or more responses in the first 14 patients. This would allow us to determine the response rate with a standard error of < 0.10.

Response was evaluated according to the WHO-criteria [20]. A complete response (CR) is the disappearance of all known disease determined by two observations not less than 4 weeks apart and partial response (PR) is defined as a decrease by at least 50% of the sum of the products of the largest perpendicular diameters of all measurable lesions, determined by two observations not less than four weeks apart. No change (NC), lasting for at least 6 weeks from start of study drug administration is defined as < 50% decrease and < 25% increase in the sum of the

products of the largest perpendicular diameters of all measurable lesions and progressive disease (PD) as > 25% increase in the size of at least one bidimensionally or unidimensionally measurable lesion or appearance of a new lesion. The occurrence of pleural effusion or ascites is also considered as progressive disease if this is substantiated by positive cytology.

Pharmacokinetics

Pharmacokinetic monitoring was performed in every patient during course 1 (week 1) and course 2 (week 9). For docetaxel, blood samples of 5 ml each were collected in heparinized tubes predosing, at 30, 60 and 90 min, and 2, 3, 4, 7, 10, 24, 30 and 48 hours after docetaxel ingestion. Blood samples were centrifuged, plasma was separated and stored at –20ºC until analysis. Docetaxel concentrations in plasma were determined using a validated high performance liquid chromatography (HPLC) assay [22]. For CsA monitoring, limited blood sampling was performed pre-dosing, at 2, 3, 10, 24 and 48 hours after CsA ingestion. Whole blood samples were stored at 4ºC and analyzed within one week using a fluorescence polarization immunoassay (TDxFLx, Abbott laboratories, Amstelveen, the Netherlands) [23].

Non-compartmental pharmacokinetic methods were applied to evaluate the results [24]. For docetaxel, the area under the concentration-time curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity using the terminal rate constant k. Other parameters to be assessed were the maximal concentration (Cmax) and the time to maximal concentration (Tmax), which were observed measured values. We calculated the interpatient variability in the docetaxel AUC by dividing the standard deviation by the mean measured values and multiplication by 100. The intrapatient variability in the AUC was defined as the AUC value in week 1 minus the AUC value in week 9 dividing by the AUC value in week 1 and multiplication by 100. All the individual values were added up and divided by the number of patients. For CsA, Cmax, Tmax and C_{24h} were determined, which were obtained directly from the experimental data.

Results (Interim Analysis)

Patient and Treatment Characteristics

Until November 1st 2000, 20 patients were enrolled into the study. Table 1 lists the clinical characteristics of the patients in this study. The median age of the patients was 48 years (range 39-64) and the median WHO score was 1 (range 0-2). Seventeen patients (85%) received one prior chemotherapy line and three patients

(15%) received earlier two lines of chemotherapy for adjuvant or metastatic disease. All patients had received prior anthracycline containing chemotherapy for (neo) adjuvant (35 %) or metastatic (65%) disease. Most patients had metastases at more than one site and most of them had liver metastases (70%).

Table 1. *Patient characteristics (n=20).*

	No of patients	%
Age (years)		
Median	48	
Range	39-64	
WHO Performance status		
0	11	55
1	5	25
2	4	20
Menopausal status		
Pre	7	35
Post	13	65
Prior treatment		
Chemotherapy	20	100
Radiotherapy	14	70
Hormonal therapy	12	60
Surgery	18	90
Number of previous chemotherapy regimens		
1	17	85
2	3	15
Previous anthracyclines		
(neo)adjuvant	7	35
metastatic	13	65
Interval from last chemotherapy to study entry		
median (months)	8	
range	1-26	
Sites of metastatic disease		
Lung	5	25
Liver	14	70
lymph nodes	10	50
bones	8	40
pleura	1	5
skin	3	15

Toxicity

All 20 patients were assessable for toxicity and the maximum severity grade of the hematological and non-hematological toxicities during treatment are shown in Tables 2 and 3.

Myelosuppression was generally mild. Rapidly reversible neutropenia grade 3 and 4 was seen in 60% of the patients. Only three patients developed a neutropenia grade 4, which was of short duration. However, neutropenia was an important reason for treatment delay (n=5) or dose reduction (n=4). One patient died after four weeks of therapy because of neutropenic fever. Blood cultures showed growth of *Staphylococcus aureus*. No serious anemia or trombocytopenia due to the therapy was observed.

Fatigue was a frequently noted toxicity (55%) and one of the most important reasons for treatment delay. Nail toxicity grade 2 was reported in about half of the patients (45%) and was very serious in some patients resulting in loss of all nails of hands and feet. Nail toxicity became more severe when treatment duration was longer, but was reversible. Stomatitis grade 2 was seen in 25% of the patients. The first three patients who entered the study received weekly a higher pretreatment scheme with dexamethason and in two of these patients oral candidiasis developed. After dose reduction of the dexamethason, less candidiasis was seen in the other patients. Fluid retention grade 2 was seen in 20% of the patients. Three of them developed pleural effusion and one patient had ankle edema, which was improving by the use of diuretics. Fluid retention in these four patients manifested after a median cumulative docetaxel dose of 1100 mg. Most patients reported mild gastro-intestinal side effects like nausea and vomiting (60-65%). This was in most of the patients of short duration and improved by using standard anti-emetics. Especially the oral intake of the many CsA capsules and the taste of the docetaxel fluid after an overnight fast was troublesome. Mild diarrhea was a frequently noted side-effect (50%). This was manageable by using loperamide. No hypersensitivity reaction was seen, but some patients complained about flushes, especially around one hour after docetaxel intake. This disappeared a few hours later.

Table 2. *Hematological toxicity profile (n= 20).*

Maximum severity grade (NCI-CTC)

	Total		1		2		3		4	
Patients	n	%	n	%	n	%	n	%	n	%
Hemoglobin	7	35	1	5	6	30				
WBC	15	75			5	25	9	45	1	5
ANC	15	75			3	15	9	45	3	15
Platelets	2	10	2	10						

ANC: absolute neutrophil count, WBC: white blood cell count.

