PHARMACOLOGIC MODULATION OF TAXANES

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Lay-out: A.A.C. Treuren-den Braber, Dept. of Medical Oncology
Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam

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FARMACOLOGISCHE MODULATIE VAN TAXANEN

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Copromotor: Dr A. Sparreboom

Overige leden: Prof. dr G. Stoter
Prof. dr J. Beijnen
Prof. dr A.M.M. Eggermont

Ik probeer niet
tot de hemel te reiken

Ik ben maar klein

Sappho van Lesbos

Voor Tanny van Zuijlen-Buijs,
mijn moeder
(†1981)

Pharmacologic modulation by Cremophor EL

Introduction to the thesis

In the 90's the taxanes paclitaxel and docetaxel, both belonging to the so-called naturally occurring drugs, because of their origin from the yew trees *Taxus brevifolia* and *Taxus baccata*, respectively, were registered for clinical use in the treatment of cancer patients. Although their mechanism of action is the same, inducing cell death by promoting polymerization of tubulin into microtubules and particularly by inhibiting depolymerization of these microtubules (1-3), they are not simply two of a kind (4). Differences in activity, toxicity and pharmacokinetic behavior have been described over the last decade (5-10). In order to increase activity they have been combined with various other (anticancer) agents (4,11). To judge combination treatment on its merits, changes in the pharmaco-dynamics as well as modulations in the pharmacokinetics of the combined drugs have to be evaluated. This thesis explores the influence of other chemical entities on the pharmacokinetics of docetaxel and paclitaxel. Intended modulation of docetaxel by co-administration of a multidrug-resistance convertor is the topic of the first part of the thesis, the unintended and underestimated pharmacokinetic implications of the choice of Cremophor EL as the formulation vehicle of paclitaxel is the subject of the second part.

Multidrug resistance is the phenomenon thought to be partly responsible for failure of cancer treatment with naturally occurring anticancer drugs such as taxanes (12-14). It is associated with overexpression of the transmembrane transport protein P-glycoprotein, which acts as an ATP-dependent drug efflux pump, yielding to a decrease in the intracellular concentrations of the substrate drugs (15-17). Abundant effort has been put into the development of agents that are able to inhibit P-glycoprotein, and when used in combination with anticancer drugs increase the intracellular exposure to these drugs (18-20). However, many clinical studies involving a wide range of P-glycoprotein inhibitors and co-administered anticancer drugs have shown that these combination treatments almost always resulted in increased toxicity of the anticancer drugs particularly because of pharmacokinetic interaction between the two agents due to competition at the level of cytochrome-P450 3A isozymes (21,22), the major metabolic pathway of many drugs including docetaxel (23,24). Recently, a new 'second generation' P-glycoprotein inhibitor, R101933, has been developed which known major metabolic route is cytochrome-P450 unrelated. Since docetaxel is known to be a more pure substrate of P-glycoprotein than paclitaxel (25), it was obvious that co-

treatment of docetaxel with R101933 could have great clinical importance. Therefore, we performed several pharmacokinetic and phase I studies with docetaxel combined with R101933, orally as well as intravenously. In order to determine the physiological and pharmacological consequences of inhibition of P-glycoprotein activity with respect to the disposition of docetaxel we monitored both plasma and fecal drug levels of docetaxel.

Modulation of P-glycoprotein, particularly in the hepatobiliary tract, by Cremophor EL, the formulation vehicle of paclitaxel, was postulated as one of the explanations of the distinctly nonlinear pharmacokinetic behavior of this taxane (26-28). The operation of Michaelis-Menten kinetics, saturable distribution in combination with saturable elimination, was hypothesized as another cause of the nonlinear disposition of paclitaxel (26-28). However, studies in mice demonstrated that in the absence of Cremophor EL the pharmacokinetic behavior of paclitaxel was linear (29). An explanation could be that, by its nature, Cremophor EL in blood is capable of forming micelles with a highly hydrophobic interior that can entrap the solubilised compound (30), consequently influencing the pharmacokinetic behavior of this drug. So we postulated that the nonlinear disposition of paclitaxel is caused by dose and time-related variations in Cremophor EL concentrations in the central compartment. To prove this hypothesis we performed a comprehensive pharmacokinetic analysis of paclitaxel and Cremophor EL *in vitro* and in patients, measuring paired whole blood and plasma levels of paclitaxel. Based on the results of these studies, we were able to develop and describe explicit modeling of the disposition of paclitaxel and free paclitaxel to permit a formal explanation of the observed phenomenon.

REFERENCES

1. Horwitz SB: Mechanism of action of taxol. TiPS 13:134-136, 1992
2. Ringel I, Horwitz SB: Studies with RP 56976 (Taxotere): a semisynthetic analogue of taxol. J Natl Cancer Inst 83:288-291, 1991
3. Gueritte-Voegelein F, Guenard D, Lavelle F, Le Goff MT, Mangatal L, Potier P: Relationships between the structure of Taxol analogues and their antimitotic activity. J Med Chem 34:992-998, 1991
4. Verweij J, Clavel M, Chevalier B: Paclitaxel (Taxol) and docetaxel (Taxotere): not simply two of a kind. Ann Oncol 5: 495-505, 1994
5. Pazdur R, Kudelka AP, Kavanagh JJ, Cohen PR, Raber MN: The taxoids: Paclitaxel (Taxol) and docetaxel (Taxotere). Cancer Treat Rev 19:351-386, 1993

6. Onetto N, Canetta R, Winograd B, Catane R, Dougan M, Grechko J, Burroughs J, Rozencweig M: Overview of Taxol safety. J Natl Cancer Inst Monogr 15:131-139, 1993
7. Cortes JE, Pazdur R: Docetaxel. J Clin Oncol 13:2643-2655, 1995
8. Pronk LC, Stoter G, Verweij J: Docetaxel (Taxotere): single agent activity, development of combination treatment and reducing side effects. Cancer Treat Rev 21:463-478, 1995
9. Kearns CM: Pharmacokinetics of the taxanes. Pharmacotherapy 17:105S-109S, 1997
10. Crown J, O'Leary M: The taxanes: an update. Lancet 355:1176-1178, 2000
11. Goldspiel BR. Clinical overview of the taxanes. Pharmacotherapy 17:110S-125S, 1997
12. Ford JM, Hait WN: Pharmacology of drugs that alter multidrug resistance in cancer. Pharm Rev 42:155-199, 1990
13. Nooter K, Herweijer H: Multidrug resistance *(mdr)* genes in human cancer. Br J Cancer 63:663-669, 1991
14. Gottesman, MM, Pastan I: Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem 62:385-427, 1993
15. Juliano RL, Ling V: A surface glycoprotein modulating drug permeability in chinese hamster ovary cell mutants. Biochem Biophys Acta 455:152-162, 1976
16. Leighton JC, Goldstein LJ: P-glycoprotein in adult solid tumors. Expression and prognostic significance. Hematology/oncology clinics of North America, 9 (2):251-271, 1995
17. Bradshaw DM, Arceci RJ: Clinical relevance of transmembrane drug efflux as a mechanism of multidrug resistance. J Clin Oncol 16:3674-3690, 1998
18. Kaye SB: Reversal of multidrug resistance. Cancer Treat Rev 17(Suppl A):37-43, 1990
19. Raderer M, Scheithauer W: Clinical trials of agents that reverse multidrug resistance. Cancer 73:3553-3563, 1993
20. Sikic BI, Fisher GA, Lum BL, Halsey J, Beketic-Oreskovic L, Chen G: Modulation and prevention of multidrug resistance by inhibitors of P-glycoprotein. Cancer Chemother Pharmacol 40:S13-S19, 1997
21. Relling MV: Are the major effects of P-glycoprotein modulators due to altered pharmacokinetics of anticancer drugs? Ther Drug Monit 18:350-356, 1996
22. Twentyman PR: Transport proteins in drug resistance: biology and approaches to circumvention. J Intern Med 242:133-137, 1997
23. Royer I, Monsarrat B, Sonnier M, Wright M, Cresteil T: Metabolism of docetaxel by human cytochromes P450: interactions with paclitaxel and other antineoplastic drugs. Cancer Res 56:58-65, 1996
24. Clarke SJ, Rivory LP: Clinical pharmacokinetics of docetaxel. Clin Pharmacokin 36:99-114, 1999
25. Vanhoefer U, Cao S, Harstrick A, Seeber S, Rustum YM: Comparative antitumor efficacy of docetaxel and paclitaxel in nude mice bearing human tumor xenografts that overexpress the multidrug resistance protein (MRP). Ann Oncol 8:1221-1228, 1997
26. Sonnichsen DS, Hurwitz CA, Pratt CB, Shuster JJ, Relling MV: Saturable pharmacokinetics and paclitaxel pharmacodynamics in children with solid tumors. J Clin Oncol 12:532-538, 1994
27. Gianni L, Kearns CM, Giani A, Capri G, Vigano L, Locatelli A, Bonadonna G, Egorin MJ: Nonlinear pharmacokinetics and metabolism of paclitaxel and its pharmacokinetic/pharmacodynamic relationships in humans. J Clin Oncol 13:180-190, 1995

28. Kearns CM, Gianni L, Egorin MJ: Paclitaxel pharmacokinetics and pharmacodynamics. Semin Oncol 22:16-23, 1995
29. Sparreboom A, Van Tellingen O, Nooijen WJ, Beijnen JH: Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle Cremophor EL. Cancer Res 56:2112-2115, 1996
30. Kessel D: Properties of Cremophor EL micelles probed by fluorescence. Photochem Photobiol 56:447-451, 1992

Pharmacologic modulation by multidrug-resistance convertors

Chapter 1

Development of multidrug-resistance convertors: Sense or nonsense?

Lia van Zuijlen, Kees Nooter, Alex Sparreboom, Jaap Verweij

Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, The Netherlands

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SUMMARY

This review describes the clinical relevance of the two drug transporters P-glycoprotein (Pgp) and multidrug resistance-associated protein (MRP) and the *in vitro* phenomenon which is referred to as multidrug resistance (MDR). The attempts to try to block these resistance mechanisms are summarized with specific attention for the intentionally designed "second generation" MDR-convertors. Potential explanations of the limited clinical success rate are given and recommendations for the design of future studies provided.

INTRODUCTION

In vitro selection of tumor cells for resistance to so-called "naturally occurring" drugs, such as anthracyclines, vinca alkaloids, epipodophyllotoxins, and taxanes, may result in the development of cross-resistance to other, structurally and functionally unrelated natural compounds. This *in vitro* phenomenon is referred to as MDR and is generally regarded to be one of the major stumbling blocks to the efficacy of chemotherapy for human malignancies. A large variety of human cell lines displaying such an MDR phenotype have been identified. The MDR-related anticancer drugs are derived from plants or micro-organisms, are structurally dissimilar and have different intra-cellular targets. What these drugs have in common is that they have a relatively low molecular weight and enter the cell by passive diffusion because they are preferentially soluble in lipids at physiological pH.

Two different drug transporters, Pgp and MRP, have been identified, that can render cells multidrug resistant. Both have a broad substrate specificity and belong to the same large superfamily of transport proteins known as adenosine triphosphate (ATP) binding cassette (ABC) transporters. From a clinical point of view it is attractive to block these drug transporters in an effort to convert drug resistance into drug sensitivity. Indeed, there has been and still is, a very extensive research concerning such resistance converting agents, but the clinical success rate of their application has untill now been very limited. Underneath we will review the putative clinical involvement of the MDR phenotypes caused by Pgp and

MRP and we will discuss the limitations of the use of resistance converting agents, the difficulties encountered, and the potential future obstacles.

PGP MEDIATED MDR

The Pgp-mediated or so-called classical MDR phenotype is characterized by reduced intracellular drug accumulation through an energy-dependent unidirectional drug-efflux pump (1). The energy required is supplied by ATP. This drug-efflux pump involves a transmembrane glycoprotein (referred to as Pgp) with a molecular weight of 170 kDa, and is encoded by the MDR1 gene on the long arm of chromosome 7. Pgp is approximately 1280 amino acids long, and is composed of two homologous halves; each half consists of six transmembrane domains and an intracellular loop with an ATP-binding motif (2). The precise mechanism by which Pgp exerts its effect is still a matter of debate. Pgp might extrude drugs directly from the plasma membrane even before they can enter the cytoplasm (model of "hydrophobic vacuum cleaner") (3). Another suggested mode of action is that a drug binds to a cytoplasmic region of Pgp, followed by an energy-dependent conformational change whereby the drug becomes transposed to the outside of the plasma membrane and is released (4-6). However, these models do not provide an explanation for the broad substrate specificity of the Pgp drug pump, which contrasts the new hypothesis recently formulated by Zhu. He proposed that Pgp functions as an energy-dependent efflux pump for certain conjugated metabolites (probably sulfates) of lipophilic anticancer drugs but not for the parent compounds (7). However, so far, data are lacking for *in vitro* conjugation of MDR-related anticancer drugs.

Pgp expression is present in normal tissues, such as kidney (proximal tubule), adrenal gland, liver (biliary surface of hepatocytes), pancreas, intestinal tract (8), and in CD34+ hematopoietic stem cells (both in bone marrow and peripheral blood), lymphocytes (particularly CD56+, natural killer cells), macrophages (9,10), and finally in capillary endothelium surrounding the brain and the testis. Although the physiologic role of Pgp is still speculative at this time, the anatomical sites of its expression suggest that Pgp is involved in general detoxification of xenobiotics in order to protect specific organs and cell types, and in transport of hormones. Experiments with Pgp-knockout mice have confirmed that a lack of either

one of the two murine drug-transporting Pgps (i.e. MDR1a or MDR1b) results in elevated drug levels in a wide variety of tissues and a reduced drug-tolerance (11,12). In other aspects these mice live quite normal showing that Pgp is not an absolute necessity for life (12,13). In line with this observation, an MDR1a Pgp-deficient subpopulation in the CF-1 mouse strain has been identified and breeding studies demonstrated that the inheritance of the markers follows a normal Mendelian autosomal pattern (14).

MRP1 MEDIATED MDR

The membrane-bound glycoprotein MRP1 has a molecular weight of 190 kDa and is encoded by the MRP1 gene located on chromosome 16 (15). It is also a member of the ABC superfamily of membrane transport proteins. MRP1 and Pgp only share 15% amino acid identity, even though they confer resistance to a similar, but not identical, range of cytotoxic agents (16). Whereas MRP1 expression also results in resistance to anthracyclines, epipodophyllotoxins, and vinca alkaloids, the resistance to taxanes, especially docetaxel, and mitoxantrone is significantly less than that observed in Pgp-mediated resistance (16,17).

Despite the overlap in substrate specificity of Pgp and MRP1, it is very likely that their pump characteristics are very different. The available data strongly suggest that MRP1 is an ATP-dependent pump for glutathione (GSH), glucuronic acid and sulfate conjugates (18,19) of endogenous substances but also of xenobiotics, such as the anticancer drugs chlorambucil and melphalan (20). Interestingly, while the unmodified drugs are not transported by MRP1, GSH conjugates of doxorubicin and daunorubicin are (21). These data suggest that some cytotoxic drugs either first have to be metabolized by conjugation reactions in order to be transported by MRP1, although there presently is no conclusive evidence for this (22), or are involved in an MRP-related co-transport system involving GSH and unmodified (that is, not covalently bound) drug. The latter has yet only been suggested for vincristine (23). GSH-conjugate transporters have also been associated with the detoxification of heavy metals, which include cisplatin and this provides an interesting potential link between MDR and cisplatin resistance (24).

MRP1 is present in normal cells in liver, kidney (25,26), adrenal gland and testes (27,28). In normal brain, liver, small intestine and colon MRP1 mRNA is present at low levels, while moderate levels of expression were found in lung, skeletal muscle, ovary and peripheral leukocytes (reviewed in: 29). The uniform expression pattern of MRP1 in normal tissues, suggests it may play a crucial role in normal cellular physiology, but much is yet unknown. MRP1 has been suggested to be involved in the regulation of intracellular redox potential and ion flux, in inflammatory responses, and in elimination of conjugated endo- and xenobiotics (30). Indeed MRP1 has been shown to protect the oropharyngeal mucosal layer and the testicular tubules against etoposide-induced damage (27). Much of the knowledge of the function of MRP1 is likely to result from investigations with specifically generated mutant mice lacking mrp1 protein (31,32).

PGP AND MRP1 EXPRESSION IN HUMAN TUMORS, AND THEIR POTENTIAL RELEVANCE

Expression of MDR1 has been detected in virtually all tumor types in man, ranging from almost absent to abundently present (Table 1) (8,33). The expression may be intrinsic or acquired. If normal cells are expressing high MDR1 levels tumors arising from such cells, such as colon and kidney cancer, usually also have relatively high intrinsic levels of MDR1 expression. Clinically, these tumors are all intrinsically drug resistant, i.e. have a very low response rate to chemotherapy. Other tumors, either initially sensitive (such as hematological malignancies, small cell lung cancer, ovarian cancer), less sensitive (breast cancer, some pediatric tumors) or insensitive (glioma) to chemotherapy, express no MDR1 before treatment, but high levels at relapse (acquired MDR) (36,38,39,44). The difference between these tumors in their initial sensitivity to chemotherapy, while negative for MDR1 expression, is one of the reasons to doubt the true relevance of Pgp in the occurrence of clinical drug resistance, even though several studies have reported a positive correlation between MDR1 gene expression and drug resistance in malignancies (45,46). Remarkably, consensus about elevated MDR1/Pgp levels and clinical drug resistance has been difficult to reach. This is probably due to differences in patient population, drug treatment and MDR1/Pgp detection methods. In the clinical correlation studies, MDR1/Pgp expression was estimated either by mRNA or protein

quantification. MDR1 mRNA expression was determined by PCR, Northern blotting, RNase protection assay or *in situ* hybridization. Pgp levels were quantitated by immunohistochemistry, flow cytometry or Western blotting. Except for *in situ* hybridization, flow cytometry and immunohistochemistry, all of these techniques were applied to RNA or protein fractions obtained by bulk destruction methods. Since tumors are heterogenous with regard to MDR1/Pgp expression and may contain a variable number of normal (matrix, epithelial and immune) cells, these assays mostly give an underestimation of the actual MDR1/Pgp levels. Despite all these difficulties, it is generally accepted now that Pgp is an adverse prognostic factor in childhood neuroblastoma, soft tissue sarcomas, colon cancer, locally advanced breast cancer and hematological malignancies (38,46-51). In osteosarcoma it is even a better prognostic marker than tumor necrosis after preoperative chemotherapy with high levels of Pgp predicting a higher relapse rate (52). The mechanisms responsible for malignant cells to increase expression after exposure to cytotoxic agents become more and more clear. In clinical samples, elevated MDR1/Pgp levels are not due to gene amplification. In acquired drug resistance alternatively, transcriptional activation (53), post-transcriptional regulation leading to mRNA stabilization (54), and posttranslational modifications, such as glycosylation and phosphorylation, that can modify Pgp expression and function (55), might all be important.