Table 3. *Non-hematological toxicity profile (n= 20).*

Maximum severity grade (NCI-CTC)

	Total		1		2		3	
Patients	n	%	n	%	n	%	n	%
Alopecia	12	60	1	5	11	55		
HSR[1]	0							
Nausea	13	65	7	35	4	20	2	10
Vomiting	12	60	7	35	3	15	2	10
Diarrhea	10	50	3	15	7	35		
Stomatitis	11	55	6	30	5	25		
Fluid retention	8	40	4	20	4	20		
Neurosensory	9	45	5	25	4	20		
Neuromotory	1	5			1	5		
Infection[2]	2	10			2	10		
Fever	4	20	1	5	2	10	1	5
Fatigue	11	55	2	10	6	30	3	15
Skin	5	25	1	5	3	15	1	5
Nail	12	60	3	15	9	45		
Tearing eyes	6	30	5	25	1	5		
Flushes	3	15	2	10	1	5		

[1]HSR: hypersensitivity reaction; [2]without neutropenia.

Efficacy

Sixteen patients were evaluable for response (Table 4). Four patients went off study prematurely because of vomiting (2), malaise (1) and one patient died after four weekly cycles in the first course because of a neutropenic fever resulting in a septic shock. The overall response rate (ORR) was 50% with 2 complete and 6 partial responses. Four patients achieved a complete or partial response already after the first course of therapy and four patients achieved a complete or partial response during the second course of therapy. Tumor responses were observed at all sites of disease. Five patients (31%) had stable disease, but three of these patients had reductions of metastases between 25% and 50%. Three patients (19%) had progressive disease after one course and went off study.

Table 4. *Response to weekly oral docetaxel 100 mg in combination with CsA 15 mg/kg after anthracycline containing chemotherapy.*

	No patients	%
Total evaluable	16	
Complete response	2	12.5
Partial response	6	37.5
Stable disease	5	31
Progressive disease	3	19

Pharmacokinetics

Table 5 summarizes the main pharmacokinetic parameters of oral docetaxel in week 1 and week 9. One patient was not evaluable for pharmacokinetics in both week 1 and week 9. This patient had lymphedema in both arms and we were not able to take blood samples. In week 9 in 12 patients blood samples were taken; 3 patients were taken off study because of progressive disease after one course and 4 patients stopped treatment earlier because of (non)-hematological toxicity. In week 9 in two patients the docetaxel dose was reduced to 75 mg and 50 mg. The mean AUC of 100 mg orally administered docetaxel was 2.50 ± 1.43 mg.h/L in week 1 and 2.12 ± 0.48 mg.h/L in week 9 with interpatient variabilities of 57% and 23%, respectively. The intrapatient variability was 35%. The AUC values of the patients who received a lower dose in week 9 were 1.19 mg.h/L and 1.60 mg.h/L with doses of 75 mg and 50 mg, respectively. Whole blood CsA concentrations were measured at two occasions (week 1: 13 patients, week 9: 9 patients). The mean maximum CsA concentration was 3.38 ± 0.69 mg/L in week 1 and 3.79 ± 0.94 mg/L in week 9 and was reached at 2.4 ± 0.4 hours and 2.5 ± 0.3 hours after intake, respectively. The mean concentration of CsA at 24 hours was 0.23 ± 0.08 mg/L in week 1 and 0.22 ± 0.09 mg/L in week 9, respectively.

Dose Intensity and Dose Reduction

As shown in Table 6 the median treatment duration of the evaluable patients was 16 weeks (range 6-32). The median dose intensity in the evaluable patients was 58 mg/week (range 33-80). Most patients (81%) had treatment delay (range 1-3 weeks) mainly because of hematological toxicity or fatigue. In seven of the patients (44%) the dose was reduced, in six patients to 75 mg and in one patient to 50 mg.

Table 5. *Pharmacokinetics of oral docetaxel: main pharmacokinetic (PK) parameters of docetaxel 100 mg represented as means (± SD) after oral intake in combination with CsA 15 mg/kg at two occasions in every patient (week 1 and week 9).*

PK parameter	Week1 n=19	Week 9 n=10
AUC (mg.h/L)	2.50 ± 1.43	2.12 ± 0.48
Cmax (mg/L)	0.66 ± 0.30	0.63 ± 0.14
Tmax (h)	1.92 ± 0.79	1.53 ± 0.56
Interpatient variability in AUC	57	23
Intrapatient variability in AUC	35	

Table 6. *Dose intensity and dose reduction in evaluable patients (n=16) during treatment with oral docetaxel and oral CsA.*

Weeks on study	
median	16
range	6-32
Dose intensity	
mean	58
sd	14
median	59
range	33-80
Dose reduction	
number of patients	7
hematological	4
fatigue	1
skin	1
stomatitis	1
Treatment delay	
number of patients	13
hematological	5
fatigue	4
skin	2
nail	1
gastro-intestinal	2
infection	1

Discussion

This interim analysis of our phase II study shows very good activity of the weekly schedule of oral docetaxel in combination with oral CsA in patients with metastatic breast cancer previously treated with anthracyclines. In the 16 evaluable patients an ORR of 50% (2 complete and 6 partial responses) was observed, which result is encouraging. Five patients had stable disease, but three of these patients had reductions of the metastases between 25-50%. Burstein described in a phase II study an ORR of 41% in 29 evaluable patients with metastatic breast cancer. They were treated with docetaxel 40 mg/m^2 weekly x 6 every 8 weeks [12]. The response rate in our study is in the upper range of results described in the literature [8-12] and promising, but evidently the number of the evaluable patients is still small and progression-free survival and overall survival data can not be evaluated yet.

Our study also shows that repeated oral administration of the i.v formulation of docetaxel plus CsA capsules is feasible. The hematological toxicity profile of this weekly schedule is less severe in comparison with the every 3-weeks regimen, and confirms other studies in which weekly schedules are used [7-12]. In our study we observed in 60% of the patients neutropenia grade 3 and 4. With the standard regimen the incidence of grade 3 and 4 neutropenia is 90–95% and neutropenic fever has also been more frequently observed [1-6,25]. In our study neutropenic fever was seen in one patient, however, resulting in septic shock and death of the patient. The non-hematological profile consisted mainly of fatigue, nail toxicity and gastro-intestinal toxicity, especially diarrhea. The latter seems to be more severe in comparison with the standard every three weeks regimen [1,25], but was manageable with loperamide. The oral intake of the docetaxel fluid (e.g. the solvent Tween 80®) and/or the co-administration of CsA may play a causative role in the occurrence of the diarrhea. The incidence of fluid retention grade 2 was 20% (n=4) and this is higher compared with a three weekly schedule [5,25], but is similar to the weekly study of Burstein [12]. The reduced corticosteroid pretreatment scheme used in our study does probably not attribute to this phenomenon, because several studies have shown that a reduced scheme is also sufficient in preventing this side effect [9,26]. Nail toxicity in our study was severe, grade 2 nail toxicity was observed in nine patients (45%), but was reversible when treatment was discontinued.

We suggest that the reason for the less severe hematological toxicity profile observed in our study and other weekly docetaxel schedules [7-11] is possibly the lower peak plasma concentration and AUC values of docetaxel in comparison with values of the 3-weekly schedule. For the every 3-week regimen Cmax and AUC

values of docetaxel of 2.6-3.3 mg/L and 3.1-4.8 mg.h/L, respectively are reported [21,27,28]. We presented earlier a phase I study showing that co-administration of the P-gp inhibitor CsA strongly enhances the systemic exposure of orally administered docetaxel [21]. The bioavailability of oral docetaxel increased up to 90% when co-administered with CsA [21]. Based on these results we calculated a weekly oral dose that would result on average in an AUC equivalent to an AUC after an intravenous dose of 40 mg/m^2. A flat dose was applied, because in particular for oral dosing there seems to be no justification to apply BSA-based adaptations. This resulted in a flat dose of 100 mg oral docetaxel. The pharmacokinetic results of the current study confirm the increased systemic exposure of docetaxel by CsA. The AUC of a flat oral dose of 100 mg docetaxel in combination with oral CsA was 2.50 ± 1.43 mg.h/L in week 1 and 2.12 ± 0.48 mg.h/L in week 9. The interpatient variabilities of 57% and 23%, respectively are in good accordance with interpatient variabilities of i.v. docetaxel previously reported in literature (29-53%) [21,28]. The intrapatient variability in our study is moderate (35%). The median dose intensity of our weekly oral docetaxel regimen is 58 mg/week (range 33-80 mg/week). Most patients experienced treatment delay (81%) or dose reduction (44%). Treatment with a slightly lower flat dose of oral docetaxel could be a better alternative in future studies to reach an optimal dose-intensity of oral docetaxel. The weekly single oral dose of CsA could have been associated with toxicity, in particular renal toxicity or infections due to immunosuppression [29]. At 24 hours after CsA ingestion we monitored CsA levels which were in the therapeutic range of immunosuppression. However, in this study, we did not observe toxicity related to CsA. This can most likely be attributed to the weekly dose administration of the drug while in the transplantation setting CsA is ingested on a continuous daily basis. Preliminary results of immunological tests performed in four patients do not show a change in T-cell counts (unpublished data).

This is the first report of a phase II study with a weekly schedule of oral docetaxel in combination with oral CsA in patients with metastatic breast cancer. The preliminary data show very promising response rates. The schedule has a favorable hematological toxicity profile and the non-hematological toxicity is acceptable. Another advantage of this scheme is that the patients receive oral medication instead of infusions, which is more convenient. Future plans are to investigate the activity and feasibility of the oral schedule in different tumor types and to explore the feasibility of oral docetaxel in combination with other P-gp blockers. In addition, exploration of the efficacy in phase III studies in advanced breast cancer is of great interest. We will continue this study and recruit 25 evaluable patients and determine also progression free survival and overall survival.

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SUMMARY AND CONCLUSIONS

Summary and Conclusions

Over the last 10 years the taxanes paclitaxel (Taxol®) and docetaxel (Taxotere®) have obtained a prominent place in cancer chemotherapy with activity against a broad range of human solid tumors. Both drugs are routinely administered intravenously. Oral administration of the drugs, however, is to be preferred for several reasons. In the first place, oral administration is convenient to patients as oral drugs can be taken at home eliminating the need for hospital admission. In addition, oral treatment avoids the discomfort of an injection and the risks of infection and extravasation that are associated with intravenous access lines. A further argument for oral treatment is that the oral route facilitates the use of more chronic treatment regimens. This seems important for paclitaxel as there are strong indications that activity of the drug is related to duration of exposure above a certain threshold concentration. Finally, in view of increasing costs of anticancer therapy, oral treatment is to be preferred, as it eliminates the need for hospitalization, physician and nursing assistance and infusion equipment.

The very low oral bioavailability of the taxanes, however, has limited development of treatment by the oral route. In preclinical studies using mdr1a P-glycoprotein knock-out mice it was shown that the low oral bioavailability of the taxanes is, at least in part, due to affinity of the drugs for the multidrug efflux pump P-glycoprotein, abundantly present in the gastro-intestinal tract. P-glycoprotein in the intestine limits the absorption of orally administered taxanes and stimulates excretion of these drugs. In addition, first-pass elimination by the cytochrome P450 metabolic enzymes in gut and liver may also contribute to the low oral bioavailability. In wild-type mice it was subsequently shown that the low oral bioavailability of paclitaxel could be significantly increased by co-administration of cyclosporin A, an efficacious inhibitor of P-glycoprotein and cytochrome P450 3A4 mediated drug metabolism. These promising preclinical results formed the basis for investigation of the feasibility of oral administration of taxanes in patients. This thesis describes the clinical development and optimization of oral therapy with the taxanes paclitaxel and docetaxel by modulation of the pharmacokinetics of the drugs after oral administration in combination with blockers of P-glycoprotein and/or cytochrome P450 3A4.