The *in vitro* identification of MRP1 as a drug-resistance gene for obvious reasons induced interest in its potential role in human tumors. MRP1 expression has been found in human tumors such as leukemias, Non-Hodgkin's lymphoma (NHL), neuroblastomas, soft tissue sarcomas, and cancers of the lung, breast, colon and esophagus (Table 1). Also for MRP1, gene amplification is not likely to be a common mechanism of MRP1 expression. In patients with squamous cell NSCLC, MRP1 expression was negatively correlated with treatment outcome (62). Analysis of paired tumor samples taken before and after chemotherapy in patients with squamous cell cancer of the esophagus showed significant increase in MRP1 expression in post-chemotherapy tumor samples from patients with partial remission or stable disease, suggesting that chemotherapy selected out drug-resistant cell clones (60). In neuroblastoma increased levels of the MRP1 gene correlate with amplification and overexpression of the N-*myc* oncogene, a negative prognostic indicator associated with a more aggressive and chemotherapy resistant phenotype (69). In breast cancer,

MRP1 expression may be of prognostic significance in the subgroups of patients with the more favorable prognosis, i.e. patients with small tumors and node-negative patients, as well as in the setting of adjuvant systemic chemotherapy (58). In patients receiving first-line chemotherapy for recurrent breast cancer, MRP1 was more often positive in non-responding tumors (50%) than in responding tumors (18%). Only 1 out of 8 (13%) MRP-positive tumors had an objective response, compared with 6 out of 15 (40%) MRP-negative tumors. Patients with MRP-positive tumors had a shorter time to progression on first-line chemotherapy than those with MRP-negative tumors. Apparently, MRP1 expression is a significant predictor of poor prognosis in patients with recurrent breast cancer (59). However, although several studies thus suggest a role of MRP1 expression, the significance in the clinical resistance of cancers is still under investigation.

Table 1 Pgp and MRP expression in human tumors

Tumor	Pgp	MRP	Reference
Colon cancer	+	+	34,56,57
Breast cancer	+	+	35,37,58,59
Ovarian cancer	+	+	36,37
Esophageal cancer	-	+	60,61
Lung cancer (SCLC +NSCLC)	-	+	62-65
Neuroblastoma	+	+	38
Glioma	+	+	39
Osteosarcoma	+	-	40
Soft tissue sarcoma	+	+	41
Melanoma	-	+	66
AML	+	+	42,67
B-CLL + B-PLL	+	+	43,68
NHL	+	+	43

IN VITRO MODULATION OF MDR

As a consequence of its broad substrate specificity, the function of Pgp can be inhibited by non-cytotoxic compounds that also have a high affinity for the drug binding site on the Pgp molecule. Probably their binding to Pgp inhibits its ability to bind cytotoxics, resulting in less efflux of co-administered anticancer agents from Pgp expressing cells (70,71). Structural similarities of these reversal agents include a ternary nitrogen, aromatic ring and amphipathic characteristics (72).

Table 2 Recently developed 'second generation' MDR-convertors

MDR-convertor	Analogue of	Inhibitor of	Stage of devol.	Reference
SDZ PSC 833	Cyclosporin	Pgp	phase III	76
GF 120918	Acridine-carboxamide	Pgp	phase I	77,78
VX-710	Pipecolinate	Pgp	phase I	79,80
R101933	-	Pgp	phase I	81,82
MS-209	Quinoline	Pgp	Preclinical	83
N276-12	Imidazothiazole	Pgp	Preclinical	84
N276-14	Imidazothiazole	Pgp	Preclinical	84
N276-17	Imidazothiazole	Pgp	Preclinical	84
LY335979	-	Pgp	Preclinical	85
XR9576(2)	Anthranilamide	Pgp	Preclinical	86
XR9051	Diketopiperazine	Pgp	Preclinical	87
B9309-068	Pyridine	Pgp	Preclinical	88
BIBW-22	Dipyridamole	Pgp + MRP	Preclinical	89,90
MK571	LTD_4 receptor Antagonist	MRP	Preclinical	91
PAK-104P	Pyridine	MRP	Preclinical	92

In 1981, the calcium channel blocker verapamil was found to restore the *in vitro* sensitivity to vinca alkaloids in Pgp-mediated MDR by competitive inhibition of the Pgp drug pump (73). Presently, a large number of such so-called MDR-reversing agents or resistance convertors have been reported, including other calcium antagonists, cyclosporin and its

analogues, steroid hormones, phenothiazines, quinidine, and quinine (reviewed in: 74,75). New modulators were developed to specifically inhibit Pgp and to be more suitable candidates for further clinical use (Table 2) (3,71).

The MDR modulator SDZ PSC 833 was reported to inhibit the development of Pgp-related drug resistance *in vitro* (93). It has therefore been suggested that clinical studies should be performed involving the early application of MDR-convertors in order to prevent Pgp-expression rather than inhibit Pgp-function. The assumption is that treatment of drug-sensitive Pgp-negative cancers (e.g. in newly diagnosed patients with leukemias, lymphomas, or myeloma or as first-line treatment of metastatic breast cancer) with effective MDR modulators might suppress the appearance of resistant Pgp-expressing subclones and result in a prolonged time to progression and, perhaps, even in the cure of an additional subset of patients (94). The major clinical endpoint of such trials will have to be time to progression (94). However, this theory does not take into account the fact that many other resistance mechanisms exist, and that it has been shown that these may immediately take on an active role. For example, a myeloma cell line was generated with the specific aim to identify mechanisms of resistance that emerge with combined treatment with MDR-convertors and cytotoxic drugs (95). In cells selected with doxorubicin only, drug resistance was mediated by over-expression of MDR1/Pgp. In contrast, in cells selected with doxorubicin plus MDR-convertor (verapamil) resistance was mediated through decreases in topoisomerase II protein levels and catalytic activity, and not by Pgp expression.

Drugs that modulate Pgp, affect MRP1 function in a much less potent way (16,96,97). In a search for agents specifically modifying MRP-mediated MDR, it was reported that the isoflavonoid genistein increased the daunorubicin and etoposide accumulation in resistant cell lines that express MRP1, but not MDR1 (98,99). However, in view of the high concentrations required and the inherent toxicity of genistein, the success of its use as a clinical resistance modifier will be negligible.

MRP1 transport of cysteinyl leukotrienes has led investigators to examine agents that can reduce cellular levels of GSH and ultimately modulate the MRP-mediated resistance phenotype. One such agent is buthionine sulfoximine (BSO), which depletes intracellular GSH by the inhibition of γ-glutamylcysteine synthetase, an important enzyme in the

GSH synthesis pathway (100,101). Depletion of cellular GSH by incubation of cells with BSO reduces the activity of MRP1 against cytotoxic drugs (102,103). However, one should be cautious when interpreting the effect of BSO on MRP1-mediated resistance, since depletion of GSH induces multiple cellular changes that may be influencing alterations in drug handling in MRP1-expressing cells.

Other efforts to modulate MRP1 activity involve the use of organic ion transport inhibitors such as probenicid and the leukotriene LTC4 receptor antagonist MK571 (4), the use of alternative substrates such as GSSG and Nonyl-GS, or use of the ATPase inhibitor sodium orthovanadate. Finally, MRP1 is a phosphoprotein, whose phosphorylation is regulated by protein kinases. Addition of protein kinase C inhibitors to MRP1 overexpressing cells, resulted in reduced levels of MRP1 phosphorylation concomitant with an elevation in drug accumulation (104) and in reversal of MRP1-mediated cellular resistance (4). A new approach could be the use of antisense oligonucleotides for the inhibition of MRP1 or Pgp-expression (105,106). With improvements in the stability of antisense oligonucleotides, this approach may one day prove valuable in reversing both MRP1- and Pgp-mediated MDR.

CLINICAL *VERSUS* PRECLINICAL

It is obvious that there are many important theoretical and actual differences between *in vitro* and *in vivo* models of MDR and the situation in patients. In models cells are selected for resistance and therefore the potential effect of the modulator, if present, will be more outspoken. *In vitro* models usually have a homogeneous pattern of cells, whereas human tumors are known to be heterogeneous. Because of this, there will also be an inhomogeneous expression of mechanisms of resistance in human tumors and the expression will even differ between cells of the same tumor. As a resultant, MDR-convertors at best will have an incomplete effect even in Pgp-positive tumors.

The limitations of presently available assays for Pgp detection in tumor cells and the variability observed in the results obtained seriously limit their use as a diagnostic tool. The apparent contradictory results of studies aiming to use Pgp expression as a marker for chemosensitiviy (44,107) can probably be explained by difficulties with the detection of Pgp

and MDR1 especially at low levels of expression in terms of reproducibility, sensitivity, and specificity of current assays, and the accessibility of tumors for biopsy (94).

Another potential concern that has arisen in extrapolating *in vitro* results to the clinical situation relates to binding of compounds to proteins in plasma. In contrast to *in vitro* studies, where MDR-convertors are usually tested in a system containing low protein concentrations (typically ≤10% plasma), the modulators are clinically exposed to high concentrations of proteins with drug binding sites, particularly albumin, in the systemic circulation. Indeed, it has been reported that in model systems the ability of MDR modulators to inhibit Pgp activity in cell cultures is significantly decreased with increasing protein concentrations (88,108). Using monolayers of polarized LLC-PK1 pig kidney cells transfected with complementary DNA containing either MDR1 or MDR1a sequences, it has been shown, for example, that inhibition of paclitaxel transport under complete (i.e., 100%) plasma conditions by SDZ PSC 833 is ~8-fold less effective than in tissue-culture medium containing 10% plasma (109). Because of this, *in vitro* studies are thought by some authors to be misleading as far as the potential clinical activity of the agents is concerned. In addition, based on these findings, it has been suggested that protein binding of MDR-convertors in the systemic circulation can reduce drug availability of the (pharmacologically active) free drug fraction at the tumor site (108). However, this view is unnecessarily simplistic and does not justify the generally accepted physiologic concept of the extent of distribution of a compound within tissues, including a tumor, involving multiple equilibria. A large body of evidence exists that only in the case of agents with high binding affinity binding will be important in a practical sense, and will impact upon the pharmacokinetic behavior of drugs in the body. In attempting to extrapolate *in vitro* data to *in vivo* expectations, many investigators tend to lose sight of the fact that the plasma comprises a relatively small fraction of the total volume available for drug distribution, and that protein-drug complexes of rather extraordinary stability must be formed [e.g. cisplatin to human serum albumin or UCN-01 to α_1-acid glycoprotein (110)] to substantially reduce the amount of drug that exists in the body in the active, diffusible, unbound form. Far outweighing a potential importance of protein binding concerns the ability of modulators to reach their target in solid tumors at sufficiently high concentrations (and

achieve inhibition of Pgp activity), a process compromized by the high cell density in patient tumors (approx. 10^9 cells/ml) that are rarely used in tissue cultures (usually 10^5 to 10^6 cells/ml) (111).

There has always been a concern that MDR modulation would reveal new side effects of the anticancer drugs due to the inhibition of Pgp in normal tissues. However, severe unexpected toxicity due to administration of MDR-convertors was never seen in patients. This is most likely because plasma levels of the MDR-convertors associated with their maximum-tolerated dose, limited by intrinsic toxicity of the compounds, were fairly lower than those required for effective Pgp inhibition. Another major contributing problem, particularly with the early MDR-convertors, is their potential to competitively inhibit cytochrome P450 (CYP) isozymes (mainly CYP3A4) involved in the metabolism of anticancer drugs (112). For this reason alone, significant interactions at the level of drug metabolism can be anticipated, that can result in pharmacokinetic interactions between the MDR-convertors and the co-administered anticancer drugs, with concomitantly significantly increases in toxicity of the anticancer drugs (Table 3). The area under the plasma concentration – time curve of the cytotoxic drug increases and the cytotoxic drug is therefore effectively dose-intensified. Pharmacokinetic studies of cyclosporin A given in combination with doxorubicin, for example, have shown that cyclosporin A can significantly increase a patient's overall drug exposure necessitating dose reductions of the anticancer drug of 40-50% because of exacerbated toxicity (118-121). The plasma concentration of doxorubicinol, the major metabolite of doxorubicin, increases more than threefold when doxorubicin is given together with cyclosporin A (119). Because doxorubicinol is less potent as an anticancer drug but may be more cardiotoxic than its parent compound, proposed studies of agents that may reverse the MDR phenotype are not without risk (122). Based on these findings, it has been suggested that administration of MDR-convertors is unlikely to improve the therapeutic index of anticancer drugs unless such agents lack significant pharmacokinetic interactions (112). Consequently, it can be postulated that in the human setting it will be very difficult for many of the MDR-convertors to reach adequate concentrations without severe toxicity.

Table 3 Pharmacokinetic interactions between 'second generation' MDR-convertors and anticancer drugs

Anticancer drug	MDR-convertor	PK-effect on anticancer drug	Toxicity/DLT	No. of patients (ref)
Paclitaxel (70mg/m^2, day 2)	SDZ PSC 833 po (5mg/kg qid, days 1-3)	Not measured	Increased hematologic toxicity	61 (113)
VAD	SDZ PSC 833 po (5-15mg/kg7days)	Elevated AUC of doxorubicin, doxorubicinol and vincrisitne	Increased Hematologic toxicity	22 (114)
Etoposide (75-100mg/m^2, days 1-5)	SDZ PSC 833 iv (1-15mg/kg, days 2-6)	Elevated AUC	Increased Hematologic toxicity	35 (115)
Mitoxantrone and etoposide (3.25mg/m^2 and 210mg/m^2 days 1 and 3-6/3-5)	SDZ PSC 833 iv (10mg/kg, days 2-6)	Elevated AUC of etoposide PK of mitoxantrone variable	Mucositis	10 (116)
Doxorubicin (35-50mg/m^2, day 3)	SDZ PSC 833 po (2.5-25mg/kg, days 1-5)	Elevated AUC	Increased Hematologic toxicity	42 (117)
Paclitaxel (80mg/m^2, day 1)	VX-710 iv (120mg/m^2/h, day 1-2)	Elevated AUC	Increased Hematologic toxicity	25 (79)
Adriamycin (45mg/m^2, day 2)	VX-710 iv (120mg/m^2/h, day 1-4)	None	No change	17 (80)
Doxorubicin (50-75mg/m^2, day 3)	GF120918 po (400 mg bid, days 1-5)	Elevated AUC of doxorubicin and doxorubicinol	Increased Hematologic toxicity	46 (77)
Doxorubicin (40mg/m^2, day 3)	GF120918 po (1000 mg bid, days 1-5)	Elevated AUC of doxorubicin and doxorubicinol	Increased Hematologic toxicity	51 (78)
Paclitaxel (200 mg/m^2, day 3)	R101933 po (200 mg bid, days 1-5)	None	No change	10 (82)
Docetaxel (100 mg/m^2, day 3)	R101933 po (300 mg bid, days 1-5)	None	No change	15 (81)

In recent years it became obvious that clinical drug resistance is a complex of many different mechanisms. For example, other MRP-related transporters have recently been identified, including cMOAT (also called MRP2), MRP3, MRP4, MRP5, and MRP6 (123), and may contribute to the resistance phenotype. The exposure of cells to particular drugs or drug combinations might induce specific patterns of resistance related to these different mechanisms. Clearly, drug resistance is multifactorial in human tumors while in models it is artificial made monofactorial by selection. As a consequence human tumors have many ways to escape the effect of cytotoxic agents and reversing one MDR-mechanism may not affect the many others and will thus not solve the problem of intrinsic or aquired resistance to many of the nowadays known anticancer agents.

Table 4 MDR modulation in hematolymphatic malignancies

Malignancy	MDR-convertor	Trial design	Response percentage	Reference
Multiple myeloma	Verapamil	Phase II	3/8 patients	124
	Verapamil	Phase III	36 vs 41%	125
	Cyclosporine	Phase II	48%	126
	SDZ PSC 833	Phase I	8/22 patients	114
Leukemia	Cyclosporin A	Phase I/II	69%	118
	Cyclosporin A	Phase III	24 vs 23%	127
	Quinine	Phase III	52.8 vs 45.5%	128
	SDZ PSC 833	Phase I	none	116
	SDZ PSC 833	Phase II	32%	76
Lymphomas	High-dose verapamil	Phase II	72%	129
	Dexverapamil	Cross-over	12%	130
	Quinine	Phase I/II	7%	131

CLINICAL MODULATION STUDIES

The initial encouraging results for MDR modulation were noted among the hematological malignancies: AML, lymphomas and multiple myeloma (Table 4). Three early studies of verapamil or cyclosporin A with a

regimen consisting of vincristine, doxorubicin and dexamethasone (VAD) in patients with drug-refractory multiple myeloma or NHL and overexpression of Pgp in tumor cells suggested that the modulator may be effective in overcoming drug resistance, with higher response rates than could be expected on the basis of historical data (124,126,129). Although a later phase III trial of VAD in combination with verapamil was negative in patients with refractory multiple myeloma this could largely be explained by fluctuations in serum levels of verapamil (125).

A controlled cross-over trial of oral dexverapamil beginning 24 hours before and continuing for 24 hours past the infusion of etoposide, vincristine, and doxorubicin in patients with drug-refractory lymphomas showed only a modest response rate to the addition of dexverapamil. In this study there was no correlation between response and the AUC of dexverapamil, but a relation between response and Pgp-expression of tumor cells was observed (130). A modest increase in hematological toxicity was found, but pharmacokinetics of the cytotoxic agents were not performed.

For patients with poor-risk acute myeloid leukemia a phase I/II trial was performed with a loading dose of cyclosporin A followed by continuous infusion given on days 6-8, administered concurrently with daunorubicin starting after cytarabine given on days 1-5 (118). A high rate of responses was seen, 62% of the patients achieved complete remisssion with a median remission duration of 13 months. Pgp expression was detected in tumor cells of 70% of the patients. Doses of cyclosporin A, yielding plasma concentrations capable of reversing Pgp-mediated anthracycline resistance *in vitro*, could be incorporated into this induction regimen with acceptable toxicity. However, mean daunorubicin concentrations were significantly higher in patients experiencing hyperbilirubinemia due to cyclosporin A. A phase III multicenter study for the same patient population with mitoxantrone, given on days 2-5, and high-dose Ara-C, given on days 1-5, with or without quinine at a dose of 30 mg/kg/d as a continuous i.v. infusion on days 1-6 showed no significant increase of the overall response rate by adding quinine, although there was a trend for quinine to improve the response rate in Pgp-positive acute leukemias (128). Possibly the achieved quinine concentrations were too low to efficiently inhibit Pgp, which explanation is supported by the low incidence of quinine-related side effects mostly seen among responding patients meaning a slight increase in hematological toxicity. Unfortunately, the pharmacokinetic parameters of

mitoxantrone were not determined. A non-randomized study in leukemia patients using mitoxantrone, etoposide and cytarabine (MEC), given on days 1-5, with or without cyclosporin A, days 1-4, could not suggest any apparent benefit in response rate, remission duration, or survival of the addition of cyclosporin A, neither was there a relation between Pgp-expression and response (127). Based on previously described drug interactions, reduced doses of mitoxantrone and etoposide were used but nevertheless excessive toxicity, in particular grade 4 stomatitis was seen. Again, appropiate pharmacokinetics were not performed in this study.

For solid tumors, the results from clinical modulation studies are even less encouraging (Table 5). Two large, randomized studies, involving untreated small-cell lung cancer patients and breast cancer patients respectively, failed to demonstrate any benefit of adding verapamil or quinidine respectively, both in terms of response and survival (132,135). Significantly more dose reductions were necessary in the verapamil treated patients due to hematological toxicity, suggesting interactions with one or more of the anticancer drugs (132). However again, pharmacokinetics were not studied. Breast cancer patients randomised for epirubicin alone or in combination with quinidine did not show any evidence of increased toxicity with quinidine suggesting that there was no pharmacokinetic interaction between quinidine and epirubicin, which was confirmed from actual pharmacokinetic parameters, obtained in only 23 of the 233 patients (135).