We first started with a proof of concept study of orally administered paclitaxel (Chapter 2.1). In this study patients received one course of oral paclitaxel, at a relative low dose of 60 mg/m^2, with or without 15 mg/kg oral cyclosporin A. For oral paclitaxel treatment the intravenous formulation, consisting of 6 mg/mL paclitaxel

dissolved in Cremophor EL:ethanol 1:1 v/v, was used. In all subsequent courses patients received standard intravenous paclitaxel 175 mg/m^2 administered as a 3-hour infusion every 3-weeks. Co-administration of cyclosporin A resulted in a significant increase in the systemic exposure of paclitaxel and plasma concentrations increased from negligible to potential therapeutic levels. The oral bioavailability of paclitaxel, calculated as the systemic exposure after oral drug administration compared to intravenous drug administration, with a correction for the difference in dose, was 4% for oral paclitaxel without cyclosporin A and 28% with cyclosporin A. However, due to the non-linear pharmacokinetics of intravenous paclitaxel, the oral bioavailability is most likely a significant underestimation of the true oral bioavailability of paclitaxel. Re-calculation of the oral bioavailability, using the systemic exposure of intravenous paclitaxel at a lower dose, at which less non-linearity is encountered, results in an apparent bioavailability of oral paclitaxel of 6% without cyclosporin A and 47% with cyclosporin A. The term apparent oral bioavailability is used as comparison between oral and intravenous paclitaxel plasma levels should be done with great caution. Intravenous paclitaxel exhibits pronounced non-linear pharmacokinetics due to the co-solvent Cremophor EL, which is thought to entrap paclitaxel in the plasma compartment. After oral paclitaxel administration, however, plasma Cremophor EL levels were undetectable. Consequently, after oral drug administration, paclitaxel plasma concentrations represent a higher fraction of free drug, which may result in enhancement of paclitaxel for the (tumor) tissues. Pharmacologically relevant plasma paclitaxel levels may therefore be lower for oral drug administration than for intravenous administration; this needs further confirmation.

Subsequently it was investigated whether dose-increment and dose-scheduling of cyclosporin A would result in a further increase in the systemic exposure to orally administered paclitaxel (Chapter 2.2). Dose-increment of cyclosporin A to 30 mg/kg and changing the schedule to two administrations of 15 mg/kg separated by 2 hours did not result in a further increase in the systemic exposure of paclitaxel. Apparently, inhibition of P-glycoprotein was maximal at a single dose of cyclosporin A of 15 mg/kg. In an attempt to further increase the systemic exposure and to determine dose limiting toxicity of the combination, dose-escalation of oral paclitaxel was investigated (Chapter 2.2). Dose limiting toxicity was reached at the dose level of 360 mg/m^2 and consisted of acute nausea and vomiting. The maximum tolerated dose was defined at 300 mg/m^2. Pharmacokinetic analysis revealed that dose-escalation of oral paclitaxel from 60 to 300 mg/m^2 resulted in significant increases in the systemic exposure of paclitaxel, however, these were moderate and not proportional with dose, indicating limited absorption of the drug. An oral paclitaxel dose of 180 mg/m^2 with a cyclosporin A dose of 15 mg/kg was considered most appropriate for further investigation.

Because of the limited oral absorption of paclitaxel, a split dose regimen was investigated to achieve a greater overall daily systemic exposure (Chapter 3.1). Oral paclitaxel was administered in two doses, seven hours apart, at dose levels of 2x 60 up to 2x 160 mg/m^2 with each dose preceded by 15 mg/kg cyclosporin A. In this study, besides the systemic exposure, duration of exposure above a threshold concentration of 0.1 μM was determined. In previous clinical work there were indications that duration of exposure above 0.1 μM is related to activity of the drug. The pharmacokinetic data revealed that twice daily dosing of oral paclitaxel also shows non-linear absorption pharmacokinetics as was observed after single dose administration of the drug. Comparison with the data after single dose administration revealed that fractionated administration of the drug resulted in higher systemic exposure and duration of exposure. Therefore, a multiple dosing regimen was considered to be a realistic option to further increase the systemic exposure after oral administration of paclitaxel. The recommended dose for further study was determined at 2x 90 mg/m^2. Because repeated administration of cyclosporin A might cause toxicities such as immunosuppression or nephrotoxicity, it felt important to minimize these effects. In a cross-over trial design with 2x 90 mg/m^2 oral paclitaxel, dose-reduction of cyclosporin A was investigated in order to determine the minimally effective dose of cyclosporin A with a maximal increase in systemic exposure to paclitaxel (Chapter 3.2). Dose-reduction of cyclosporin A from 10 to 5 mg/kg resulted in a significant decrease in the systemic exposure to orally administered paclitaxel. Cyclosporin A doses of 10 and 15 mg/kg resulted in comparable paclitaxel plasma levels. It was concluded that the minimally effective dose of cyclosporin A to maximally enhance the bioavailability of oral paclitaxel is 10 mg/kg.

To obtain better insight into the mechanisms of uptake, disposition and excretion of orally administered paclitaxel, plasma, urine and feces of the patients were analyzed at the maximal tolerated dose of 300 mg/m^2 (Chapter 2.5). In feces a high fraction of the dose was recovered as unchanged drug, suggesting incomplete absorption at this dose level. Moreover, high amounts of the co-solvent Cremophor EL were also recovered in feces and the fractions of the dose of Cremophor EL and paclitaxel excreted in feces were strongly correlated. This raised the hypothesis that the co-solvent Cremophor EL might play an important role in the limited absorption of orally administered paclitaxel. This was subsequently tested in a randomized cross-over trial design, in which patients received oral paclitaxel formulated in Cremophor EL at one occasion and in polysorbate 80 at the other (Chapter 2.6). The selection of polysorbate 80 was based on the following findings 1) the fast degradation of polysorbate 80 *in vivo* and 2) the very high oral bioavailability of docetaxel, different from paclitaxel in its formulation in polysorbate

80. Formulation of paclitaxel in polysorbate 80 resulted in a significant increase in the systemic exposure to the drug and a significant decrease in the excretion of unchanged paclitaxel, indicating increased absorption of the drug when formulated in polysorbate 80. These results highlight the need for designing a better, non-Cremophor EL based drug formulation of paclitaxel.