Table 5 MDR modulation in solid tumors

Malignancy	MDR-convertor	Trial design	Response percentage	Reference
SCLC	Verapamil	Phase III	82 vs 80%	132
NSCLC	Cyclosporin A	Phase I/II	22.7%	133
Breast cancer	*r*-Verapamil	Phase I	-	134
	Quinidine	Phase III	43 vs 44%	135
	Dexverapamil	Phase II	10%	136
Ovarian cancer	SDZ PSC 833	Phase II	7%	113
Colorectal cancer	Cyclosporin A	Phase II	1/24 patients	137

The modifiers used in these trials have been 'off the shelf' compounds, developed initially for other indications and were incidentally found to have resistance modifying properties. As outlined, they have considerable intrinsic toxicity at required concentrations and clinical application is further compromised due to the occurrence of profound pharmacokinetic interactions with the anticancer agent, which confound the interpretation of toxicity and response data, if any. Almost without exception studies have shown that at the maximum-tolerated dose modulators yielded serum concentrations considerably lower than the concentrations required to effectively inhibit Pgp *in vitro* (reviewed in: 4). Early clinical studies with either quinine or high-dose cyclosporin A and various cytotoxic drugs, disclosed two potential consequences of MDR modulation on the central nervous system: an increase in nausea and vomiting (possibly due to increased anticancer drug distribution to the brain) and greater than expected obtundation when lorazepam was used as an antiemetic (119,120,128,138). Because of these inherent problems associated with the early MDR-modulators, more recent studies have focussed on potentially less toxic 'second generation' modifiers, i.e. pharmaceutical agents developed specifically for their resistance modifying properties such as SDZ PSC 833, GF120918, VX-710, and R101933.

SDZ PSC 833 (valspodar) is the non-immunosuppressive cyclosporin D analogue with increased inhibitory activity as compared to cyclosporin A. Currently, SDZ PSC 833 is undergoing phase II and III evaluation in drug resistant myeloma in combination with VAD since pilot experiments suggested promising results with this treatment approach in this tumor type (114). A multicenter phase II trial in patients with refractory or relapsed acute leukemia of the combination of SDZ PSC 833 given as a continuous infusion of 10 mg/kg/d on days 1-5 and mitoxantrone, etoposide, and cytarabine given on days 1-5 suggested encouraging antileukemic effects with a complete remission rate of 32% (76). Despite the use of reduced doses of mitoxantrone and etoposide, the doses of the anticancer drugs had to be further reduced due to substantial hematological and gastrointestinal toxicity. In line with this, pharmacokinetic studies showed 57% decrease in plasma clearance of etoposide and a 1.8-fold longer $t_{1/2}$ of mitoxantrone as compared with historical controls without SDZ PSC 833. The combination of 3-weekly paclitaxel 70 mg/m^2, day 2, and SDZ PSC 833 5 mg/kg given orally q.i.d. on days 1-3, yielded some responses in a few patients with

paclitaxel and cisplatin refractory ovarian cancer (113). The low paclitaxel dose was selected based on results from an unpublished phase I trial identifying this dose in combination with the MDR-convertor as having similar tolerability to standard single agent paclitaxel dosing. Pharmacokinetics were not performed in this study, but previously 3 phase I studies involving etoposide or doxorubicin demonstrated an increased toxicity of the anticancer drug due to a markedly decreased clearance of the agent (115-117). Although the exact mechanism responsible for the increased drug exposure in combinations with SDZ PSC 833 are not known, these studies strongly suggest that the pharmacokinetic interactions result primarily at the level of elimination pathways. These may involve inhibition of endogenous Pgp expressed in bile canaliculi and intestinal epithelial cells, although metabolic interference due to competitive inhibition of hepatic CYP3A4 can not be ruled out. Recent work has also raised important issues regarding the specificity of SDZ PSC 833 for Pgp, as the compound has been demonstrated to inhibit a bile-acid transporter in the canalicular membrane that may be involved in active secretion of anticancer agents (139). In addition, the pharmaceutical formulation vehicle Cremophor EL for i.v. use of SDZ PSC 833 in itself dramatically affects the pharmacokinetic behavior of numerous compounds, including paclitaxel, doxorubicin and etoposide (140), thereby potentially nonselectively increasing the incidence or severity of toxic side effects. As a consequence of all pharmacological issues associated with SDZ PSC 833, it is unlikely this MDR-convertor will improve the therapeutic index of any of the used anticancer drugs, unless the systemic exposure to the anticancer drug is monitored and adjusted to that obtained without SDZ PSC 833.

VX-710 (biricodar), a pipecolinate derivative, is the prototype of a series of non-macrocyclic high-affinity ligands that are potent modulators of Pgp mediated MDR *in vitro*. A recent phase I study combined 3-weekly paclitaxel 3-hour iv infusions in increasing doses with increasing concentrations of VX-710 given as a 24-hour iv infusion, started 8 hours before the start of paclitaxel infusion (79). The overall qualitative toxicity profile was similar to the toxicitity profile of single-agent paclitaxel therapy and VX-710 was well tolerated, with minimal clinical toxicity. However, a major alteration of the pharmacokinetic parameters of paclitaxel was observed, characterized by a disproportional increase in AUC with increasing doses of VX-710, the mean paclitaxel AUC increased

disproportional. This pharmacokinetic interaction was speculated to be caused by inhibition of P-450-dependent processes that are responsible for the metabolism of paclitaxel. As expected based on the pharmacokinetic interaction, neutropenia was the principal DLT of the regimen already observed at a paclitaxel dose of 80 mg/m^2 in combination with 120 mg/m^2/h VX-710. In contrast, a phase I study of the combination of doxorubicin at a dose of 45 mg/m^2 given on day 2, with VX-710 given as a 96-hour infusion on days 1-4, showed that the pharmacokinetics of doxorubicin remained relatively constant among the various VX-710 dose levels and that dose limiting toxicity at doses up to 120 mg/m^2/h VX-710 was not found (80).

GF120918 (elacridar), another novel agent derived from acridinecarboxamide, fully reverses Pgp-mediated resistance *in vitro* and restores sensitivity of various MDR tumors to doxorubicin *in vivo* without adverse pharmacokinetic interaction in mice (141). Based on these results, phase I trials of orally administered GF120918 at increasing doses twice daily, days 1-5, in combination with doxorubicin 50-75 mg/m^2 given on day 3, were performed (77,78). In line with the mouse-model, the pharmacokinetic behavior of the anthracycline was not altered by GF120918, although in 3 of 47 patients (6%) a significantly increased exposure to doxorubicinol was observed, which was likely related to inhibition of reductive NADPH glycosidase and/or CYP-450-mediated demethylation (142). A significant relationship was established between the AUC of doxorubicinol and the decrease in neutrophils and platelets, that was identical in the presence and absence of GF120918, indicating no pharmacodynamic potentiation of the hematological toxicity.

Recently, a new orally administered potent inhibitor of Pgp *in vitro*, R101933, was investigated in two phase I studies in combination with either docetaxel or paclitaxel (81,82). Interestingly, the major metabolic route of R101933 is CYP450 unrelated and in line with this, it was observed that the plasma pharmacokinetic characteristics of docetaxel and paclitaxel were not substantially influenced by R101933 enabling studies to investigate the contribution of the MDR-convertor to the toxicity and activity of an anticancer drug independent of pharmacokinetic interactions (81).

Crucial for the effect of the MDR-convertors is that sufficient tumor levels are achieved. To assess this process, two elegant *ex vivo* methods

have been developed and used in various trials. (*i*) Tc-99m-sestamibi is a known substrate of Pgp and it can be used as a functional imaging agent for Pgp inhibition *in vivo* (143,144). In the phase I study of doxorubicin with biricodar Tc-99m-sestamibi scans suggested an *in vivo* inhibition of hepatic Pgp during biricodar infusion (80). A recent phase I trial of vinblastine in combination with SDZ PSC 833 demonstrated that sestamibi retention by tumor and liver was altered in the presence of the MDR-convertor and that tumor visualization was enhanced (145). However, it is of importance to realise that Tc-99m sestamibi efflux was also shown to be influenced by MRP expression *in vitro* (146). (*ii*) The CD56(+) subset of peripheral blood lymphocytes has been shown to express functional Pgp and has been suggested as a surrogate target for monitoring MDR-convertor activity *ex vivo* (147). A study with patients receiving SDZ PSC 833 showed a decreased rhodamine efflux from CD56(+) cells *ex vivo* within 15 minutes of treatment and with a clear dose-response relationship (148). In future studies, an effort should be made to relate the measured plasma levels of the MDR-convertor with the efficacy of the modulator on tumor by one of the two surrogate markers. This also could give an indication of the time needed to administer the MDR-convertor before and after treatment with the anticancer drug to reach the optimal effect of both.

At present, no specific, highly effective, non-toxic modulator of MRP1 is available yet for studies in patients, although some MDR-convertors, such as biricodar, have been shown to inhibit both Pgp and MRP1 *in vitro* (149).

CONCLUSIVE REMARKS

More than two decades ago, the discovery of the MDR molecule Pgp was inducing great expectations. It was postulated that if the effects of this drug transporter could be reversed, many more cancer patients might benefit from treatment. Still, despite numerous studies there is no indisputable evidence about Pgp as a prognostic indicator of response and survival after treatment with cytotoxic drugs. In addition a plethora of clinical studies involving a wide range of MDR-convertors and co-administered anticancer drugs have shown little convincing evidence for clinical benefit. The most plausible explanations for this failure include (*i*) insufficient local concentrations of the MDR-convertor, (*ii*) the negative

impact of pharmacokinetic interactions, and (*iii*) the multiple other existing mechanisms of intrinsic or acquired clinical drug resistance that render it less likely that circumventing one of them would already restore drug sensitivity.

So where do we go from here? Clearly we need to change gears. First of all we should be more selective in the choice of the cytotoxic agents. It has been shown that the taxane drugs are much better substrates for Pgp than any of the other cytotoxic agents known at present (150,151). Also they are among the most active anti-cancer agents in a wide variety of different tumors, so selecting taxane drugs for studies on MDR-modulation would make sense.

Secondly, we should focus on those MDR-convertors that lack a pharmacokinetic interaction with anticancer agents. Pharmacokinetic interactions have frequently necessitated major dose reductions for the anticancer agents, thereby loosing all the potential benefits in terms of antitumor activity as compared to the normal doses given without the addition of the modulator.

We will also have to be more restrictive in study design. Non-controlled phase II studies will never provide an answer to the question if MDR-convertors have any value. Therefore there should be more focus on different designs. Phase I studies should without any exception include pharmacokinetic analyses of both the modulator and the anti-cancer drug involved, in order to exclude a potential pharmacokinetic interaction. The study design should aim for applying the anticancer agent at normal doses and adding escalating doses of the modifier. Alternatively, if a positive interaction could be exploited they should aim for at least achieving plasma levels of the anticancer agent that are similar to those achieved with the normal single agent dose. In other words, if the dose of the anticancer agent has to be tapered because of a pharmacokinetic interaction, this should not lead to a coinciding decrease in plasma levels. If such a decrease would be noted, development would better be stopped. Phase I studies should also if possible already include studies with surrogate markers of effect. Performing Tc-99m sestamibi scans or measuring rhodamine efflux from CD56(+) cells *ex vivo* are examples of tests that could provide evidence of an effect at Pgp level. One should also aim on involving these markers in pharmacokinetic/pharmacodynamics analyses.

As said, standard phase II studies are not informative. Only a phase II study using the combination of a modifier and an anticancer agent, performed in patients that have shown proven progression to the same dose of the anticancer agent previously, would provide useful information. However these studies have been proven difficult in accrual. Randomized phase II studies could provide some insight in differences in side effects as compared to the single agent anticancer agent, but can never be used for proof of concept.

Because of the above in most instances adequately powered phase III studies should follow the phase I studies. Data on pharmacokinetic interactions should be taken into account. In preference only those modifiers lacking a pharmacokinetic interaction or where the systemic exposure to the anticancer drug is monitored and adjusted to that obtained without the modulator, should enter phase III studies. Specific attention should be given to adequate dosing.

The early use of Pgp modifiers to prevent Pgp-expression rather than inhibit Pgp-function deserves further attention. However, we should keep a close eye on the obvious limitations related to modification of only one resistance mechanism in human tumors where tumor cells are known to escape the effect of anticancer agents by means of many different mechanisms of resistance.

REFERENCES

1. Juliano RL, Ling V: A surface glycoprotein modulating drug permeability in chinese hamster ovary cell mutants. Biochem Biophys Acta 455:152-162, 1976
2. Sharom FJ: The P-glycoprotein efflux pump: how does it transport drugs? J Membr Biol 160:161-175, 1997
3. Gottesman MM, Pastan I: Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem 62:385-427, 1993
4. Twentyman PR: Transport proteins in drug resistance: biology and approaches to circumvention. J Intern Med 242:133-137, 1997
5. Germann UA: P-glycoprotein – a mediator of multidrug resistance in tumour cells. Eur J Cancer 32A:927-944, 1996
6. Higgins CF, Gottesman MM: Is the multidrug transporter a flippase? Trends Biochem Sci 17:18-21, 1992
7. Zhu BT: A novel hypothesis for the mechanism of action of P-glycoprotein as a multidrug transporter. Mol Carcinog 25:1-13, 1999
8. Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR, Melamed MR: Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. J Histochem Cytochem 38:1277-1287, 1990

9. Chaudhary PM, Roninson IB: Expression and activity of P-glycoprotein, a multidrug efflux pump, in human hematopoietic stem cells. Cell 66:85-94, 1991
10. Drach D, Zhao S, Drach J, Mahadevia R, Gattringer C, Huber J, Andreeff M: Subpopulations of normal peripheral blood and bone marrow cells express a functional multidrug resistant phenotype. Blood 80:2729-2734, 1992
11. Schinkel AH, Smit JJ, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol CAAM, van der Valk MA, Robanus-Maandag EC, te Riele HPJ, Berns AJM, Borst P: Disruption of the mouse *MDR1a* P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. Cell 77:491-502, 1994
12. Schinkel AH, Mayer U, Wagenaar E, Mol CAAM, van Deemter L, Smit JJM, van der Valk MA, Voordouw AC, Spits H, van Tellingen O, Zijlmans JMJM, Fibbe WE, Borst P: Normal viability and altered pharmacokinetics in mice lacking *MDR1*-type (drug-transporting) P-glycoproteins. Proc Natl Acad Sci U S A 94:4028-4033, 1997
13. Schinkel AH, Mol CA, Wagenaar E, van Deemter L, Smit JJM, Borst P: Multidrug resistance and the role of P-glycoprotein knockout mice. Eur J Cancer 31A:1295-1298, 1995
14. Lankas GR, Cartwright ME, Umbenhauer D: P-glycoprotein deficiency in a subpopulation of CF-1 mice enhances avermectin-induced neurotoxicity. Toxic Appl Pharmacol 143:357-365, 1997
15. Cole SPC, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AMV, Deeley RG: Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science 258:1650-1654, 1992
16. Cole SPC, Sparks KE, Fraser K, Loe DW, Grant CE, Wilson GM, Deeley RG: Pharmacological characterization of multidrug resistant MRP-transfected human tumor cells. Cancer Res 54:5902-5910, 1994
17. Vanhoefer U, Cao S, Harstrick A, Seeber S, Rustum YM: Comparative antitumor efficacy of docetaxel and paclitaxel in nude mice bearing human tumor xenografts that overexpress the multidrug resistance protein (MRP). Ann Oncol 8:1221-1228, 1997
18. Jedlitschky G, Leier I, Buchholz U, Center M, Keppler D: ATP-dependent transport of glutathione *S*-conjugates by the multidrug resistance-associated protein. Cancer Res 54:4833-4836, 1994
19. Jedlitschky G, Leier I, Buchholz U, Barnouin K, Kurz G, Keppler D: Transport of glutathione, glucuronate, and sulfate conjugates by the *MRP* gene-encoded conjugate export pump. Cancer Res 56:988-994, 1996
20. Barnouin K, Leier I, Jedlitschky G, Pourtier-Manzanedo A, König J, Lehmann W-D, Keppler D: Multidrug resistance protein-mediated transport of chlorambucil and melphalan conjugated to glutathione. Br J Cancer 77:201-209, 1998
21. Priebe W, Krawczyk M, Tien Kuo M, Yamane Y, Savaraj N, Ishikawa T: Doxorubicin- and daunorubicin-glutathione conjugates, but not unconjugated drugs, competitively inhibit leukotriene C_4 transport mediated by MRP/GS-X pump. Biochem Biophys Res Commun 247:859-863, 1998
22. Zaman GJ, Lankelma J, van Tellingen O, Beijnen J, Dekker H, Paulusma C, Oude Elferink RP, Baas F, Borst: Role of glutathione in the export of compounds from cells by the multidrug-resistance-associated protein. Proc Natl Acad Sci U S A 92:7690-7694, 1995

23. Loe DW, Deeley RG, Cole SPC: Characterization of vincristine transport by the *M*, 190,000 multidrug resistance protein (MRP): Evidence for cotransport with reduced glutathione. Cancer Res 58:5130-5136, 1998
24. Ishikawa T, Bao JJ, Yamana Y, Akimaru K, Frindrich K, Wright CD, Kuo MT: Coordinated induction of MRP/GS-X pump and gamma-glutamylcysteine synthetase by heavy metals in human leukemia cells. J Biol Chem 271:14981-14988, 1996
25. Roelofsen H, Vos TA, Schippers IJ, Kuipers F, Koning H, Moshage H, Jansen PLM, Muller M: Increased levels of the multidrug resistance protein in lateral membranes of proliferating hepatocyte-derived cells. Gastroenterology 112:511-521, 1997
26. Evers R, Zaman GJR, van Deemster L, Jansen H, Calafat J, Oomen LCJM, Oude Elferink RPJ, Borst P, Schinkel AH: Basolateral localization and export activity of the human multidrug resistance-associated protein in polarized pig kidney cells. J Clin Invest 97:1211-1218, 1996
27. Wijnholds J, Scheffer GL, van der Valk M, van der Valk P, Beijnen JH, Scheper FJ, Borst P: Multidrug resistance protein 1 protects the oropharyngeal mucosal layer and the testicular tubules against drug-induced damage. J Exp Med 188 (5):797-808, 1998
28. Nooter K, Stoter G: Molecular mechanisms of multidrug resistance in cancer chemotherapy. Pathol Res Pract 192:768-780, 1996
29. Loe DW, Deeley RG, Cole SPC: Biology of the multidrug resistance-associated protein, MRP. Eur J Cancer 32A:945-957, 1996
30. Deeley RG, Cole SPC: Function, evolution and structure of multidrug resistance protein (MRP). Semin Cancer Biol 8:193-204, 1997
31. Wijnholds J, Evers R, van Leusden MR, Mol CA, Zaman GJ, Mayer U, Beijnen JH, van der Valk M, Krimpenfort P, Borst P: Increased sensitivity to anticancer drugs and decreased inflammatory response in mice lacking the multidrug resistance-associated protein. Nat Med 3:1275-1279, 1997
32. Rappa G, Lorico A, Flavelli RA, Sartorelli AC: Evidence that the multidrug resistance protein (MRP) functions as a co-transporter of glutathione and natural product toxins. Cancer Res 57:5232-5237, 1997
33. Nooter K, Herweijer H: Multidrug resistance *(MDR)* genes in human cancer. Br J Cancer 63:663-669, 1991
34. Hotta T. Tanimura H, Tsunoda T, Iwahashi M, Tani M, Noguchi K, Mizobata S, Arii K, Terasawa H, Nakamori M, Yamaue H: P-glycoprotein expression and chemosensitivity in highly purified fresh human gastrointestinal cancer cells. Hepatogastroenterology 46:316-321, 1999
35. Filipits M, Suchomel RW, Dekan G, Haider K, Valdimarsson G, Depisch D, Pirker R: MRP and MDR1 gene expression in primary breast carcinomas. Clin Cancer Res 2:1231-1237, 1996
36. Arao S, Suwa H, Mandai M, Tashiro H, Miyazaki K, Okamura H, Nomura H, Hiai H, Fukumoto H: Expression of multidrug resistance gene and localization of P-glycoprotein in human primary ovarian cancer. Cancer Res 54:1355-1359, 1994
37. Kavallaris M, Leary JA, Barrett JA, Friedlander ML: MDR1 and multidrug resistance-associated protein (MRP) gene expression in epithelial ovarian tumors. Cancer Lett 102:7-16, 1996
38. Chan HS, Haddad G, Thorner PS, DeBoer G, Lin YP, Ondrusek N, Yeger H, Ling V: P-glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. N Engl J Med 325:1608-1614, 1991