The oral combination of paclitaxel and cyclosporin A was in all clinical studies well tolerated. Dose limiting toxicity consisted of acute nausea and vomiting, which rarely occurred at the doses recommended for further study. Toxicities clearly related to cyclosporin A administration were not observed. In addition, no toxicities were observed which could be related to inhibition of the physiological protective function of P-glycoprotein by cyclosporin A. Importantly, after oral administration of paclitaxel the co-solvent Cremophor EL is not absorbed. Cremophor EL is suspect of causing hypersensitivity reactions requiring extensive premedication. Absence of systemic Cremophor EL justified oral drug administration without the premedication regimen. In several studies described in this thesis no premedication was given prior to oral paclitaxel administration and no hypersensitivity reactions were observed. As use of repeated doses of cyclosporin A might result in undesirable side-effects, a potent non-immunosuppressive and furthermore specific blocker of P-glycoprotein, GF120918, was tested (chapter 2.4). Co-administration of GF120918 resulted in a similar systemic exposure to oral paclitaxel compared to cyclosporin A. Therefore, GF120918 was considered to be a good alternative for cyclosporin A. Because of the very good safety profile of GF120918, the drug may even be a better candidate for clinical use, especially for repeated administration.

The mechanism by which cyclosporin A increases the systemic exposure to oral paclitaxel is most likely due to inhibition of P-glycoprotein in the gut. In addition, inhibition of paclitaxel metabolism may also have contributed. Metabolism of paclitaxel is mediated by the cytochrome P450 isoenzymes 2C8 and 3A4, resulting in the metabolites 6a-hydroxypaclitaxel and 3'p-hydroxypaclitaxel, respectively. Both metabolites are substantially less active than the parent compound. Cyclosporin A is also metabolized by cytochrome P450 3A4. By analyzing the metabolites it was found that after oral drug administration in combination with cyclosporin A, the relative contribution of 3'p-hydroxypaclitaxel was substantially lower than after intravenous administration, indicating inhibition of cytochrome P450 3A4 mediated paclitaxel metabolism by cyclosporin A. These data were further supported by the relative lower contribution of 3'p-hydroxypaclitaxel after cyclosporin A co-administration compared to GF120918 administration, which is a selective blocker of P-glycoprotein. Interpretation of the metabolite data should, however, be done with caution because

of the very large interpatient variability in the data. Furthermore, it is important to note that inhibition of the cytochrome 3A4 mediated pathway will not necessarily result in prolonged exposure of active parent compound because drug not handled by cytochrome P450 3A4 enters into the cytochrome P450 2C8 pathway, which is, in general, the predominant metabolic pathway of paclitaxel.

Similar to paclitaxel, a proof of concept study of orally administered docetaxel was initiated (Chapter 4.1). Patients received one course of oral docetaxel at a dose of 75 mg/m^2 with or without 15 mg/kg cyclosporin A. Patients continued on intravenous docetaxel 100 mg/m^2 given as a 1-hour infusion every three weeks. Co-administration of cyclosporin A strongly enhanced the systemic exposure of orally administered docetaxel. Docetaxel administered as a single agent exhibited poor oral bioavailability of only 8%, whereas oral docetaxel in combination with cyclosporin A exhibited a bioavailability of 90%. Furthermore, the interpatient variability in the systemic exposure after oral drug administration was of the same order as after intravenous administration. The mechanism by which cyclosporin A increases the oral bioavailability of docetaxel is most likely based on inhibition of intestinal P-glycoprotein. In addition, inhibition of cytochrome P450 3A4 mediated docetaxel metabolism may also contribute.

Recent clinical studies have shown that administration of docetaxel on a weekly schedule compared to the standard 3-weekly schedule, decreases hematologic toxicity of the drug while therapeutic activity is increased or maintained. Oral drug treatment facilitates the use of this more frequent dosing regimen. Activity and toxicity of weekly oral docetaxel in combination with cyclosporin A was investigated in a phase II study in patients with metastatic breast cancer (Chapter 4.3). Up to this moment twenty patients received a flat oral dose of 100 mg docetaxel in combination with 15 mg/kg cyclosporin A weekly x 6, every 8 weeks, with a maximum of 3 cycles. Sixteen patients were evaluable for response. There were 2 complete (12.5%) and 6 partial (37.5%) responses with an overall response rate of 50%. Five patients (31%) had stable disease and 3 patients (19%) progressive disease. The most frequently recorded toxicities were leucocytopenia, neutropenia, diarrhea, stomatitis, nail toxicities, fatigue and fluid retention, which were mostly manageable. Importantly, the hematologic toxicity of this treatment regimen appeared to be less severe than observed for intravenous docetaxel administered every three weeks. Thus, weekly oral docetaxel plus cyclosporin A is feasible with manageable toxicity and is clinically active.

Conclusions and Future Perspectives

This thesis describes the feasibility of oral administration of paclitaxel and docetaxel by concomitant administration of cyclosporin A and GF120918. For docetaxel, the results of the proof of concept study have led to a phase II activity study of weekly oral docetaxel in patients with metastatic breast cancer. This study shows very promising results with an overall response rate of 50% and acceptable toxicity. For paclitaxel, activity and toxicity of weekly bi-daily oral dosing is currently investigated in phase II studies in patients with metastatic lung, stomach or breast cancer. Our future plans are to investigate activity and toxicity of oral treatment schedules in other tumor types and with other inhibitors of P-glycoprotein. Furthermore, in case of paclitaxel, research will focus on the development of a new, non-Cremophor EL based formulation in order to further increase the oral bioavailability of the drug.

SAMENVATTING EN CONCLUSIES

Samenvatting en Conclusies

De afgelopen 10 jaar hebben de taxanen paclitaxel (Taxol®) en docetaxel (Taxotere®) een belangrijke plaats verworven in de chemotherapeutische behandeling van kanker. Beide geneesmiddelen worden doorgaans intraveneus toegediend. De orale toediening van deze stoffen brengt een aantal voordelen met zich mee. In de eerste plaats het gemak voor de patiënt. De geneesmiddelen kunnen thuis worden ingenomen zonder een bezoek aan het ziekenhuis. Bovendien is er geen intraveneuze injectie nodig met de daarbij behorende risico's van infectie en extravasatie. Een ander voordeel van orale toediening is dat het de behandeling in een continu doseringsschema vergemakkelijkt. Dit is met name belangrijk voor paclitaxel aangezien er sterke aanwijzingen zijn dat de effectiviteit ervan gerelateerd is aan de tijdsduur van blootstelling. Tenslotte is orale toediening vanuit economisch oogpunt aantrekkelijk daar een reductie in kosten verwacht mag worden nu behandeling in het ziekenhuis minder frequent nodig zal zijn.