39. Abe T, Mori T, Wakabayashi Y, Nakagawa M, Cole SPC, Koike K, Kuwano M, Hori S: Expression of multidrug resistance protein gene in patients with glioma after chemotherapy. J Neurooncol 40:11-18, 1998
40. Stein U, Walther W, Wunderlich V: Point mutations in the *MDR1* promoter of human osteosarcomas are associated with *in vitro* responsiveness to multidrug resistance relevant drugs. Eur J Cancer 10:1541-1545, 1994
41. Oda Y, Schneider-Stock R, Rys J, Gruchala A, Niezabitowske A, Roessner A: Expression of multidrug-resistance protein gene in human soft-tissue sarcomas. J Cancer Res Clin Oncol 122:161-165, 1996
42. Broxterman HJ, Lankelma J, Pinedo HM, Eekman CA, Wahrer DC, Ossenkoppele GJ, Schuurhuis GJ: Theoretical and practical considerations for the measurement of P-glycoprotein function in acute myeloid leukemia. Leukemia 11:1110-1118, 1997
43. Webb M, Brun M, McNiven M, Le Couteur D, Craft P: MDR1 and MRP expression in chronic B-cell lymphoproliferative disorders. Br J Haematol 102:710-717, 1998
44. Holzmayer TA, Hilsenbeck S, Von Hoff DD, Roninson IB: Clinical correlates of *MDR1* (P-glycoprotein) gene expression in ovarian and small-cell lung carcinomas. J Natl Cancer Inst 84:1486-1491, 1992
45. Willman CL: The prognostic significance of the expression and function of multidrug-resistance transporter proteins in acute myeloid leukemia: Studies of the Southwest Oncology Group Leukemia Research Program. Semin Hematol 34:25-33, 1997
46. Leith C, Kopecky K, Godwin J, McConnell T, Slovak ML, Chen I-M, Head DR, Appelbaum FR, Willman CL: Acute myeloid leukemia in the elderly: Assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. Blood 89:3323-3329, 1997
47. Weinstein RS, Jakate SM, Dominguez JM, Lebovitz MD, Koukoulis GK, Kuszak JR, Klusens LF, Grogan TM, Saclarides TJ, Roninson IB, Coon JS: Relationship of the expression of the multidrug resistance gene product (P-glycoprotein) in human colon carcinoma to local tumor aggressiveness and lymph node metastasis. Cancer Res 51:2720-2726, 1991
48. Campos L, Guyotat D, Archimbaud E, Calmard-Oriol P, Tsuruo T, Troncy J, Treille D, Fiere D: Clinical significance of multidrug resistance P-glycoprotein expression on acute non-lymphocytic leukemia cells at diagnosis. Blood 79:473-476, 1992
49. Goasguen JE, Dossot J-M, Fardel O, Le Mee F, Le Gall E, Leblay R, LePrise PY, Chaperon J, Fauchet R: Expression of the multidrug resistance-associated P-glycoprotein (P-170) in 59 cases of de novo acute lymphoblastic leukemia: prognostic implications. Blood 81:2394-2398, 1993
50. Zochbauer S, Gsur A, Brunner R, Kryle PA, Lechner K, Pirker R: P-glycoprotein expression as unfavorable prognostic factor in acute myeloid leukemia. Leukemia 8:974-977, 1994
51. Guerci A, Merlin JL, Missoum N, Feldmann L, Marchal S, Witz F, Rose C, Guerci O: Predictive value for treatment outcome in acute myeloid leukemia of cellular daunorubicin accumulation and p-glycoprotein expression simultaneously determined by flow cytometry. Blood 85:2147-2153, 1995
52. Baldini N, Scotlandi K, Barbanti-Brodano G, Manara MC, Maurici D, Bacci G, Bertoni F, Picci P, Sottili S, Campanacci M, Serra M: Expression of P-glycoprotein in high-grade osteosarcomas in relation to clinical outcome. N Engl J Med 333:1380-1385, 1995

53. Chin K-V, Kazumitsu U, Pastan I, Gottesman MM: Modulation of activity of the promoter of the human MDR1 gene by *ras* and p53. Science 255:459-462, 1992
54. Thorgeirsson SS, Huber BE, Sorrell S, Fojo A, Pastan I, Gottesman MM: Expression of the multidrug-resistant gene in hepatocarcinogenesis and regenerating rat liver. Science 236:1120-1122, 1987
55. Schinkel AH, Kemp S, Dolle M, Rudenko G, Wagenaar E: N-glycosylation and deletion mutants of the human MDR1 P-glycoprotein. J Biol Chem 268:7474-7481, 1993
56. Nooter K, Westerman AM, Flens MJ, Zaman GJR, Scheper RJ, Van Wingerden KE, Burger H, Oostrum R, Boersma T, Sonneveld P, Gratama JW, Kok T, Eggermont AMM, Bosman FT, Stoter G: Expression of the multidrug resistance-associated (*MRP*) gene in human cancers. Clin Cancer Res 1:1301-1310, 1995
57. Filipits M, Suchomel RW, Dekan G, Stiglbauer W, Haider K, Depisch D, Pirker R: Expression of the multidrug resistance-associated protein (MRP) gene in colorectal carcinomas. Br J Cancer 75:208-212, 1997
58. Nooter K, Brutel de la Riviere G, Look MP, van Wingerden KE, Henzen-Logmans SC, Scheper RJ, Flens MJ, Klijn JGM, Stoter G, Foekens JA: The prognostic significance of expression of the multidrug resistance-associated protein (MRP) in primary breast cancer. Br J Cancer 76:486-493, 1997
59. Nooter K, Brutel de la Riviere G, Klijn J, Stoter G, Foekens J: Multidrug resistance protein in recurrent breast cancer. Lancet 349:1885-1886, 1997
60. Nooter K, Kok, T, Bosman FT, van Wingerden KE, Stoter G: Expression of the multidrug resistance protein (MRP) in squamous cell carcinoma of the oesophagus and response to pre-operative chemotherapy. Eur J Cancer 34:81-86, 1998
61. Takebayashi Y, Akiyama S, Natsugoe S, Hokita S, Niwa K, Kitazono M, Sumizawa T, Tani A, Furukawa T, Aikou T: The expression of multidrug resistance protein in human gastrointestinal tract carcinomas. Cancer 82:661-666, 1998
62. Ota E, Abe Y, Oshika Y, Ozeki Y, Iwasaki M, Inoue H, Yamazaki H, Ueyama Y, Takagi K, Ogata T, Tamaoki N, Nakamura M: Expression of the multidrug resistance-associated protein (*MRP*) gene in non-small-cell lung cancer. Br J Cancer 72:550-554, 1995
63. Narasaki F, Matsuo I, Ikuno N, Fukuda M, Soda H, Oka M: Multidrug resistance-associated protein (MRP) gene expression in human lung cancer. Anticancer Res 16:2079-2082, 1996
64. Nooter K, Bosman FT, Burger H, van Wingerden KE, Flens MJ, Scheper RJ, Oostrum RG, Boersma AWM, van der Gaast A, Stoter G: Expression of the multidrug resistance-associated protein (MRP) gene in primary non-small-cell lung cancer. Ann Oncol 7:75-81, 1996
65. Campling BG, Young LC, Baer KA, Lam YM, Deeley RG, Cole SP, Gerlach JH: Expression of the MRP and MDR1 multidrug resistance gene in small cell lung cancer. Clin Cancer Res 3:115-122, 1997
66. Berger W, Hauptmann E, Elbling L, Vetterlein M, Kokoschka EM, Micksche M: Possible role of the multidrug resistance-associated protein (MRP) in chemoresistance of human melanoma cells. Int J Cancer 71:108-115, 1997
67. Burger H, Nooter K, Zaman GJ, Sonneveld P, van Wingerden KE, Oostrum RG, Stoter G: Expression of the multidrug resistance-associated protein (MRP) in acute and chronic leukemias. Leukemia 8:990-997,1994

68. Burger H, Nooter K, Sonneveld P, van Wingerden KE, Zaman GJ, Stoter G: High expression of the multidrug resistance-associated protein (MRP) in chronic and prolymphocytic leukaemia. Br J Haematol 88:348-356, 1994
69. Norris MD, Bordow SB, Marshall GM, Haber PS, Cohn SL, Haber M: Expression of the gene for multidrug-resistance-associated protein and outcome in patients with neuroblastoma. N Engl J Med 334:231-238, 1996
70. Nooter K, Oostrum R, Jonker R, van Dekken H, Stokdijk W, van den Engh G: Effect of cyclosporin A on daunorubicin accumulation in multidrug-resistant P388 leukemia cells measured by real-time flow cytometry. Cancer Chemother Pharmacol 23:296-300, 1989
71. Twentyman PR: MDR1 (P-glycoprotein) gene expression – Implications for resistance modifiers trials. J Natl Cancer Inst 84:1458-1460, 1992
72. Fine RL: Multidrug resistance. Cancer Chemother Biol Response Modif 10:73-84, 1989
73. Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y: Overcoming of vincristine resistance in P388 leukemia *in vivo* and *in vitro* through enhanced cytotoxicity of vincristine and vinblastine by verapamil. Cancer Res 41:1967-1972, 1981
74. Ford JM, Hait WN: Pharmacology of drugs that alter multidrug resistance in cancer. Pharmacol Rev 42:155-199, 1990
75. Bradshaw DM, Arceci RJ: Clinical relevance of transmembrane drug efflux as a mechanism of multidrug resistance. J Clin Oncol 16(11):3674-3690, 1998
76. Advani R, Saba H, Tallman MS, Rowe JM, Wiernik PH, Ramek J, Dugan K, Lum B, Villena J, Davis E, Paietta E, Litchman M, Sikic BI, Greenberg PL: Treatment of refractory and relapsed acute myelogenous leukemia with combination chemotherapy plus the multidrug resistance modulator PSC 833 (Valspodar). Blood 93:787-795, 1999
77. Planting AST, van der Gaast A, Sparreboom A, van der Burg MEL, de Boer M, Wissel PS, Jewell RC, Paul EM, Verweij J: Phase I and pharmacokinetic (PK) study targeting a 100 ng/mL plasma concertration of the potent multidrug resistance (MDR) modulator GF120918 (GF) with doxorubicin (dox) in patients with advanced solid tumors. Proc Am Soc Clin Oncol 17:199a, 1998 (abstract)
78. Ferry D, Moore M, Bartlett NL, Fyfe D, Oza A, Fracasso PM, Kersey K, Wissel PS, Jewell RC, Paul EM: Phase I and pharmacokinetic study targeting a 500 ng/ml plasma concentration of the potent multidrug resistance modulator GF120918 with doxorubicin in patients with advanced solid tumors. Proc Am Soc Clin Oncol 17:922, 1998 (abstract)
79. Rowinsky EK, Smith L, Wang Y-M, Chaturvedi P, Villalona M, Campbell E, Aylesworth C, Eckhardt SG, Hammond L, Kraynak M, Drengler R, Stephenson Jr J, Harding MW, Von Hoff DD: Phase I and pharmacokinetic study of paclitaxel in combination with biricodar, a novel agent that reverses multidrug resistance conferred by overexpression of both *MDR1* and MRP. J Clin Oncol 16:2964-2976, 1998
80. Peck RA, Marshall J, Ziessman H, Hewett J, Schmalbach T, Chatuvedi P, Hawkins M: A phase I trial of doxorubicin and VX-710. Proc Am Assoc Cancer Res 37:165, 1996 (abstract)
81. van Zuijlen L, Sparreboom A, van der Gaast A, van der Burg MEL, van Beurden V, Bol CJ, Woestenborghs R, Palmer PA, Verweij J: The orally administered P-glycoprotein inhibitor R101933 does not alter the pharmacokinetics of docetaxel. Clin Cancer Res 6:1365-1371, 2000

82. Awada A, Bleiberg H, Gil T, Di Leo A, Geurs F, de Valeriola D, McCabe S, Palmer PA, Woestenborghs R, Bol CJJG, Piccart MJ: Oral R101933, an inhibitor of MDR1 P-glycoprotein does not significantly alter the pharmacokinetics and pharmacodynamics of paclitaxel. Proc Am Ass Cancer Res 40:4373, 1999 (abstract)
83. Naito M, Tsuruo T: New multidrug-resistance-reversing drugs, MS-209 and SDZ PSC 833. Cancer Chemother Pharmacol 40:S20-S24, 1997
84. Naito S, Koike K, Ono M, Machida T, Tasaka S, Kiue A, Koga H, Kumazawa J: Development of novel reversal agents, imidazothiazole derivatives, targeting MDR1- and MRP-mediated multidrug resistance. Oncol Res 10:123-132, 1998
85. Dantzig AH, Shepard RL, Cao J, Law KL, Ehlhardt WJ, Baughman TM, Bumol TF, Starling JJ: Reversal of P-glycoprotein-mediated multidrug resistance by a potent cyclopropyldibenzosuberane modulator, LY335979. Cancer Res 56:4171-4179, 1996
86. Roe M, Folkes A, Ashworth P, Brumwell J, Chima L, Hunjan S, Pretswell I, Dangerfield W, Ryder H, Charlton P: Reversal of P-glycoprotein mediated multidrug resistance by novel anthranilamide derivatives. Bioorg Med Chem Lett 9:595-600, 1999
87. Mistry P, Plumb J, Eccles S, Watson S, Dale I, Ryder H, Box G, Charlton P, Templeton D, Bevan PB: *In vivo* efficacy of XR9051, a potent modulator of P-glycoprotein mediated multidrug resistance. Br J Cancer 79:1672-1678, 1999
88. Beck JF, Buchholz F, Ulrich W-R, Boer R, Sanders KH, Niethammer D, Gekeler V: Rhodamine 123 efflux modulation in the presence of low or high serum from CD56+ hematopoietic cells or CD34+ leukemic blasts by B9309-068, a newly designed pyridine derivative. Cancer Lett 129:157-163, 1998
89. Chen H, Bamberger U, Heckel A, Guo X, Cheng Y: BIBW-22 , a dipyridamole analogue, acts as a bifunctional modulator on tumor cells by influencing both P-glycoprotein and nucleoside transport. Cancer Res 53:1974-1977, 1993
90. List AF, Chakos H, Glinsmann-Gibson B, Bamberger U: Reversal of classical and non-P-glycoprotein (PGP)-mediated multidrug resistance (MDR) by BIBW-22. Blood 86:516a, 1995 (abstract)
91. Gekeler V, Ise W, Sanders KH, Ulrich W, Beck J. The leukotreine LTD_4 receptor antagonist MK571 specifically modulates MRP associated multidrug resistance. Biochem Biophys Res Commun 208:345-352, 1995
92. Vanhoefer U, Cao S, Minderman H, Tóth K, Scheper RJ, Slovak ML, Rustum YM: PAK-104P, a pyridine analogue reverses paclitaxel and doxorubicin resistance in cell lines and nude mice bearing xenografts that overexpress the multidrug resistance protein. Clin Cancer Res 2:369-377, 1996
93. Beketic-Oreskovic L, Duran GE, Chen G, Dumontet C, Sikic BI: Decreased mutation rate for doxorubicin resistance and suppression of *MDR1* gene activation by PSC 833. J Natl Cancer Inst 87:1593-1602, 1995
94. Beck WT, Grogan TM, Willman CL, Cordon-Cardo C, Parham DM, Kuttesch JF, Andreeff M, Bates SE, Berard CW, Boyett JM, Brophy NA, Broxterman HJ, Chan HSL, Dalton WS, Dietel M, Fojo AT, Gascoyne RD, Head D, Houghton PJ, Srivastava DK, Lehnert M, Leith CP, Paietta E, Pavelic ZP, Rimsza L, Roninson IB, Sikic BI, Twentyman PR, Warnke R, Weinstein R: Methods to detect P-glycoprotein-associated multidrug resistance in patients' tumors: consensus recommendations. Cancer Res 56:3010-3020, 1996

95. Dalton WS, Grogan T, Salmon SE: Drug resistance in multiple myeloma. Cancer Res Ther Control 6:189-190, 1998
96. Zaman GJR, Flens MJ, van Leusden MR, de Haas M, Mülder HS, Lankelma J, Pinedo HM, Scheper RJ, Baas F, Broxterman HJ, Borst P: The human multidrug-resistance-associated protein MRP is a plasma membrane drug-efflux pump. Proc Natl Acad Sci U S A 91:8822-8826, 1994
97. Breuninger LM, Paul S, Gaughan K, Miki T, Chan A, Aaronson SA, Kruh GD: Expression of multidrug resistance-associated protein in NIH/3T3 cells confers multidrug resistance associated with increased efflux and altered intracellular drug distribution. Cancer Res 55:5342-5347, 1995
98. Versantvoort CH, Schuurhuis GJ, Pinedo HM, Eekman CA, Kuiper CM, Lankelma J, Broxterman HJ: Genistein modulates the decreased drug accumulation in non-P-glycoprotein mediated multidrug resistant tumour cells. Br J Cancer 68:939-946, 1993
99. Versantvoort CH, Broxterman HJ, Lankelma J, Feller N, Pinedo HM: Competitive inhibiton by genistein and ATP-dependence of daunorubicin transport in intact MRP overexpressing human small-cell lung cancer cells. Biochem Pharmacol 48:1129-1136, 1994
100. Versantvoort CHM, Broxterman HJ, Bagrij T, Scheper RJ, Twentyman PR: Regulation of glutathione of drug transport in multidrug resitant human lung tumour cell lines overexpressing *MRP*. Br J Cancer 72:82-89, 1995
101. Zaman GJR, Lankelma J, van Tellingen O, Beijen J, Dekker H, Paulusma C, Oude Elferink RPJ, Baas F, Borst P: Role of glutathione in the export of compounds from cells by the multidrug-resitance-associated protein. Proc Natl Acad Sci U S A 92:7690-7694, 1995
102. Ford JM, Yang JM, Hait WN: Effect of buthionine sulfoximine on toxicity of verapamil and doxorubicin to multidrug resistant cells and to mice. Cancer Res 51:67-72, 1991
103. Lai GM, Moscow JA, Alvarez MG, Fojo AT, Bates SE: Contribution of glutathione and glutathione-dependent enzymes in the reversal of Adriamycin resistance in colon carcinoma cell lines. Int J Cancer 49:688-695, 1991
104. Ma L, Krishnamachary N, Center MS: Phosporylation of the multidrug resistance associated protein gene encoded protein P190. Biochemistry 34:3338-3343, 1995
105. Bertram J, Paifner K, Killian M, Brysch W, Schlingensiepen K-H, Hiddemann W, Kneba M: Reversal of multiple drug resistance *in vitro* by phosphorothioate olignucleotides and ribozymes. Anticancer Drugs 6:124-134, 1995
106. Stewart AJ, Canitrot Y, Baracchini E, Dean NM, Deeley RG, Cole SPC: Reduction of expression of the multidrug resistance protein (MRP) in human tumor cells by antisense phosphorothioate olignucleotides. Biochem Pharmacol 51:461-469, 1996
107. Rubin SC, Finstad CL, Hoskins WJ, Saigo PF, Provencher DM, Federici MG, Hakes TB, Markman M, Reichman BS, Lloyd KO, Lewis JL: Expression of P-glycoprotein in epithelial ovarian cancer: Evaluation as a marker of multidrug resistance. Am J Obstet Gynecol 163:69-73, 1990
108. Lehnert M, de Giuli R, Kunke K, Emerson S, Dalton WS, Salmon SE: Serum can inhibit reversal of multidrug resistance by chemosensitisers. Eur J Cancer 32A:862-867, 1996
109. Smith AJ, Mayer U, Schinkel AH, Borst P: Availability of PSC833, a substrate and inhibitor of P-glycoproteins, in various concentrations of serum J Natl Cancer Inst 90:1161-1166, 1998

110. Fuse E, Tanii H, Kurata N, Kobayashi H, Shimada Y, Tamura T, Sasaki Y Tanigawara Y, Lush RD, Headlee D, Figg WD, Arbuck SG, Senderowicz AM, Sausville EA, Akinaga S, Kuwabara T, Kobayashi S: Unpredicted clinical pharmacology of UCN-01 caused by specific binding to human alpha1-acid glycoprotein. Cancer Res 58:3248-3253, 1998
111. Tunggal JK, Ballinger JR, Tannock IF: Influence of cell concentration in limiting the therapeutic benefit of P-glycoprotein reversal agent. Int J Cancer 81:741-747, 1999
112. Relling MV: Are the major effects of P-glycoprotein modulators due to altered pharmacokinetics of anticancer drugs? Ther Drug Monit 18:350-356, 1996
113. Fields A, Hochster H, Runowicz C, Speyer J, Goldberg G, Cohen C, Dottino P, Wadler S, Berk G, Gretz H, Mandeli J, Holland J, Letvak L: PSC833: initial results in refractory ovarian cancer patients. Curr Opin Oncol 10:S21, 1998
114. Sonneveld P, Marie JP, Huisman C, Vekhoff A, Schoester M, Faussat AM, van Kapel J, Groenewegen A, Charnick S, Zittoun R, and Löwenberg: reversal of multidrug resistance by SDZ PSC 833, combined with VAD (vincristine, doxorubicin, dexamethasone) in refractory multiple myeloma. Leukemia 10:1741-1750, 1996
115. Boote DJ, Dennis IF, Twentyman PR, Osborne RJ, Laburte C, Hensel S, Smyth JF, Brampton MH, Bleehen NM: Phase I study of etoposide with SDZ PSC 833 as a modulator of multidrug resistance in patients with cancer. J Clin Oncol 14:610-618, 1996
116. Kornblau SM, Estey E, Madden T, Tran HT, Zhao S, Consoli U, Snell V, Sanchez-Williams G, Kantarjian H, Keating M, Newman RA, Andreeff M: Phase I study of mitoxantrone plus etoposide with multidrug blockade by SDZ PSC-833 in relapsed or refractory acute myelogenous leukemia. J Clin Oncol 15:1796-1802, 1997
117. Giaccone G, Linn SC, Welink J, Catimel G, Stieltjes H, van der Vijgh WJF, Eeltink C, Vermorken JB, Pinedo HM: A dose-finding and pharmacokinetic study of reversal of multidrug resistance with SZ PSC 833 in combination with doxorubicin in patients with solid tumors. Clin Cancer Res 3:2005-2015, 1997
118. List AF, Spier C, Greer J, Wolff S, Hutter J, Dorr R, Salmon SE, Futcher B, Baier M, Dalton W: Phase I/II trial of cyclosporin as a chemotherapy resistance modifier in acute leukemia. J Clin Oncol 11:1652-1660, 1993
119. Bartlett NL, Lum BL, Fisher GA, Brophy NA, Ehsan MN, Halsey J, Sikic BI: Phase I trial of doxorubicin with cyclosporin as a modulator of multidrug resistance. J Clin Oncol 12:835-842, 1994
120. Yahanda AM, Adler KM, Fisher GA, Brophy NA, Halsey J, Hardy RI, Gosland MP, Lum BL, Sikic BI: A phase I trial of etoposide with cyclosporin as a modulator of multidrug resistance. J Clin Oncol 10:1624-1634, 1992
121. Erlichman C, Moore M, Thiessen JJ, Kerr IG, Walker S, Goodman P, Bjarnason G, DeAngelis C, Bunting P: Phase I pharmacokinetic study of cyclosporin A combined with doxorubicin. Cancer Res 53:4837-4842, 1993
122. Pinedo HM, Giaccone G: P-glycoprotein – a marker of cancer-cell behavior. N Engl J Med 333:1417-1419, 1995 (editorial)
123. Kool M, de Haas M, Scheffer GL, Scheper RJ, van Eijk MJT, Juijn JA, Baas F, Borst P: Analysis of expression of *cMOAT* (*MRP2*), *MRP3*, *MRP4*, and *MRP5*, homologues of the multidrug resistance-associated protein gene (*MRP1*), in human cancer cell lines. Cancer Res 57:3537-3547, 1997

124. Dalton WS, Grogan TM, Meltzer PS, Scheper RJ, Durie BGM, Taylor CW, Miller TP, Salmon SE: Drug resistance in multiple myeloma and non-Hodgkin's lymphoma: detection of P-glycoprotein and potential circumvention by addition of verapamil to chemotherapy. J Clin Oncol 7:415-424, 1989
125. Dalton WS, Crowley JJ, Salmon S, Grogan TM, Laufman LR, Weiss G, Bonnet JD: A phase III randomized study of oral verapamil as a chemosensitizer to reverse drug resistance in patient with refractory myeloma: A Southwest Oncology Group Study. Cancer 75:815-820, 1995
126. Sonneveld P, Durie BGM, Lokhorst HM, Marie J-P, Solbu G, Suciu S, Zittoun R, Löwenberg B, Nooter K: Modulation of multidrug-resistant multiple myeloma by cyclosporin. Lancet 340:255-259, 1992
127. Tallman MS, Lee S, Sikic BI, Paietta E, Wiernik PH, Bennett JM, Rowe JM: Mitoxantrone, etoposide, and cytarabine plus cyclosporine for patients with relapsed or refractory acute myeloid leukemia. An Eastern Cooperative Oncology Group Pilot Study. Cancer 85:358-367, 1999
128. Solary E, Witz B, Caillot D, Moreau P, Desablens B, Cahn J-Y, Sadoun A, Pignon B, Berthou C, Maloisel F, Guyotat D, Casassus P, Ifrah N, Lamy Y, Audhuy B, Colombat P, Harousseau J-L: Combination of quinine as a potential reversing agent with mitoxantrone and cytarabine for the treatment of acute leukemias: A randomized multicenter study. Blood 88:1198-1205, 1996
129. Miller TP, Grogan TM, Dalton WS, Spier CM, Scheper RJ, Salmon SE: P-glycoprotein expression in malignant lymphoma and reversal of clinical drug resistance with chemotherapy plus high-dose verapamil. J Clin Oncol 9:17-24, 1991
130. Wilson WH, Bates SE, Fojo A, Bryant G, Zhan Z, Regis J, Wittes RE, Jaffe ES, Steinberg SM, Herdt J, Chabner BA: Controlled trial of dexverapamil, a modulator of multidrug resistance, in lymphomas refractory to EPOCH chemotherapy. J Clin Oncol 13:1995-2004, 1995
131. Miller TP, Chase EM, Dorr R, Dalton WS, Lam KS, Salmon SE: A phase I/II trial of paclitaxel for non-Hodgkin's lymphoma followed by paclitaxel plus quinine in drug-resistant disease. Anticancer Drugs 9:135-140, 1998
132. Milroy R: A randomised clinical study of verapamil in addition to combination chemotherapy in small cell lung cancer. Br J Cancer 68:813-818, 1993
133. Ross HJ, Cho J, Osann K, Wong SF, Ramsinghani N, Williams J, Downey-Hurtado N, Slater LM: Phase I/II trial of low dose cyclosporin A with EP for advanced non-small cell lung cancer. Lung Cancer 18:189-198, 1997
134. Tolcher AW, Cowan KH, Solomon D, Ognibene F, Goldspiel B, Chang R, Noone MH, Denicoff AM, Barnes CS, Gossard MR, Fetsch PA, Berg SL, Balis FM, Venzon DJ, O'Shaughnessy JA: Phase I crossover study of paclitaxel with r-verapamil in patients with metastatic breast cancer. J Clin Oncol 14:1173-1184, 1996
135. Wishart G, Bisset D, Paul J, Harnett JA, Habeshaw T, Kerr DJ, Macham MA, Soukop M, Leonard RCF, Knepil J, Kaye SB: Quinidine as a resitance modulator of epirubicin in advanced breast cancer: Mature results of a placebo-controlled randomized trial. J Clin Oncol 12:1771-1777, 1994
136. Warner E, Hedley D, Andrulis I, Myers R, Trudeau M, Warr D, Pritchard KI, Blackstein M, Goss PE, Franssen E, Roche K, Knight S, Webster S, Fraser RA, Oldfield S, Hill W, Kates R: Phase II study of dexverapamil plus anthracycline in patients with metastatic breast cancer who have progressed on the same anthracycline regimen. Clin Cancer Res 4:1451-1457, 1998

137. Verweij J, Herweijer H, Oosterom R, van der Burg MEL, Planting ASTh, Seynaeve C, Stoter G, Nooter K: A phase II study of epidoxorubicin in colorectal cancer and the use of cyclosporin-A in an attempt to reverse multidrug resistance. Br J Cancer 64:361-364, 1991
138. Rodenburg CJ, Nooter K, Herweijer H, Seynaeve, Oosterom R, Stoter G, Verweij J: Phase II study of combining vinblastine and cyclosporin-A to circumvent multidrug resistance in renal cell cancer. Ann Oncol 2:305-306, 1991
139. Mayer U, Wagenaar E, Dorobek B, Beijnen JH, Borst P, Schinkel AH: Full blockade of intestinal P-glycoprotein and extensive inhibition of blood-brain barrier P-glycoprotein by oral treatment of mice with PSC833. J Clin Invest 100:2430-2436, 1997
140. Sparreboom A, van Zuijlen L, Brouwer E, Loos WJ, de Bruijn P, Gelderblom H, Pillay M, Nooter K, Stoter G, Verweij J: Cremophor EL-mediated alteration of paclitaxel distribution in human blood: clinical pharmacokinetic implications. Cancer Res 59:1454-1457, 1999
141. Hyafil F, Vergely C, Du Vignaud P, Grand-Perret T: *In vitro* and *in vivo* reversal of multidrug resistance by GF120918, an acridinecarboxamide derivative. Cancer Res 53:4595-4602, 1993
142. Sparreboom A, Planting AST, Jewell RC, van der Burg MEL, van der Gaast A, de Bruijn P, Loos WJ, Nooter K, Chandler LH, Paul EM, Wissel PS, Verweij J: Clinical pharmacokinetics of doxorubicin in combination with GF120918, a potent inhibitor of MDR1 P-glycoprotein. Anti-Cancer Drugs 10:719-728, 1999
143. Piwnica-Worms D, Chiu ML, Budding M, Kronauge JF, Kramer RA, Croop JM: Functional imaging of multi-drug resistant P-glycoprotein with an organtechnetium complex. Cancer Res 53:977-984, 1993
144. Del Vecchio S, Ciarmiello A, Pace L, Potena MI, Carriero MV, Mainolfi C, Thomas R, D'Aiuto G, Tsuruo T, Salvatore M: Fractional retention of Technetium-99m-Sestamibi as an index of P-glycoprotein expression in untreated breast cancer patients. J Nucl Med 38:1348-1351, 1997
145. Chen CC, Meadows B, Regis J, Kalafsky G, Fojo T, Carrasquillo JA, Bates SE: Detection of *in vivo* P-glycoprotein inhibition by PSC 833 using Tc-99m Sestamibi. Clin Cancer Res 3:545-552, 1997
146. Hendriks NH, Franssen EJF, van der Graaf WTA, Meijer C, Piers DA, Vaalburg W, de Vries EGE: 99m-Tc-sestamibi is a substrate for P-glycoprotein and the multidrug resistance-associated protein. Br J Cancer 77:353-358, 1998
147. Whitherspoon SM, Emerson DL, Kerr BM, Lloyd TL, Dalton WS, Wissel PS: Flow cytometric assay of modulation of P-glycoprotein function in whole blood by the multidrug resistance inhibitor GG918. Clin Cancer Res 2:7-12, 1996
148. Robey R, Bakke S, Stein W, Meadows B, Litman T, Patil S, Smith T, Fojo T, Bates S: Efflux of rhodamine from CD56+ cells as a surrogate marker for reversal of P-glycoprotein-mediated drug efflux by PSC 833. Blood 93:306-314
149. Ruetz S, Brault M, Dalton W, Gros P: Functional interactions between synthetic alkyl phospholipids and the ABC transporters P-glycoprotein, Ste-6, MRP, and Pgh 1. Biochemistry 36:8180-8188, 1997
150. Ringel I, Horwitz SB: Studies with RP 56976 (taxotere): a semisynthetic analogue of taxel. J Natl Cancer Inst 83:288-291, 1991

151. Schinkel AH, Wagenaar E, Mol CA, van Deemter L: P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. J Clin Invest 97:2517-2524, 1996

Chapter 2

The orally administered P-glycoprotein inhibitor R101933 does not alter the plasma pharmacokinetics of docetaxel

Lia van Zuijlen[1], Alex Sparreboom[1], Ate van der Gaast[1], Maria E. L. van der Burg[1], Vera van Beurden[1], Cornelis J. Bol[2], Robert Woestenborghs[2], Peter A. Palmer[2], and Jaap Verweij[1]

[1]Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, The Netherlands and [2]Janssen Research Foundation, Beerse, Belgium

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ABSTRACT

This phase I study was performed to assess the feasibility of combining docetaxel with the new P-glycoprotein inhibitor R101933 and to determine the dose limiting toxicity of this combination. Fifteen patients received oral R101933 alone at a dose escalated from 200 to 300 mg twice daily (cycle 0), an escalating i.v. dose of docetaxel (60, 75, 100 mg/m^2) as a 1-hour infusion (cycle 1), and the combination (cycle 2 and further). Dose limiting toxicity consisting of mucositis and neutropenic fever was reached at the combination docetaxel 100 mg/m^2 and R101933 300 mg twice daily, and the maximum tolerated dose was established at docetaxel 100 mg/m^2 and R101933 200 mg twice daily. Plasma concentrations of R101933 achieved in patients were in the same range as required in preclinical rodent models to overcome paclitaxel resistance. The plasma pharmacokinetics of docetaxel were not influenced by the R101933 regimen at any dose level tested, as indicated by plasma clearance values of 26.5 ± 7.78 L/h/m^2 and 23.4 ± 4.52 L/h/m^2 (P=0.15) in cycle 1 and 2, respectively. These findings indicate that the contribution of a P-glycoprotein inhibitor to the activity of anticancer chemotherapy can now be assessed in patients for the first time independent of its effect on drug pharmacokinetics.

INTRODUCTION

Acquired or intrinsic resistance of malignant cells to taxanes and other naturally occurring drugs has been linked to the so called "classical" mechanism of multidrug resistance (MDR) resulting in decreased intracellular concentrations of these anticancer drugs. This MDR phenotype is characterized by increased levels of P-glycoprotein, a member of the ATP binding cassette superfamily of transmembrane transport proteins with a molecular weight of 170 kD encoded by the MDR1 gene, and acting as an energy dependent drug efflux pump with broad substrate specificity (1,2).

Since the first observation that verapamil could reverse MDR *in vitro*, similar properties have been shown for a wide range of drugs (3). These agents are thought to be competitive substrates for P-glycoprotein and thus can increase the intracellular concentration of a co-administered anticancer agent and consequently restore the antitumoral activity (4). Initially, a

number of drugs, marketed for other indications than inhibiting P-glycoprotein, have entered clinical trials (5). However, it became evident that pharmacokinetic interactions occurred between these P-glycoprotein inhibitors and the co-administered anticancer drugs due in part to competitive inhibition of cytochrome P-450 enzymes resulting in significantly increased toxicity of the anticancer drug (6). By rational design, new modulators were developed to specifically inhibit P-glycoprotein and to be more suitable candidates for further clinical evaluations (7,8). The results of most of these clinical studies have been rather disappointing and the pharmacokinetic interaction between the cytotoxic and the P-glycoprotein inhibiting agent remains a confounding problem (6,9).

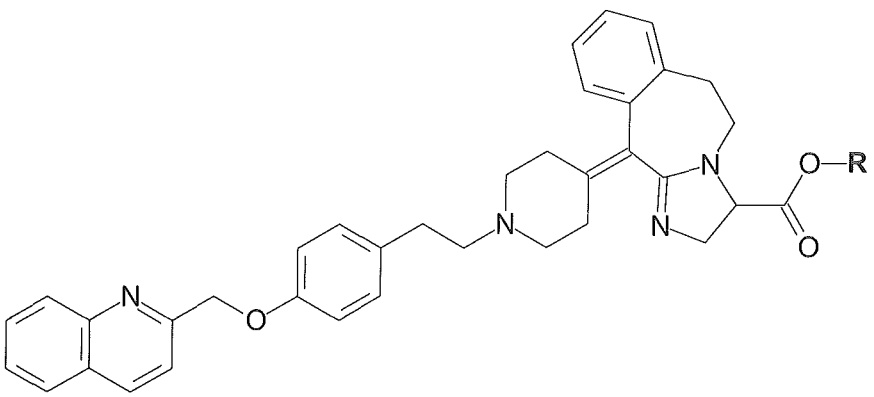

Figure 1 Structure of R101933 (R = CH_3) and its main metabolite R102207 (R = H)

R101933 (Fig. 1) is a new orally administered compound that inhibits P-glycoprotein as demonstrated by various *in vitro* and *in vivo* models (10,11). The tolerability, cardiovascular and laboratory safety, and the pharmacokinetics were investigated in healthy subjects.[1] Nausea and vomiting were the dose limiting adverse events and were reported above 400 mg single oral dose. Drowsiness was also mentioned as side effect. No clinically relevant changes in laboratory and cardiovascular safety parameters were observed. *In vitro* metabolism studies showed that the major metabolic pathway is not cytochrome P450 3A4 dependent.[1] Plasma

[1] Janssen Research Foundation, data on file

levels of R101933 at 200 mg b.i.d. are in the range of concentrations that are active in paclitaxel and adriamycin resistant human tumor xenograph rodent models.[1]

Docetaxel is a known substrate of P-glycoprotein and has shown to have higher affinity for the protein than the related compound paclitaxel (12,13). It also lacks the problems associated with i.v. use of paclitaxel caused by the presence of the formulation vehicle Cremophor EL, which is known to (a) alter the pharmacokinetics of the anticancer drug by entrapment in micelles (14) and (b) masks the effects, if any, of endogeneously expressed P-glycoprotein on the plasma levels of paclitaxel (15). Therefore, the development of agents that could reverse or prevent development of resistance to docetaxel is of great interest.

The principal objectives of this phase I and pharmacokinetic study of R101933 and docetaxel were to determine the clinical utility of the combination and to investigate the potential lack of pharmacokinetic interactions.

PATIENTS AND METHODS

Eligibility

Patients with a histologically confirmed diagnosis of a solid tumor for whom docetaxel as monotherapy was a viable therapeutic option or for whom other treatment options were not available, were candidates for this study. Additional eligibility criteria were: age $\geq$18 and $\leq$75 years; Eastern Cooperative Oncology Group performance status $<$3; life expectancy of at least 3 months; off previous anticancer therapy for at least 4 weeks; no previous treatment with taxanes or high dose chemotherapy requiring progenitor cell support; adequate bone marrow function (WBC count $>$3.5 x 10^9/L, platelet count $>$100 x 10^9/L), renal function (serum creatinine $\leq$2 times upper limit of normal), and liver function (bilirubin level normal, aspartate/alanine aminotransferase $\leq$2 times upper limit of normal and alkaline phosphatase $\leq$2.5 times upper limit of normal); and symptomatic peripheral neuropathy less than grade 2 (NCI criteria). Written informed consent was obtained from all patients, and the study was approved by the Rotterdam Cancer Institute Ethics Board.