De zeer lage orale biologische beschikbaarheid van de taxanen maakt echter dat orale toediening geen geschikte route is. In preklinische studies met mdr1a P-glycoproteïne 'knock-out' muizen is aangetoond dat de lage orale biologische beschikbaarheid van de taxanen deels wordt veroorzaakt door affiniteit van deze stoffen voor de geneesmiddel efflux pomp P-glycoproteïne. P-glycoproteïne in het maagdarmkanaal inhibeert de absorptie van oraal toegediende taxanen en stimuleert tevens de excretie van de middelen. Mogelijk speelt ook 'first-pass' metabolisme door cytochroom P450 enzymen een rol bij de lage orale biologische beschikbaarheid. In wild-type muizen is vervolgens aangetoond dat de orale biologische beschikbaarheid van paclitaxel sterk wordt verhoogd door de gelijktijdige toediening van ciclosporine A, een blokker van het P-glycoproteïne en cytochroom P450 3A4 gemedieerd geneesmiddel metabolisme. Deze veelbelovende preklinische resultaten vormden de basis voor onderzoek naar de mogelijkheid van orale toediening van taxanen in patiënten. Dit proefschrift beschrijft de klinische ontwikkeling en optimalisering van orale toediening van de taxanen paclitaxel en docetaxel gebaseerd op modulatie van de farmacokinetiek van de middelen na orale toediening in combinatie met blokkers van P-glycoproteïne en/of cytochroom P450 3A4.

In de kliniek is gestart met een 'proof of concept' studie van oraal toegediend paclitaxel (Hoofdstuk 2.1). In deze studie kregen patiënten 1 kuur oraal paclitaxel, in een relatief lage dosering van 60 mg/m^2, toegediend met of zonder oraal ciclosporine A 15 mg/kg. Voor orale toediening werd de intraveneuze formulering,

bestaande uit paclitaxel 6 mg/mL Cremophor EL:alcohol 1:1 v/v, gebruikt. In de vervolgkuren kregen de patiënten intraveneus paclitaxel 175 mg/m^2 toegediend als 3-uurs infuus. Gelijktijdige toediening van ciclosporine A leidde tot een sterke toename in de systemische blootstelling van oraal paclitaxel. De orale biologische beschikbaarheid van paclitaxel, berekend als de systemische blootstelling na orale toediening gedeeld door die na intraveneuze toediening met een correctie voor het verschil in dosis, was 4% voor oraal paclitaxel zonder ciclosporine A en 28% met ciclosporine A. Kanttekening echter hierbij is dat de niet-lineaire kinetiek van intraveneus paclitaxel zorgt voor een onderschatting van de orale biologische beschikbaarheid. Herberekening van de orale biologische beschikbaarheid, gebruikmakend van de systemische blootstelling van een lagere dosis intraveneus paclitaxel, waarin minder niet-lineariteit van de kinetiek wordt gezien, leidt dan ook tot een hogere waarde van de biologische beschikbaarheid van oraal paclitaxel met ciclosporine A van 47%. Een ander belangrijk punt is dat vergelijking tussen orale en intraveneuze paclitaxel spiegels met grote voorzichtigheid moet worden gedaan. Bij intraveneuze toediening zorgt systemische blootstelling aan het oplosmiddel Cremophor EL voor een toename in de affiniteit van paclitaxel voor het plasma compartiment en veroorzaakt op deze manier de niet-lineaire kinetiek. Na orale toediening echter zijn de Cremophor EL spiegels in het plasma compartiment niet detecteerbaar. De paclitaxel plasma spiegels vertegenwoordigen dus hogere vrije concentraties. Deze hogere vrije concentraties kunnen mogelijk tot een betere penetratie van paclitaxel in de (tumor) weefsels leiden. Farmacologisch relevante plasma paclitaxel concentraties zijn wellicht lager voor orale toediening dan voor intraveneuze toediening. Dit laatste zal verder onderzocht moeten worden.

Na de ‘proof of concept’ studie is vervolgens onderzocht of dosis-verhoging en verandering van toedieningsschema van ciclosporine A zou leiden tot een toename in de systemische blootstelling aan oraal paclitaxel (Hoofdstuk 2.2). Dosis-verhoging van ciclosporine A tot 30 mg/kg en verandering van toedieningsschema naar 2x 15 mg/kg, met een tussenpoze van 2 uren, leidde niet tot een toename in de systemische blootstelling van oraal paclitaxel. Inhibitie van P-glycoproteïne door ciclosporine A is kennelijk maximaal bij een dosis van 15 mg/kg. Om de systemische blootstelling van oraal toegediend paclitaxel verder te verhogen en tegelijkertijd de dosis limiterende toxiciteit te bepalen is dosis-escalatie van paclitaxel onderzocht (Hoofdstuk 2.2). Dosis limiterende toxiciteit werd bereikt bij 360 mg/m^2 en bestond uit acute misselijkheid en braken. De maximaal tolereerbare dosis werd bepaald op 300 mg/m^2. Farmacokinetische analyse liet zien dat dosis-escalatie van oraal paclitaxel van 60 tot 300 mg/m^2 leidt tot een toename in de systemische blootstelling van paclitaxel, echter deze is niet proportioneel met de

dosis. Dit duidt op beperkte opname van oraal toegediend paclitaxel vanuit het maagdarmkanaal. De aanbevolen dosis voor verder onderzoek werd bepaald op 180 mg/m^2 met 15 mg/kg ciclosporine A.