Pretreatment and Follow-up

Pretreatment evaluation consisted of recording the history of the patient, physical examination, laboratory studies, electrocardiography, and chest-X-ray. Computer tomographic scans were performed for tumor measurements. Laboratory studies included a complete blood-cell count analysis, and measurement of WBC differential, electrolytes (including sodium, potassium, chloride, calcium and inorganic phosphate), creatinine, urea, alkaline phosphatase, aspartate aminotransfer-ase, alanine aminotransferase, lactate dehydrogenase, bilirubin, total plasma proteins, serum albumin, glucose, uric acid, and urinalysis. History, physical examination, and toxicity scoring (according to the NCI Expanded CTC) were repeated once a week. Complete blood cell counts including WBC differential were performed twice a week, and the other laboratory tests were done once a week. Electrocardiography was repeated as clinically indicated. A final assessment was to be made after patients went off study. Formal tumor measurements and chest-X-ray were performed at 6-weeks intervals until documentation of progressive disease. Standard WHO response criteria were used.

Drug Administration

Docetaxel was administered every 3 weeks on day 3 as a 1-h infusion and was started 1 h after intake of R101933. All patients received pre-medication with dexamethasone orally 8 mg twice daily (b.i.d.), starting one day prior to each infusion of docetaxel for 5 days. R101933 (Janssen Research Foundation, Belgium) was supplied as a 10-mg/mL oral solution in 15% hydroxypropyl-β-cyclodextrin. It had to be taken with water at least 1 h after a meal. The drug was administered twice daily from days 1-5. From studies with healthy volunteers it was known that the terminal half-life of R101933 averaged about 24 h, with peak plasma concentrations attained within 2 h after intake. The maximum tolerated dose after 7-days b.i.d. dosing was 300 mg in healthy volunteers.[1] Seven days dosing at 200 mg b.i.d. appeared to be safe and well tolerated. Pharmacokinetic data revealed that plasma levels of R101933 at 200 mg b.i.d achieve concentrations that are in the same range as required in *in vivo* models to overcome paclitaxel resistance. Hence, the starting dose for our study was set at this dose level. In view of the terminal disposition half life of docetaxel, a simultaneous exposure to both R101933 and docetaxel for a 3-day period

was considered sufficient. This led to the choice of the 5 day R101933 regimen. In the first stage of the study, the dose of docetaxel was escalated and the dose of R101933 was fixed. In the second stage of the study, the dose of docetaxel was fixed and the dose of R101933 was escalated.

First, the patients received 5 doses of R101933 alone every 12 h (cycle 0) followed by a 48 h wash-out to allow assessment of the terminal half-life of R101933. One week later, cycle 1 was initiated with docetaxel alone. Thereafter, the combination was given 3-weekly until progressive disease (PD) or dose limiting toxicity (DLT) occurred.

In each cohort 3 patients were treated unless DLTc (DLT from chemotherapy) or DLTr (DLT from R101933) was observed. In that case, the accrual of 3 additional patients was required. DLTc was defined as grade 3 non-hematological toxicity (with the exception of non-hematological toxicity that was still manageable in an out-patient setting, such as nausea/vomiting) or grade 4 neutropenia lasting >8 days, grade 4 thrombocytopenia or required delay >2 weeks to a subsequent cycle due to toxicity. Febrile neutropenia and neutropenia with severe infection (≥ grade 3 infection) was also considered as DLTc. DLTr was defined as any non-hematological toxicity >grade 2 in the first 2 days of treatment before chemotherapy was given. For dose-escalation decisions only DLTs in cycles 0 and 2 were taken into account. The DLT of the combination of R101933 with docetaxel was reached when greater or equal to three of six patients experienced DLTc. The DLT of R101933 alone was reached when greater or equal to one of three (or greater or equal to two of six) patients experienced DLTr. The maximum tolerated dose (MTD) was defined as the dose level below DLT.

Sample Collection and Processing

Blood specimens were taken in all patients during the first, second and third courses of treatment. Blood volumes of 6 mL were drawn directly into Vacutainer tubes containing lyophilized sodium heparin (Becton Dickinson, Meylan, France) from a peripheral venous access device. In each patient, sufficient plasma was obtained before drug administration to evaluate possible interfering peaks in the chromatographic analysis. Samples for docetaxel analysis were collected immediately before infusion, and at 0.5, 1, 1.25, 1.5, 2, 3, 7, 11, 23, and 31 h after start of infusion.

For determination of R101933 concentrations, blood samples were obtained on day 1 (prior to the first dosing), day 2 (prior to the second dosing and 12 h thereafter), and day 3 (prior to the third dosing and 2, 4, 8, 12, 24, 32, and 48 h thereafter). All blood samples were centrifuged immediately for 10 min at 1000 x *g* to yield plasma, which was stored frozen in polypropylene vials (Eppendorf, Hamburg, Germany) until the time of analysis.

Analytical Methods

A pure reference standard of docetaxel (batch: 14PROC9230; purity: 98.0% by reversed-phase HPLC) and the clinical docetaxel formulation in polysorbate 80 (Taxotere; 40 mg/mL) were kindly supplied by Rhône-Poulenc Rorer (Vitry-sur-Seine, France), and were used as received. Plasma concentrations of docetaxel were determined by a validated liquid chromatographic/tandem mass spectrometric assay, with a lowest limit of quantitation of 1 ng/mL. Samples (200 µL) were pretreated by solid-phase extraction using endcapped Bond Elut nitrile micro-columns (Varian, Harbor City, CA), based on an earlier procedure described for paclitaxel (16). A stainless steel analytical column (100 × 4.6 mm internal diameter) packed with 3 µm Hypersil BDS C18 material (Alltech, Breda, The Netherlands) was used for chromatographic separation, and gradient elution was performed with a mixture of acetonitrile and 0.02 *M* ammonium acetate (pH 4.0) at a flow rate of 0.8 mL/min. Paclitaxel (50 µL of 20 µg/mL in acetonitrile) was used as internal standard. Triple quadrupole mass-spectrometric detection was performed with a turboionspray interface used in the positive ion mode with selective monitoring at *m/z* 808.5 (molecular ion docetaxel parent) and *m/z* 854.5 (molecular ion paclitaxel parent) and at *m/z* 527.0 (docetaxel taxane ring fragment) and *m/z* 569.0 (paclitaxel taxane ring fragment) in the first and third quadrupole, respectively. Calibration curves spanning a range of 1-5000 ng/mL were calculated by regression analysis of peak area ratios of docetaxel and the internal standard *versus* the spiked drug concentration of the standard.

Blood samples collected and processed to plasma were also analyzed, as appropriate, for R101933 and its esterase-mediated carboxylic acid metabolite R102207 using a validated HPLC method. This assay used a selective solid-phase extraction with Bond-Elut Certify microcolumns (Varian). The columns were conditioned with 3 mL of ethanol, 3 mL of

deionized water and 1 mL of 1 *M* aqueous acetic acid. Plasma samples (1 mL) were mixed with 3 volumes of 1 *M* acetic acid and 100 µL of the internal standard (R125026; 10 µg/mL in acetonitrile), and then loaded on the extraction columns. Consecutive washing steps with 3 mL of deionized water, 1 mL of 1 *M* acetic acid and 3 mL of ethanol were performed prior to elution in 3 mL of ethanol-ammonia (98:2, v/v). Samples were dried under nitrogen at 65°C and reconstituted in 100 µL of 0.02 *M* ammonium formate (pH 4.0)-acetonitrile-ethanol (50:25:25, v/v/v). The analytes were separated on a 3-µm Hypersil BDS C8 column (100 × 4.6 mm internal diameter; Alltech) using a mobile phase comprising 0.02 *M* ammonium formate (pH 4.0), acetonitrile and ethanol, delivered with gradient elution at 0.8 mL/min. Detection was performed by UV absorption measurements at 270 nm. The concentrations of R101933 and R102207 were determined from calibration curves constructed in blank human plasma in the range of 2-10,000 ng/mL. The ratio of the log-transformed peak areas of each of the analytes and the internal standard were plotted *versus* nominal concentrations for quantitative computations.

Pharmacokinetic Data Analysis

Individual plasma concentration-time profiles of R101933 and its inactive metabolite R102207 were analyzed model-independently using a validated macro in the EXCEL software package. The actual times of drug intake and blood sampling were taken into account. Peak plasma concentration (C_{max}) was determined by visual inspection of the data. The area under the plasma concentration-time curve (AUC) within a 12-hour dosing interval was calculated by the trapezoidal rule. In all cases, the AUC was extrapolated to infinity by addition of C_{last}/λ, in which C_{last} is the last quantifiable concentration in the curve and λ the terminal elimination rate constant, determined by linear regression analysis of the terminal points of the ln-linear plasma concentration-time curve. The terminal disposition half-life [$t_{1/2(z)}$] was defined as $\ln 2/\lambda$. Individual plasma concentration-time curves of docetaxel were analyzed using the software package WinNonlin (Pharsight, Mountain View, CA), by determination of slopes and intercepts of the plotted curves with multi-exponential functions. All curves were fitted using the actual infusion duration and blood sampling times. In all cases, concentration-time profiles of docetaxel were best fitted to a bi-exponential equation after zero-order input with weighting according the

square of the model predictions of the concentrations. Final values of the iterated parameters of the best-fit function were used to calculate the pharmacokinetic parameters using standard equations (17).

Statistical Considerations

Pharmacokinetic parameters for docetaxel and R101933 are reported as mean values ± SD. Variability in dose-normalized parameters between the various docetaxel dose levels was evaluated by the Kruskal-Wallis statistic followed, if required, by a Dunn's test to determine which group differed. To test pharmacodynamic and pharmacokinetic parameter difference for statistical significance among treatment courses, a two-tailed paired Student's *t* test was performed. Probability values of less than 0.05 were regarded as statistically significant. All statistical calculations were performed using the Number Cruncher Statistical System version 5.X (Dr. Jerry Hintze, Kaysville, UT; 1992) or using Statgraphics Plus version 2 (Manugistics Inc., Rockville, MA).

Table 1 Patient characteristics

Characteristic		No. of patients
Patients included		17
Sex		
Male		10
Female		7
Age, years		
Median	57.5	
Range	42-72	
Performance score (WHO)		
0		8
1		9
Primary tumor		
Urogenital tract		6
Gastrointestinal tract		5
Respiratory tract		2
Melanoma		2
Sarcoma		1
Unknown primary		1
Prior therapy		
Surgery		14
Radiotherapy		7
Chemotherapy		11
None		1

RESULTS

Seventeen patients were entered into this study. Patient characteristics are listed in Table 1; all patients were eligible. Two patients were considered not evaluable for toxicity and response. They did not receive the combination therapy, one because of unexpected rapid deterioration of the clinical condition and another because of development of liver enzyme abnormalities due to the malignant disease that would have precluded administration of docetaxel within normal safety limits. At the dose level R101933 300 mg b.i.d., one patient was not evaluable for pharmacokinetics, because it was not possible to take blood samples during the second cycle for technical reasons.

Toxicity and Pharmacodynamics

A total of 59 cycles, including 44 cycles of combined docetaxel and R101933, were given. Table 2 lists the number of cycles at each dose level and the main toxicities at each dose level. In the first part of the study, the dose of docetaxel was escalated and the dose of R101933 was fixed at 200 mg b.i.d. Each dose level of docetaxel, 60, 75 and 100 mg/m^2 respectively, consisted of 3 patients and no DLT was experienced at this stage. One patient at the dose level 60 mg/m^2 was hospitalized because of neutropenic fever after cycle 3, and was diagnosed to have pneumonia and sinusitis. Because of this clearly unrelated focus, the patient received 2 further cycles which were uneventful. Two patients had grade 2 diarrhea starting one week after docetaxel infusion lasting not more than 4 days. Due to infection occuring in the area of the primary head/neck tumor after cycle 0, one patient treated with docetaxel at a dose level of 100 mg/m^2 was given prophylactic antibiotics during both cycles 1 and 2. From all 3 dose levels of docetaxel studied with R101933 200 mg b.i.d., it was concluded that the combination was feasible with docetaxel given up to 100 mg/m^2, which is the single agent MTD for docetaxel (12).

In the second part of the study the dose of docetaxel was fixed at 100 mg/m^2 and the dose of R101933 was escalated to 300 mg b.i.d. Paired analysis of hematological pharmacodynamic parameters indicated that R101933 co-administration had no significant influence of the observed myelotoxicity (Table 3), including the percent decrease in WBC and absolute neutrophil count. DLTs were seen in four out of six patients,

Table 2 Main toxicities (worst per cycle) at each dose level expressed in number of cycles in which they occurred

Docetaxel mg/m^2	R101933 mg bid	Number pts/cycles	Neutropenic Fever	Neutropenia CTC grade*					Vomiting CTC grade					Mucositis CTC grade				Fatigue CTC grade			DLTc
				0	1	2	3	4	0	1	2	3	4	0	1	2	3	0	1	2	
60	200	3/11	1	4	-	5	-	2	10	-	1	-	-	11	-	-	-	6	-	5	0
75	200	3/18	-	1	1	6	3	7	17	1	-	-	-	16	2	-	-	10	6	2	0
100	200	3/7	2	-	-	2	-	5	7	-	-	-	-	5	1	1	-	2	1	4	0
100	300	6/23	4	1	1	2	3	16	20	-	1	-	2	17	3	2	1	12	7	4	4

* NCI Common Toxicity Criteria

but in two of these already with single treatment of docetaxel, and thus could not be attributed to the combination (Table 4). DLT consisted of neutropenic fever ($n = 2$), mucositis ($n = 1$) and vomiting ($n = 1$).

Table 3 Summary of hematological pharmacodynamics*

Parameter	Cycle 1	Cycle 2 and further	Mean difference	95% C.L.**	*P****
Leukocytes					
Nadir (x10^9/L)	1.51±1.00 (0.43–3.40)	2.23±1.82 (0.20–7.30)	-0.72±0.35	-1.48, 0.038	0.061
%decrease WBC	75.2±18.0 (33.3–96.6)	70.9±17.7 (37.9–96.9)	4.28±4.41	-5.18, 13.7	0.348
Neutrophils					
Nadir (x10^9/L)	0.44±0.49 (0.04–1.50)	0.64±0.86 (0.05–2.60)	-0.20±0.17	-0.57, 0.18	0.274
%decrease ANC	90.6±9.48 (75.6–99.3)	88.6±15.7 (44.8–98.7)	2.06±3.89	-6.50, 10.6	0.607

* Data were obtained from patients after treatment with a 1-h i.v. infusion of docetaxel at a dose level of 60, 75 or 100 mg/m^2 given either alone (cycle 1) or in the presence of oral R101933 at a dose level of 200 or 300 mg b.i.d. (cycle 2 and further). The relative hematological toxicity (i.e., the percentage decrease in blood cell count) was defined as: % decrease = [(pretherapy value – nadir value)/(pretherapy value)] x 100%. Data are presented as mean values ± SD, with the observed range shown in parenthesis.

** C.L., 95% confidence limits for the mean difference; ANC, absolute neutrophil count.

*** Paired Student's *t* test.

Table 4 Neutropenia (highest CTC grade*) and DLT as per protocol in cycle 1 *versus* cycle 2 and further

Taxotere (mg/m^2)	R101933 (mg bid)	Patient	Cycle 1		Cycle 2 and further	
			Neutropenia	DLT	Neutropenia	DLT
60	200	1	2	-	0	-
		2	2	-	4	-
		3	2	-	0	-
75	200	4	4	-	4	-
		5	4	-	4	-
		6	4	-	3	-
100	200	7	4	-	4	-
		8	4	-	4	-
		9	4	-	2	-
100	300	10	4	-	4	+
		11	4	-	4	-
		12	4	+	4	-
		13	0	-	4	+
		14	4	+	4	+
		15	4	-	4	-

* NCI Common Toxicity Criteria

DLTr was not reached at any of the investigated dose levels. Nausea/vomiting and drowsiness, known to be side effects of R101933 in healthy subjects[1], were not seen in our patients after administration of R101933 alone. Fatigue was often mentioned as side effect after docetaxel but never exceeded grade 2. Nevertheless, for one patient given docetaxel at 100 mg/m^2 and R101933 200 mg b.i.d., it was a reason to refuse further treatment after cycle 3, although an ongoing partial response was noted. Two patients at docetaxel 100 mg/m^2 and R101933 300 mg b.i.d. went off study after cycle 2 because of the observed DLT consisting of mucositis and vomiting, respectively. All other patients went off study

because of PD. Two patients achieved a partial response, and seven had stable disease.

Plasma Pharmacokinetics

For the evaluation of docetaxel pharmacokinetics, only the patients who had sampling and complete kinetic data during both treatment courses were included (n = 14 of 15). The results of paired plasma concentration-time profiles of unchanged docetaxel given with and without co-treatment were remarkably similar for all patients studied (Fig. 2).

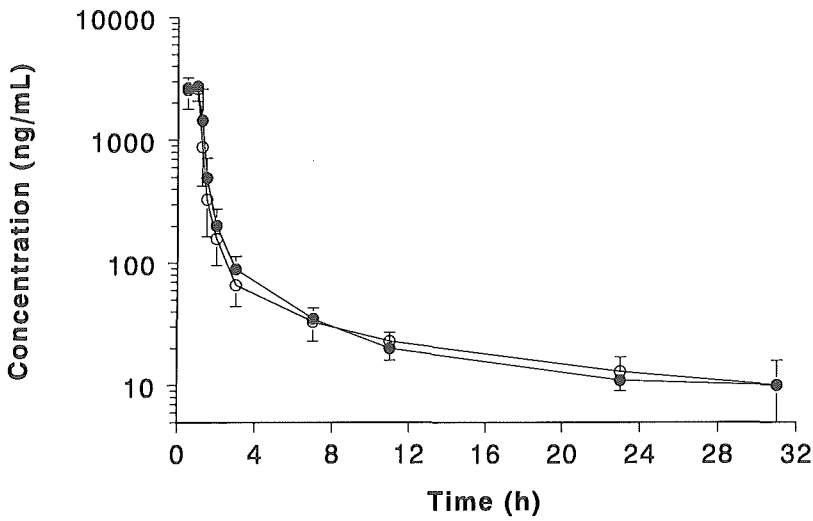

Figure 2 Plasma concentration *versus* time profiles of docetaxel in patients treated with 100 mg/m^2 of docetaxel alone (○) or in combination with 300 mg b.i.d. oral R101933 (●). Data are presented as mean values (symbols) ± SD (error bars).

During both treatment courses, disposition phases appeared to be very typical of a bi-exponential profile, with plasma concentrations of docetaxel decreasing very rapidly immediately after cessation of the infusion, followed by a more prolonged terminal disposition phase of

approximately 11 h, in line with previous observations (18). The mean pharmacokinetic parameters of docetaxel for both treatment courses are summarized as a function of the study cohort in Table 5. The docetaxel total body clearance was normally distributed as judged by the D'Agostino-Pearson omnibus K^2-test, and was independent of the administered dose (Kruskal-Wallis, $P=0.396$), and averaged 26.5 ± 7.78 L/h/m^2 (mean±SD) without R101933 and 23.4 ± 4.52 L/h/m^2 with R101933 (Kruskal-Wallis, $P=0.608$), which is within the same range as described for this compound previously (18). There were no statistically significant differences in any of the studied docetaxel pharmacokinetic parameters, including the clearance ($P=0.15$), between the two treatment courses (Table 5), suggesting that R101933 administration did not influence the disposition of the taxane at the dose levels tested. At the final dose level, combining docetaxel at 100 mg/m^2 and R101933 300 mg b.i.d, statistical analysis indicated that a 1.3-fold change in docetaxel clearance could have been detected with $(1-\beta)=0.80$ at the observed standard deviation of the mean difference between cycles ($s_d=3.13$), and a calculated standardized difference of $2\delta/s_d$ (19).

Similarly, docetaxel did not significantly alter the absorption and elimination routes of R101933 (Table 6). In addition, dose-normalized AUC values for R101933 were similar with or without docetaxel co-treatment. Overall, substantial interpatient variability in R101933 kinetic parameters was apparent, with upto 10-fold variation in peak plasma levels. Over the total dose range studied, the peak plasma levels of R101933 did not increase with values of 133 ± 74 ng/mL (mean±SD; $n=10$) and 136 ± 45 ng/mL ($n=7$) respectively, suggesting a dose-dependent kinetic behavior of the compound with saturable absorption characteristics. For this reason no attempt was made to further increase the dose of R101933.

In all patients, there was extensive formation of the pharmacologically inactive compound R102207, the principal circulating metabolite of R101933, reaching AUC values approximately 80-fold higher than that of the parent compound. Concentrations of this compound were also not substantially influenced by the administration of docetaxel at any dose level tested (Table 6). Of particular note, plasma levels of R101933 capable of reversal of daunorubicin resistance in A2780 cell cultures with and without P-glycoprotein expression were achieved in all patients (20).

Table 5 Plasma pharmacokinetic parameters of docetaxel in the absence or presence of R101933*

Cohort	AUC** (μg.h/mL)	C_{max} (μg/mL)	$t_{1/2(\alpha)}$ (h)	$t_{1/2(z)}$ (h)	V_{dss} (L/m²)	MRT (h)
Docetaxel 60 mg/m² ($n=3$)						
Without R	3.18±0.95	2.06±0.58	0.17±0.02	12.5±3.8	120±43	6.21±2.78
With R 200 mg	2.88±0.52	1.77±0.27	0.20±0.01	11.3±0.4	119±35	5.54±1.08
Docetaxel 75 mg/m² ($n=3$)						
Without R	2.83±1.03	1.68±1.01	0.37±0.36	12.0±2.4	253±227	7.49±3.34
With R 200 mg	3.59±0.30	2.20±0.53	0.32±0.12	12.1±2.5	112±29	5.31±1.22
Docetaxel 100mg/m² ($n=3$)						
Without R	3.93±0.67	2.47±0.21	0.15±0.06	11.1±2.3	137±27	5.51±1.92
With R 200 mg	4.33±1.13	2.95±0.26	0.24±0.06	9.4±4.4	86±41	3.78±2.05
Docetaxel 100mg/m²($n=5$)						
Without R	3.62±0.71	2.54±0.54	0.18±0.03	11.0±2.0	129±32	4.59±0.95
With R 300 mg	4.04±0.84	2.86±0.55	0.23±0.07	10.9±4.8	104±50	4.27±2.02

* Data were obtained from patients after the first (without R101933) and second treatment cycle (with R101933) of a 1-h infusion of docetaxel. The kinetic terms are mean values ± SD.

** AUC, area under the plasma concentration *versus* time curve; C_{max}, maximum plasma concentration of drug; $t_{1/2(i)}$, half life of the *i*-th disposition phase; V_{dss}, volume of distribution at steady state; MRT, mean residence time; *n*, number of patients evaluated at both treatment courses; R, R101933.

Table 6 Plasma pharmacokinetic parameters of R101933 and its metabolite R102207 in the absence or presence of docetaxel*

Parameter	200 mg R101933 ($n=9$) Without D	With D	300 mg R101933 ($n=5$) without D	with D
R101933				
C_{max}** (ng/mL)	120 ± 66	94.3 ± 26.9	127 ± 50	144 ± 42
$t_{1/2(z)}$ (h)	19.6 ± 7.4	NA	23.0 ± 9.2	NA
AUC (μg.h/mL)	0.55 ± 0.24	0.53 ± 0.15	0.91 ± 0.46	0.86 ± 0.49
R102207				
AUC (μg.h/mL)	50.5 ± 14.2	52.4 ± 26.7	37.9 ± 20.2	45.7 ± 11.0

* Data were obtained from patients after cycle 0 (without docetaxel) and after cycle 2 (with docetaxel). The kinetic terms are mean values ± SD.

** AUC, area under the plasma concentration *versus* time curve; C_{max}, maximum plasma concentration of drug; $t_{1/2(z)}$, terminal disposition half-life; *n*, number of patients evaluated; D, docetaxel; NA, not available.

DISCUSSION

In the present study, we observed that the plasma pharmacokinetic characteristics of docetaxel were not substantially influenced by R101933, a new orally administered P-glycoprotein inhibitor. The lack of a pharmacokinetic interaction between docetaxel and R101933 is an important finding, that makes it possible to study the contribution of an inhibitor of P-glycoprotein to the toxicity and activity of an anticancer drug independently of its effects on drug pharmacokinetics. Previous clinical studies have shown that drug interactions between modulator and anticancer drugs occur (even with dose reductions of the anticancer drug), resulting in increased toxicity that can be accounted for by pharmacokinetic interactions alone (reviewed in Ref. 6). These clinical investigations indicate that P-glycoprotein inhibitors [verapamil, cyclosporin A, PSC833 (valspodar), and VX-710 (biricodar)] increase the anticancer drug's systemic exposure, thereby potentially (nonselectively) increasing exposure to normal and malignant cells, resulting in increased severity or incidence of toxic

adverse effects. Based on these findings, it has been proposed that the administration of P-glycoprotein inhibitors is unlikely to improve the therapeutic index of docetaxel (or any other drug) without dose adjustment unless such agents lack a pharmacokinetic interaction (6,9).

Our findings show that previously observed drug pharmacokinetic interactions between anticancer drugs and modulators, such as those between the docetaxel analogue paclitaxel and *r*-verapamil (21), cyclosporin A (22), PSC833 (23) or VX-710 (24), are most likely more related to an overlap in specificity of enzymes responsible for metabolism of the compounds, than to modulation of P-glycoprotein activity. Although few clinical data are available, several *in vitro* studies have shown that docetaxel is extensively metabolized in humans by the cytochrome P450 3A4 system (25,26). The main pathway of docetaxel metabolism in humans consists of successive oxidations of the *tert*-butyl proprionate group on the C13 side chain, with spontaneous cyclization occurring for the putative aldehyde and acid derivatives. All metabolites so far characterized have been found to be many-fold less cytotoxic than docetaxel itself (27,28). In this context, it is noteworthy that R101933 did not influence the *in vitro* metabolism of docetaxel even at concentrations as high as 1 μg/mL, and that the major metabolic route to R102207 is cytochrome-P450 unrelated.[1] Clearly, further experiments are needed to establish the relevance of this principle in humans, and to determine for what drugs it will apply. In addition, when given in combination with docetaxel, biologically relevant R101933 concentrations could be achieved and sustained for several hours, similating optimal pharmacologic conditions required for complete reversal of the MDR phenotype in *in vitro* systems.

Clinically, we observed that single treatment with R101933 given orally at the tested dosages was associated with minimal toxicity. The toxicologic profile of the combination appeared to be very similar to that reported for docetaxel alone, and included neutropenic fever and mucositis as the principal dose limiting toxicities. Febrile neutropenia requiring hospitalization has been reported in approximately 15% and severe mucositis in approximately 10% of cases treated with docetaxel alone (29). In fact, the incidence of neutropenia observed with other inhibitors of P-glycoprotein in studies with anticancer drugs is greater than that observed with the cytotoxic agent alone (24). Fatigue was often mentioned by the

patients in this study as a side effect, but never after R101933 alone and asthenia is also a known side effect of docetaxel.

In conclusion, we have shown that the studied combination of oral R101933 and i.v. docetaxel is safe, and at the achieved dose levels lacks the significant kinetic interaction with the anticancer drug as observed previously with other modulators. In case of a phase II/III study with the combination of R101933 and docetaxel 100 mg/m^2, and in view of the pharmacokinetic data on R101933 presently presented, the recommended dose of R101933 will be 200 mg b.i.d. orally.

REFERENCES

1. Leighton JC, and Goldstein LJ: P-glycoprotein in adult solid tumors. Expression and prognostic significance. Hematology/oncology clinics of North America 9:251-271, 1995
2. Bradshaw DM, and Arceci RJ: Clinical relevance of transmembrane drug efflux as a mechanism of multidrug resistance. J Clin Oncol 16:3674-3690, 1998
3. Ford JM, and Hait WN: Pharmacology of drugs that alter multidrug resistance in cancer. Pharm Rev 42:155-199, 1990
4. Kaye SB: Reversal of multidrug resistance. Cancer Treat Rev 17(Suppl A): 37-43, 1990
5. Raderer M and Scheithauer W: Clinical trials of agents that reverse multidrug resistance. Cancer 73:3553-3563, 1993
6. Relling MV: Are the major effects of P-glycoprotein modulators due to altered pharmacokinetics of anticancer drugs? Ther Drug Monit 18:350-356, 1996
7. Pastan I, Gottesman MM: Multidrug resistance. Ann Rev Med 42:277-286, 1991
8. Twentyman PR: MDR1 (P-glycoprotein) gene expression – Implications for resistance modifier trials. J Natl Cancer Inst 84:1458-1460, 1992
9. Van Zuijlen L, Nooter K, Sparreboom A, and Verweij J: Development of multidrug-resistance convertors: Sense or nonsense? Invest New Drugs 18: 205-220, 2000
10. Wouters W, Janssens F, van Dun J, Krekels M, and Smets G: R101933 is an antagonist of Pgp and modulates resistance to different chemotherapy agents. Proc Am Ass Cancer Res 40:4374 (abstract), 1999
11. Van Ginckel R, Floren W, Moelans P, Janssens B, Moelenberghs K, and Wouters W: Oral treatment with R101933 restores the sensitivity towards paclitaxel and adriamycin in resistant human tumors xenografted in athymic mice. Proc Am Ass Cancer Res 40:4375 (abstract), 1999
12. Fulton B, and Spencer CM: Docetaxel: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in the management of metastatic breast cancer. Drugs 5:1075-1092, 1996
13. Ringel I, and Horwitz SB: Studies with RP 56976 (Taxotere): a semisynthetic analogue of taxol. J Natl Cancer Inst 83:288-291, 1991

14. Sparreboom A, Van Zuijlen C, Brouwer E, Loos WJ, De Bruijn P, Gelderblom AJ, Pillay M, Nooter K, Stoter G, and Verweij J: Cremophor EL-mediated alteration of paclitaxel distribution in human blood: clinical pharmacokinetic implications. Cancer Res 59:1454-1457,1999
15. Van Asperen J, Sparreboom A, Van Tellingen O, and Beijnen JH: Cremophor EL masks the effect of MDR1a P-glycoprotein on the plasma pharmacokinetics of paclitaxel in mice. *In:* J. van Asperen, The pharmacological role of drug-transporting P-glycoproteins in mice, PhD thesis, Chap. 3.2, pp. 109-124, Utrecht University, 1998
16. Willey TA, Bekos EJ, Gaver RC, Duncan GF, Tay LK, Beijnen JH, and Farmen RH High-performance liquid chromatographic procedure for the quantitative determination of paclitaxel (Taxol) in human plasma. J Chromatogr 621:231-238, 1993
17. Gibaldi M and Perier D: Pharmacokinetics. Second edition. New York: Marcel Dekker, Inc. 1982
18. Clarke SJ, and Rivory LP Clinical pharmacokinetics of docetaxel. Clin Pharmacokin 36:99-114, 1999
19. Altman DG Practical statistics for medical research, pp. 440-476. London: Chapman & Hall, 1994
20. Van Zuijlen L, Verweij J, Nooter K, Brouwer E, Stoter G, and Sparreboom A: Role of Intestinal P-glycoprotein in the Plasma and Fecal Disposition of Docetaxel in Humans. Clin Cancer Res 5(S):abstract #518, 1999
21. Berg SL, Tolcher A, O'Shaughnessy JA, Denicoff AM, Noone M, Ognibene FP, Cowan KH, and Balis FM Effect of r-verapamil on the pharmacokinetics of paclitaxel in women with breast cancer. J Clin Oncol 13:2039-2042, 1995
22. Sarris AH, Younes A, McLaughlin P, Moore D, Hagemeister F, Swan F, Rodriguez MA, Romaguera J, North L, Mansfield P, Callendar D, Mesina O, and Cabanillas F: Cyclosporin A does not reverse clinical resistance to paclitaxel in patients with relapsed non-Hodgkin's lymphoma. J Clin Oncol 14:233-239, 1996
23. Fields A, Hochster H, Runowicz C, Speyer J, Goldberg G, Cohen C, Dottino P, Wadler S, Berk G, Gretz H, Mandeli J, Holland J, and Letvak L: PSC833: initial clinical results in refractory ovarian cancer patients. Curr Opin Oncol 10(S1):S21, 1998
24. Rowinsky EK, Smith L, Wang Y-M, Chaturvedi P, Villalona M, Campbell E, Aylesworth C, Eckhardt SG, Hammond L, Kraynak M, Drengler R, Stephenson J, Harding MW, and Von Hoff DD: Phase I and pharmacokinetic study of paclitaxel in combination with biricodar, a novel agent that reverses multidrug resistance coferred by overexpression of both *MDR1* and MRP. J Clin Oncol 16:2964-2976, 1998
25. Royer I, Monsarrat B, Sonnier M, Wright M, and Cresteil T: Metabolism of docetaxel by human cytochromes P450: interactions with paclitaxel and other antineoplastic drugs. Cancer Res 56:58-65, 1996
26. Marre F, Sanderink GJ, De Souza G, Martinet M, and Rahmanui R: Hepatic biotransformation of docetaxel (Taxotere) *in vitro*: involvement of the CYP3A subfamily in humans. Cancer Res 56:1296-1302, 1996
27. Sparreboom A, Van Tellingen O, Scherenburg EJ, Boesen JJB, Huizing MT, Nooijen WJ, Versluis C, and Beijnen JH: Isolation, purification and biological activity of major docetaxel metabolites from human feces. Drug Metab Dispos 24:655-661, 1996
28. Commerçon A, Bourzat JD, Bézard D, and Vuilhorgne M: Partial synthesis of major metabolites of docetaxel. Tetrahedron, 50:10289-10298, 1994

29. Trudeau ME: Docetaxel (Taxotere): An overview of first line monotherapy. Sem Oncol, 22(suppl 13):17-21, 1995

Chapter 3

Role of intestinal P-glycoprotein in the plasma and fecal disposition of docetaxel in humans

Lia van Zuijlen, Jaap Verweij, Kees Nooter, Eric Brouwer, Gerrit Stoter, and Alex Sparreboom

Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, The Netherlands

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ABSTRACT

MDR1 P-glycoprotein (Pgp) is a drug-transporting protein that is abundantly present in biliary ductal cells and epithelial cells lining the gastrointestinal tract. Here, we have determined the role of Pgp in the metabolic disposition of the antineoplastic agent docetaxel (Taxotere) in humans. Pharmacokinetic profiles were evaluated in five cancer patients receiving treatment cycles with docetaxel alone (100 mg/m^2 i.v. over a 1-h period) and in combination with a new potent inhibitor of Pgp activity, R101933 (200-300 mg b.i.d.). The terminal disposition half-life and total plasma clearance of docetaxel were not altered by treatment with oral R101933 ($P \geq 0.27$). The cumulative fecal excretion of docetaxel, however, was markedly reduced from 8.47±2.14% (mean±SD) of the dose with the single agent to less than 0.5% in the presence of R101933 ($P = 0.0016$). Levels of the major cytochrome P450 3A4-mediated metabolites of docetaxel in feces were significantly increased after combination treatment with R101933 ($P = 0.010$), indicating very prominent and efficient detoxification of reabsorbed docetaxel into hydroxylated compounds before reaching the systemic circulation. It is concluded that intestinal Pgp plays a principal role in the fecal elimination of docetaxel by modulating re-absorption of the drug after hepatobiliary secretion. In addition, the results indicate that inhibition of Pgp activity in normal tissues by effective modulators, and the physiological and pharmacological consequences of this treatment, cannot be predicted based on plasma drug monitoring alone.

INTRODUCTION

Acquired or intrinsic resistance of malignant cells to taxanes and other naturally occurring anticancer drugs has been linked to the so called "classical" mechanism of multidrug resistance (MDR) which can cause decreased intracellular concentrations of these drugs (1). This MDR phenotype is characterized by increased levels of Pgp, a member of the ATP binding cassette superfamily of transmembrane transport proteins with a molecular weight of 170 kD encoded by the *MDR1* gene, that acts as an energy dependent drug-efflux pump with broad substrate specificity (1). Pgp expression has been found in nearly all tumor types, ranging from leukemia to carcinoma, and it has also been reported in a number of normal

tissues, mainly in specialized epithelial cells with secretory functions including the apical biliary surface of hepatocytes and epithelial cells of the intestinal tract (2). The physiologic functions of Pgp are still speculative at this time, but possibilities include involvement in the protection of epithelial mucosal cells in the gastrointestinal tract from xenobiotics, in transport of steroids in the adrenals and bile salts in the liver, in protein secretion in the kidneys (3), and most recently discovered, in migration of dendritic cells and T-lymphocytes out of the skin (4). In addition, the expression of Pgp in the capillary endothelial cells forming the blood-brain and the blood-testis barrier apparently protects these organs from exogenous toxins, and thus contributes to the role of these anatomic sites as drug sanctuaries (reviewed in Ref. 5).

Several studies have shown that Pgp-mediated MDR is of clinical significance, and this observation has stimulated a search for noncytotoxic agents that can reverse this resistance phenomenon (6,7). Clinical trials performed thus far with identified Pgp inhibitors given in combination with anticancer drugs, however, have raised important issues regarding the safety of the chemotherapeutic treatment because of drug interactions that increased or changed the spectrum of associated toxic side effects (8,9). The results of these clinical trials in combination with the poorly defined pharmacologic and physiologic function of Pgp in humans emphasize the need to perform studies to further evaluate the role of Pgp in the disposition of substrate drugs. The importance of these studies is further underscored by the previous use of anticancer drugs that are rather poor substrates of Pgp given with nonspecific inhibitors of Pgp (9). Thus, in the present study, we evaluated the role of Pgp in the plasma disposition and fecal elimination pathways of docetaxel, one of the best known substrates of Pgp (10), in humans using a combined treatment with the new orally administered Pgp inhibitor R101933 as a model compound.

MATERIALS AND METHODS

Drug Administration

Docetaxel (Rhône-Poulenc Rorer, Antony Cedex, France) was supplied as a concentrated solution in polysorbate 80 (40 mg/mL; Taxotere) and was administered to 5 cancer patients at a dose of 100 mg/m^2 as a 1-h i.v. infusion. Three weeks later, the same dose was administered to each

patient 1 h after intake of R101933 on day 3. All patients received pre-medication with dexamethasone (8 mg b.i.d.), starting 24 h prior to infusion of docetaxel and continuing for 4 days thereafter. R101933 (methyl 6,11-dihydro-11-[1-[2-[4-(2-quinolinyl-methoxy)phenyl]ethyl]-4-piperidinylidene]-5*H*-imidazo[2,1-b][3]benzazepine-3-carboxylate), an agent acting as a specific antagonist of Pgp, was supplied by Janssen Research Foundation (Beerse, Belgium) as an oral solution containing 10 mg/mL of the active compound in 15% hydroxypropyl-β-cyclodextrin. The drug was administered orally twice daily for 5 consecutive days (*i.e.*, days 1-5) with 200 mL water, at least 1 h after a meal. The clinical protocol was approved by the institutional ethics committee, and signed informed consent was obtained from each participant prior to study entry.