Om de dagelijkse systemische blootstelling aan oraal paclitaxel te verhogen, is een 2x daags toedieningsschema onderzocht (Hoofdstuk 3.1). Oraal paclitaxel werd toegediend in twee doses van 2x 60 tot 2x 160 mg/m^2 met een tussenpoze van 7 uren. Elke paclitaxel dosis werd voorafgegaan door 15 mg/kg ciclosporine A. In deze studie werd naast de systemische blootstelling aan paclitaxel ook gekeken naar de duur van blootstelling boven de therapeutische drempelwaarde van 0.1 µM. In voorgaand klinisch onderzoek was een duidelijke relatie gevonden tussen de cytotoxische activiteit van paclitaxel en de duur van blootstelling boven 0.1 µM. De farmacokinetische data van deze studie lieten zien dat in navolging van het 1x daags schema, het 2x daags behandelschema ook een beperkte absorptie van paclitaxel vertoonde. De vergelijking met het 1x daags behandelschema liet zien dat 2x daags doseren tot een hogere systemische blootstelling en een langere duur van blootstelling boven de 0.1 µM leidt. Geconcludeerd werd dat een gefractioneerde toediening van oraal paclitaxel een realistische optie is om de systemische blootstelling te verhogen. De aanbevolen dosis voor verder onderzoek werd bepaald op 2x 90 mg/m^2. Aangezien herhaalde toediening van ciclosporine A zou kunnen leiden tot bijwerkingen zoals onderdrukking van het immuunsysteem en niertoxiciteit, is in een gerandomiseerd ‘cross-over’ onderzoek gekeken naar het effect van dosis-reductie van ciclosporine A op de systemische blootstelling van oraal paclitaxel 2x 90 mg/m^2 (Hoofdstuk 3.2). Dosis-reductie van ciclosporine A van 10 naar 5 mg/kg resulteerde in een significante afname in de blootstelling van oraal paclitaxel. Ciclosporine A doses van 10 en 15 mg/kg leidde tot vergelijkbare blootstellingen aan paclitaxel. De minimaal effectieve dosis van ciclosporine A met een maximale toename in de blootstelling aan oraal paclitaxel werd derhalve bepaald op 10 mg/kg.

Om meer inzicht te krijgen in de mechanistische processen achter opname, dispositie en uitscheiding van oraal toegediend paclitaxel zijn, op het maximaal tolereerbare dosisniveau van oraal paclitaxel van 300 mg/m^2, plasma, urine en ontlasting van de patiënten geanalyseerd (Hoofdstuk 2.5). In de ontlasting werd een hoge fractie van de toegediende paclitaxel dosis teruggevonden als onveranderd geneesmiddel, hetgeen incomplete absorptie op dit dosisniveau suggereert. In de ontlasting werd tevens een grote fractie van het oplosmiddel Cremophor EL teruggevonden, die bovendien sterk gecorreleerd was aan de hoeveelheid uitgescheiden paclitaxel. Dit leidde tot de hypothese dat het

oplosmiddel Cremophor EL wellicht een cruciale rol speelt in de beperkte absorptie van oraal toegediend paclitaxel. Deze hypothese is vervolgens onderzocht in een gerandomiseerde 'cross-over' studie waarbij patiënten oraal paclitaxel kregen opgelost in Cremophor EL of in polysorbaat 80 (Hoofdstuk 2.6). De keuze van de laatste was gebaseerd op de volgende bevindingen 1) de snelle afbraak van polysorbaat 80 in *in vivo* studies in muizen en 2) de zeer hoge orale biologische beschikbaarheid van docetaxel, onder meer verschillend van paclitaxel door formulering in polysorbaat 80. Formulering van paclitaxel in polysorbaat 80 leidde tot een toename in de systemische blootstelling aan paclitaxel en bovendien tot een afname in de uitscheiding van paclitaxel in de ontlasting, beiden indicatief voor een toename in absorptie vanuit het maagdarmkanaal. Deze resultaten onderstrepen de behoefte aan onderzoek naar een betere, niet op Cremophor EL gebaseerde formulering van paclitaxel.

De orale combinatie van paclitaxel en ciclosporine A werd in alle klinische studies goed verdragen. Dosis limiterende toxiciteit bestond uit acute misselijkheid en braken, wat echter zelden voorkwam bij de aanbevolen doses. Bijwerkingen gerelateerd aan ciclosporine A toediening werden niet waargenomen. Ook reacties gerelateerd aan de inhibitie van de fysiologische beschermingsfunctie van P-glycoproteïne door ciclosporine A werden niet gezien. Belangrijk is dat na orale toediening van paclitaxel het oplosmiddel Cremophor EL niet wordt geabsorbeerd. Cremophor EL is verantwoordelijk voor overgevoeligheidsreacties die kunnen optreden na intraveneuze toediening van paclitaxel. Om deze te voorkomen moet uitvoerige premedicatie worden gegeven. Orale toediening van paclitaxel zou dus zonder premedicatie gegeven kunnen worden. Dit is gedaan in verscheidene studies beschreven in dit proefschrift en overgevoeligheidsreacties werden niet gezien. Aangezien frequente toediening van ciclosporine A zou kunnen leiden tot ongewenste bijwerkingen als onderdrukking van het immuunsysteem en niertoxiciteit, is gekeken naar het effect van toediening van GF120918, een middel specifiek ontwikkeld voor blokkade van P-glycoproteïne en met een zeer mild bijwerkingenprofiel (Hoofdstuk 2.4). Toediening van GF120918 aan oraal paclitaxel 120 mg/m^2 leidde tot een vergelijkbare systemische blootstelling aan paclitaxel als bij ciclosporine A. GF120918 kan dus worden beschouwd als een goed alternatief voor ciclosporine A om de biologische beschibaarheid van oraal paclitaxel te verhogen.

Het mechanisme achter de toename in systemische blootstelling aan oraal paclitaxel door ciclosporine A is hoogstwaarschijnlijk inhibitie van P-glycoproteïne in het maagdarmkanaal. Daarbij zou remming van paclitaxel metabolisme door

ciclosporine A mogelijk ook een rol kunnen spelen. Metabolisme van paclitaxel vindt plaats door de cytochroom P450 enzymen 2C8 and 3A4, welke leiden tot de metabolieten 6a-hydroxypaclitaxel en 3'p-hydroxypaclitaxel. Beide metabolieten zijn veel minder cytotoxisch dan paclitaxel zelf. Ciclosporine A wordt ook door cytochroom P450 3A4 gemetaboliseerd. Analyse van paclitaxel metabolieten in plasma liet zien dat na orale toediening van paclitaxel in combinatie met ciclosporine A, de relatieve contributie van de metaboliet 3'p-hydroxypaclitaxel aanzienlijk lager is dan na intraveneuze toediening. Dit suggereert inhibitie van cytochroom P450 3A4 gemedieerd paclitaxel metabolisme door ciclosporine A. Ondersteunende data werden gevonden in de vergelijking tussen oraal paclitaxel met GF120918, een selectieve blokker van P-glycoproteïne, en oraal paclitaxel met ciclosporine A. Toediening van ciclosporine A leidde tot lagere hoeveelheden van 3'p-hydroxypaclitaxel dan toediening van GF120918. Interpretatie van de metaboliet data moet echter met voorzichtigheid worden gedaan door de zeer grote interpatient variabiliteit. Bovendien is het belangrijk te vermelden dat remming van cytochroom P450 3A4 veroorzaakte afbraak van paclitaxel niet noodzakelijkerwijs zal resulteren in verlengde blootstelling aan paclitaxel, aangezien paclitaxel niet alleen gemetaboliseerd wordt door cytochroom P450 3A4, maar zelfs voor een belangrijk deel door cytochroom P450 2C8.

In navolging van de 'proof of concept' studie van oraal paclitaxel is een zelfde studie opgezet voor oraal docetaxel (Hoofdstuk 4.1). Patiënten kregen 1 kuur oraal docetaxel in een dosis van 75 mg/m^2 met of zonder 15 mg/kg ciclosporine A. Vervolgkuren bestonden uit intraveneus docetaxel 100 mg/m^2 toegediend als een 1-uurs infuus. Gelijktijdige toediening van ciclosporine A leidde tot een sterke toename in de systemische blootstelling van oraal toegediend docetaxel. De orale biologische beschikbaarheid van docetaxel was 8% zonder ciclosporine A en 90% met ciclosporine A. De interpatient variabiliteit in de systemische blootstelling na orale docetaxel toediening was vergelijkbaar met die na intraveneuze toediening. Het mechanisme achter de toename in biologische beschikbaarheid van docetaxel door ciclosporine A is hoogstwaarschijnlijk gebaseerd op inhibitie van P-glycoproteïne in het maagdarmkanaal. Daarbij speelt mogelijk, als voor paclitaxel, remming van cytochroom P450 3A4 gemedieerd docetaxel metabolisme een rol.

Recente klinische studies hebben aangetoond dat toediening van docetaxel in een wekelijks schema ten opzichte van het standaard 3-wekelijks schema leidt tot minder hematologische toxiciteit terwijl de therapeutische activiteit toeneemt of behouden blijft. De mogelijkheid van orale toediening van docetaxel vergemakkelijkt deze meer frequente toediening. Activiteit en toxiciteit van

wekelijks oraal docetaxel met ciclosporine A is onderzocht in een fase II studie in patiënten met gemetastaseerde borstkanker (Hoofdstuk 4.3). Tot op heden kregen twintig patiënten wekelijks maal 6, elke 8 weken, een orale dosis van 100 mg docetaxel in combinatie met 15 mg/kg ciclosporine A. Zestien patiënten waren evalueerbaar voor respons. Er waren 2 complete (12.5%) en 6 partiële (37.5%) responsen met een totaal reponspercentage van 50%. Vijf patiënten (31%) hadden stabiele ziekte en 3 patiënten (19%) progressieve ziekte. De meest voorkomende toxiciteiten waren leukocytopenie, neutropenie, diarree, stomatitis, nageltoxiciteit, moeheid en vochtretentie, welke in het algemeen acceptabel en behandelbaar waren. De hematologische toxiciteit leek minder dan bij het standaard 3-wekelijks intraveneuze schema. Conclusie van het onderzoek was dat wekelijks oraal docetaxel met ciclosporine A zeer goed mogelijk is met acceptabele toxiciteit en klinische werkzaamheid.

Conclusies en Toekomstperspectieven

Dit proefschrift toont de mogelijkheid van orale toediening van paclitaxel en docetaxel door gelijktijdige toediening met ciclosporine A en GF120918. Voor docetaxel hebben de eerste resultaten geleid tot een fase II activiteits-studie van wekelijks oraal docetaxel met ciclosporine A in patiënten met gemetastaseerde borstkanker. Deze laatste studie laat vooralsnog zeer goede resultaten zien met een totaal responspercentage van 50% en acceptabele toxiciteit. Voor paclitaxel wordt momenteel de activiteit en toxiciteit van een wekelijks 2x daags schema onderzocht in fase II activiteits-studies in patiënten met gemetastaseerde long-, maag- of borstkanker. In de toekomst zal ons onderzoek zich richten op het bepalen van de activiteit van orale behandelschema's van paclitaxel en docetaxel in meer tumorsoorten en in combinatie met andere blokkers van P-glycoproteïne. Voor paclitaxel zal het onderzoek zich bovendien richten op de ontwikkeling van een niet op Cremophor EL gebaseerde formulering ter verdere verhoging van de orale biologische beschikbaarheid.

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Mirte Malingré
Utrecht, april 2001

Curriculum Vitae

Mirte Malingré werd op 14 maart 1972 geboren te Groningen. In 1989 behaalde zij het Gymnasium-ß diploma aan het Willem Lodewijk Gymnasium te Groningen. Van 1989 tot 1990 volgde zij het eindexamenjaar aan Craigmont Highschool te Memphis (USA). In 1990 werd begonnen met de studie Farmacie aan de Universiteit Groningen. Na het behalen van het propaedeusediploma (cum laude) in augustus 1991, werd het doctoraalexamen (cum laude) afgelegd in november 1996. Tijdens de doctoraalopleiding werd onderzoek gedaan naar de rol van polyaminen bij tumorgroei aan het Department of Physiology and Biophysics, University of Lund (Zweden). Na het behalen van het doctoraaldiploma werd aangevangen met de opleiding tot apotheker aan de Faculteit Farmacie van de Universiteit Groningen. In december 1997 werd het apothekersdiploma behaald. Januari 1998 werd begonnen met het promotie-onderzoek “Orale toediening van taxanen” in de apotheek van het Slotervaartziekenhuis en de kliniek van het Antoni van Leeuwenhoek ziekenhuis, te Amsterdam, onder begeleiding van promotores Prof. Dr J.H. Beijnen en Prof. Dr J.H.M. Schellens en co-promotor Dr W.W. Ten Bokkel Huinink, hetgeen geleid heeft tot dit proefschrift. Tegelijkertijd volgde zij de opleiding voor de aantekening van klinisch farmacoloog onder auspiciën van de Nederlandse Vereniging voor Klinische Farmacologie en Biofarmacie.

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