Sample Collection and Processing

Blood specimens were acquired in all patients during both treatment courses. Sample volumes of ~6 mL were drawn directly from a peripheral venous access device into tubes containing lyophilized sodium heparin as an anticoagulant. Blood samples were collected immediately before infusion, and at 0.5, 1, 1.25, 1.5, 2, 3, 7, 11, 23, and 31 h after start of infusion. All samples were centrifuged immediately for 10 min at 1000 x *g* to yield plasma, which was stored frozen at -20°C in polypropylene vials until the time of analysis.

Complete stool collections for docetaxel analysis were obtained for the duration of the study (*i.e.*, up to 31 h after start of drug administration). This space of time was chosen based on previous fecal excretion studies with the structural related agent paclitaxel (11). Fecal specimens were collected in polystyrene containers and stored immediately at -20°C. Weighted feces samples were homogenized individually in 3 volumes of a phosphate buffer [containing 0.01 M potassium phosphate, 0.137 M sodium chloride and 2.7 mM potassium chloride in the presence of 0.05% (w/v) glucose at pH 7.4] using five 1-min bursts of an Ultra-Turrax T25 homogenizer (IKA-Labortechnik, Dottingen, Germany) operating at 20,500 rpm. Aliquots of 500 μL feces homogenates were diluted with human plasma (1:1, v/v) and stored frozen at -80°C prior to further processing.

Analytical Methods

A pure reference standard of docetaxel (batch: 14PROC9230; purity: 98.0% by HPLC) was kindly supplied by Rhône-Poulenc Rorer, and was used as received. Plasma concentrations of docetaxel were determined by a validated HPLC assay with mass-spectrometric detection, with a lowest limit of quantitation of 1 ng/mL. Samples (200 μL) were pretreated by solid-phase extraction using endcapped Bond Elut nitrile micro-columns (Varian, Harbor City, CA). A stainless steel analytical column (100×4.6 mm, internal diameter) packed with 3 μm Hypersil BDS C18 material (Alltech, Breda, the Netherlands) was used for chromatographic separation, and gradient elution was performed with a mixture of acetonitrile and 0.02 M ammonium acetate (pH 4.0) at a flow rate of 0.8 mL/min. Paclitaxel (Bristol-Myers Squibb, Wallingford, CT) was used as internal standard.

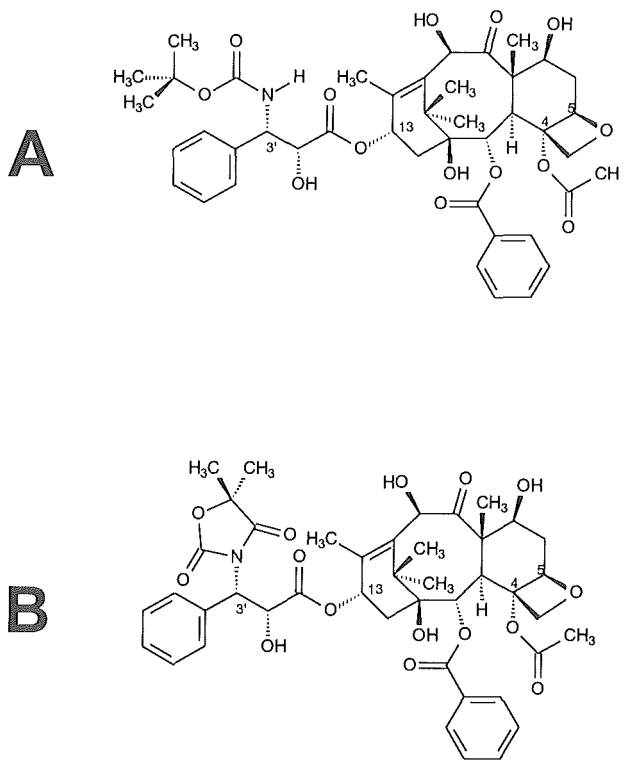

Figure 1 Chemical structures of docetaxel (**A**) and its major cytochrome P450 3A4-mediated metabolite M4 (3'-de-*tert*-butoxy-carbonylamino-3'-[3-(5,5-dimethyl-2,4-dioxo-1,3-oxazoli-dinyl)]-docetaxel) (**B**).

Authentic reference standards of the docetaxel metabolites M1, M2, M3 and M4 (Fig. 1) were obtained following isolation and purification of a patient fecal sample, as described (12), and their concentrations in feces homogenates were determined by reversed-phase HPLC with UV detection using a modification of a procedure described elsewhere (13). In brief, quantitative extraction was achieved by a single solvent extraction of 0.5-mL samples with a mixture of acetonitrile-*n*-butyl chloride (1:4, v/v). Chromatography was performed at 60°C using an Inertsil ODS-80A column (150×4.6 mm; 5 μm particle size; GL Science, Tokyo, Japan) protected by a Lichrospher 100 RP-18 endcapped-guard column (4.0×4.0 mm; 5 μm particle size; Merck, Darmstadt, Germany), and a 1-h exponential gradient elution (45 to 75%; 1 mL/min) of methanol in water-tetrahydrofuran-aqueous ammonium hydroxide (97.4:2.5:0.1, v/v/v) at pH 6.0. The column effluent was monitored at a wavelength of 230 nm.

Concentrations of R101933 and its esterase-mediated carboxylic acid metabolite, R102207, were determined by a validated HPLC method involving solid-phase extraction on Bond-Elut Certify micro-columns (Varian). Standard curves were prepaired in drug-free human plasma and were expanded to encompass concentrations between 2 and 10,000 ng/mL. A 1-mL aliquot of standard or plasma sample was mixed with 3-mL of 1 M aqueous acetic acid and 100 μL of internal standard solution (R125026 in acetonitrile at 10 μg/mL). Prior to loading, columns were preconditioned with 3 mL of ethanol, 3 mL of water and 1 mL of 1 M of aqueous acetic acid. Consecutive washing steps involved 3 mL of water, 1 mL of aqueous acetic acid, and 3 mL of ethanol, and elution was performed with 3 mL of a mixture of ethnanol-ammonia (98:2, v/v). Samples were dried under nitrogen at 65°C and redissolved in 100 μL of 0.02 M ammonium formate (pH 4.0)-acetonitrile-ethanol (50:25:25, v/v/v). Chromatography was performed on a column (100×4.6 mm) packed with 3-μm Hypersil BDS C8 material (Alltech), and gradient elution (0.8 mL/min) with a mixture of 0.02 M ammonium formate (pH 4.0), acetonitrile and ethanol. UV absorption measurements were performed at a wavelength of 270 nm. Determination of analyte concentrations was based on log-transformed peak areas of R101933 and R102207 and the internal standard *versus* nominal concentrations by interpolation using linear regression analysis.

Pharmacokinetic Analysis

Individual plasma concentration-time curves of docetaxel were analyzed by a two-compartimental model using the software package WinNonlin (Pharsight, Mountain view, CA). All curves were fitted using the actual infusion duration and blood sampling times. Pharmacokinetic parameters were calculated by standard methods. To test parameter differences for statistical significance among treatment courses, a two-tailed paired Student's *t* test was performed. Probability values of less than 0.05 were considered statistically significant.

In Vitro Studies

A functional *in vitro* study was performed to evaluate whether oral R101933 in humans yields plasma levels which are capable of inhibiting Pgp function in tumor cells. The parental human ovarian carcinoma cell line A2780 (Pgp negative) and the derived anthracycline-resistant lines $A2780_{T100}$ and 2780AD (both Pgp positive), developed by transfection of Pgp and step-wise exposure to doxorubicin, respectively, were grown and maintained in colorless RPMI1640 medium (Brunschwig, Amsterdam, The Netherlands). Cells were kept in continuous logarithmic growth at 37°C in a humidified atmosphere in 5% CO_2/95% air in medium supplemented with 10% (w/v) heat-inactivated bovine calf serum (Hyclone, Logan, UT), penicillin, streptomycin and L-glutamine. Exponentially grown cells were trypsinized, packed by centrifugation for 5 min at 1500×*g* and washed twice with medium. The cells were counted microscopically and transferred to protein-free RPMI to dilutions containing 4.0×10^5 cells per mL. Inhibition of Pgp-mediated drug efflux in each of the cell lines was evaluated using a daunorubicin (final concentration, 1 μg/mL) retention assay, in the presence of various concentrations of R101933, ranging from 1 to 1000 ng/mL. Preliminary time-course experiments revealed that at a given R101933 concentration, daunorubicin uptake in the cells did not change after 90 min, at which time equilibrium was reached. Thus experiments were carried out with a 2-h incubation period at 37°C, using 0.5-mL aliquots of the cell suspension in 4.5-mL polypropylene screw-cap tubes (Greiner, Alphen aan den Rijn, The Netherlands). The addition of R101933 was done following extraction of the compound from a human plasma matrix or patient sample using a mixture of acetonitrile and *n*-butyl chloride (1:4, v/v), and reconstitution of dried extracts in neat medium by agitation.

During the incubation period, tubes were gently vortex-mixed at 30-min intervals to prevent clogging of the cells. Intracellular levels of daunorubicin were measured using flow cytometry with the aid of a fluorescence-activated cell sorter. The fluorescence intensity in the resistant cell lines relative to that observed in the A2780 parental cell line in the absence of R101933, expressed as percentage, was plotted relative to the R101933 concentration.

RESULTS

Plasma Pharmacokinetics

The results of paired plasma concentration-time profiles of unchanged docetaxel given with and without co-treatment of oral R101933 were remarkable similar for all patients studied (Fig. 2).

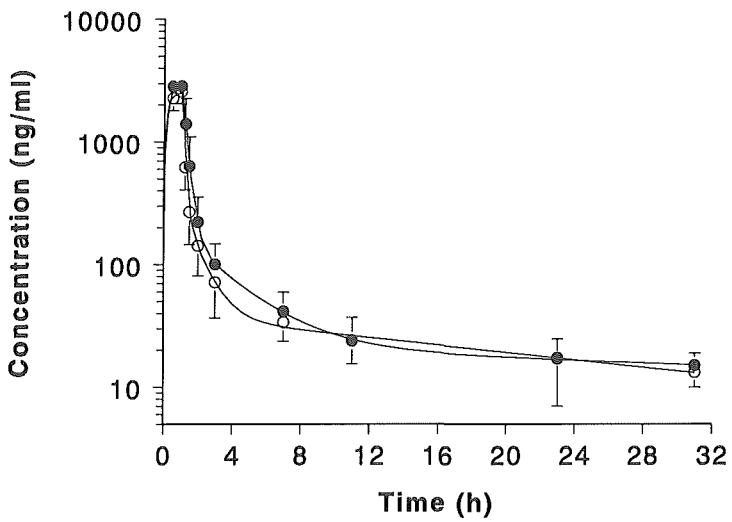

Figure 2 Plasma concentration *versus* time profiles of docetaxel in 5 patients treated with docetaxel alone (100 mg/m^2 as a 1-h i.v. infusion) (○) or in combination with oral R101933 (200 or 300 mg b.i.d.) (●). Data are presented as mean values (symbols) ± SD (error bars).

The mean pharmacokinetic parameters of docetaxel for both treatment courses are summarized in Table 1. There were no statistically significant differences in any of the studied docetaxel kinetic parameters, including the AUC and terminal disposition half-life, suggesting that R101933 did not influence the disposition of the taxane in the systemic circulation. The observed peak plasma concentrations of R101933 were in the range of 25.3 to 168 ng/mL and 83.1 to 142 ng/mL in the absence and presence of docetaxel, respectively. Concentrations of R101933 in this range were observed to inhibit Pgp-mediated daunorubicin efflux in 2 cell lines with known expression of Pgp and resistance to anthracyclines (Fig. 3). In all patients, there was extensive formation of the pharmacologically inactive compound, R102207, resulting from an ester hydrolysis, with peak levels approximately 75-fold higher (range, 33 to 124) than that of the parent drug.

Table 1 Disposition and elimination kinetics of docetaxel in the absence or presence of oral R101933*

Parameter	Without R101933	With R101933	*P***
Plasma			
Docetaxel			
AUC*** (μg.h/mL)	3.62 ± 0.66	4.22 ± 0.97	0.33
	(3.04 - 4.50)	(3.09 - 5.63)	
$t_{1/2(\beta)}$ (h)	11.2 ± 2.10	8.70 ± 4.80	0.27
	(8.60 - 13.3)	(3.15 - 14.4)	
Feces			
Docetaxel			
fe_f (%)	8.47 ± 2.14	0.45 ± 0.24	0.0016
	(5.92 - 11.3)	(0.13 - 0.72)	
M4	13.3 ± 1.81	20.1 ± 4.39	0.010
fe_f (%)	(11.3 - 15.5)	(16.8 - 27.4)	

* Data were obtained from 5 cancer patients receiving a 1-h i.v. infusion of docetaxel at 100 mg/m^2 either alone or in combination with 200 or 300 mg R101933 given orally. The kinetic terms are mean values ± SD, with ranges in parentheses.

** Probability value *vs.* control without R101933 (paired two-sided Student's *t* test).

*** AUC, area under the plasma concentration of docetaxel *vs* time curve; $t_{1/2(\beta)}$, terminal disposition half-life of docetaxel; fe_f, percentage of the absolute docetaxel dose excreted in the feces within 31 h after drug administration as indicated drug.

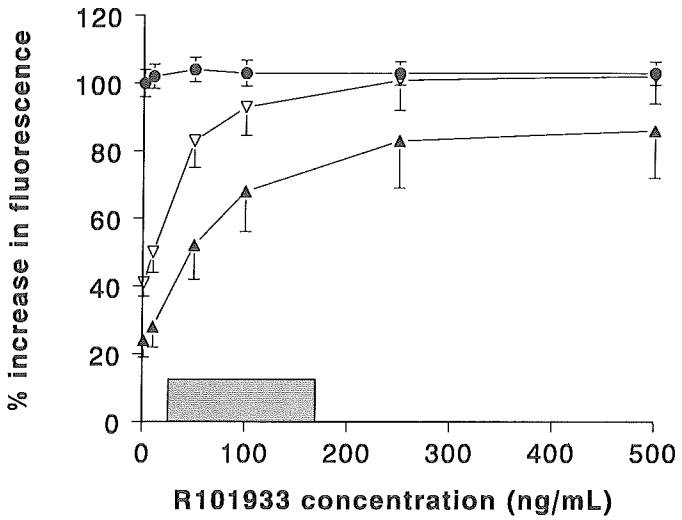

Figure 3 Modulation of cellular daunorubicin uptake by R101933 as measured by a change in the fluorescence intensity in the P-glycoprotein expressing and anthracycline-resistant cell lines $A2780_{T100}$ (▽) and 2780AD (▲) relative to that observed in the A2780 parental cell line (●) in the absence of R101933. Experiments were carried out with a 2-h incubation period at 37°C with R101933 present at various concentrations. Data points, expressed as percentage, are mean values of at least 3 independent experiments performed in duplicate, and bars represent standard errors. The grey box indicates the range of R101933 peak levels observed in the 5 patients following treatment with an oral dose of 200 or 300 mg. Over the entire drug concentration range, substantial increases in intracellular fluorescence, induced by R101933-mediated inhibition of P-glycoprotein activity, could be detected.

Fecal Elimination

In humans, excretion of docetaxel via the feces is a major route of drug elimination (12,14). To evaluate the effects of Pgp inhibition on elimination pathways of docetaxel, feces samples were collected over a period of 31 h from all 5 patients during both treatment courses with and without R101933. Preliminary insight obtained from work of our labaratory into the composition of docetaxel metabolites present in feces samples indicated that apart from docetaxel, 7 chromatographic peaks absorbed at

230 nm, the peak wavelength of taxane derivatives. Structural identification using HPLC and off-line mass spectrometry showed that the major peak was M4, a known metabolite of docetaxel resulting from hydroxylation reactions on the *tert*-butyl propionate side chain (15). Using reference standards, 3 of the minor peaks were identified as metabolites resulting from oxidation of one of the methyl groups on the *tert*-butyl propionate side chain (M2), and spontaneous cyclization of this alcohol derivative to the 2 diastereoisomers M1 and M3.

In patients treated with docetaxel alone, 8.47±2.14% (mean±SD, $n=5$) of the administered dose was excreted in the first 31 h as the parent drug, whereas 13.3±1.81% of the dose could be accounted for by metabolite M4 (Table 1). The total fecal recovery of the metabolites M1, M2, and M3 was very low in all patients (less than 0.5% of the dose; data not shown) and apparently the compounds play only a minor role in the overall drug disposition. In the same group of patients, co-administration with R101933 resulted in markedly reduced fecal excretion of unchanged drug to less than 0.5% of the administered dose ($P=0.0016$). In contrast, however, the excretion of metabolite M4 was significantly increased in all patients to 20.1±4.39% of the dose ($P=0.010$). These data clearly show that R101933 administered orally causes a profound alteration of Pgp-mediated intestinal re-absorption of docetaxel, without modifying the drug's kinetic behavior in plasma.

DISCUSSION

In the present study we have shown that intestinal Pgp plays a principal role in the fecal elimination pathways of docetaxel without modifying the pharmacokinetic behavior of the drug in the systemic circulation. Previous investigations have shown a major role of the cytochrome P450 3A4 isozymes in docetaxel metabolism. In humans, the principal biotransformation routes involve hydroxylation of the *tert*-butoxy function in the C13 side chain, followed by a spontaneous cyclization reaction (15). The 4 principal metabolites resulting from this pathway have substantially less cytotoxic activity on tumor and nonmalignant hematopoietic cells as compared to the parent drug (12). Fecal excretion of docetaxel and its metabolites has been previously reported in 2 other cases (14,15), and in both M4 was the main metabolite, very similar to our

current findings, and accounted for a larger fraction of the recovered dose than docetaxel itself. Interestingly, this metabolite can only be detected in the systemic circulation in about 30% of the patients treated with docetaxel at a dose of 100 mg/m^2, and reaches peak plasma levels ranging from only 22-230 ng/mL (16). This makes it very likely that metabolite M4 is very efficiently excreted through a biliary secretion pathway into the intestinal lumen immediately following its formation, and that re-absorption is low. In our patients, we observed that co-administration with R101933 had a marked effect on the fecal elimination of docetaxel, changing from excretion partly as unchanged drug in case of single agent administration to almost exclusively through metabolic breakdown (mainly to M4) in the combination treatment. The similarity of the terminal disposition phases in plasma of docetaxel between treatment courses indicate that the reduced fecal excretion of the parent drug in the patients receiving R101933 is unlikely related to diminished (Pgp-mediated) biliary secretion. This is also in line with recent preclinical studies performed in mice lacking MDR1 (drug-transporting) Pgp, indicating that these proteins are not essential *per se* for normal hepatobiliary secretion of the related compound, paclitaxel (17). Following biliary secretion of docetaxel given in combination with R101933, re-absorption of drug from the intestinal lumen appears to be a very efficient process. This does not, however, result in increased plasma levels due to a virtually complete first-pass extraction and/or docetaxel metabolism in the liver and intestinal mucosa, which also has significant expression of cytochrome P450 3A isozymes (9) (see Fig. 4 for a schematic representation of the role of intestinal Pgp in docetaxel metabolism and excretion). These findings lend further support to previous observations that the absorption of substrate drugs from the intestines can be increased by concomitant administration with Pgp inhibitors (reviewed in Ref. 18).

The present data also raise important questions regarding the role of endogenously expressed Pgp (for instance in the bile canaliculi, kidneys and intestinal epithelial cells) in the pharmacokinetics of substrate drugs in plasma. It has been shown previously that R101933 does not interfere with docetaxel metabolism in preclinical systems, it does not induce cytochrome P450 isozymes, and the major metabolic routes of the modulator itself is ester hydrolysis, which is independent of cytochrome P450 activity[1]. Our results of unaltered plasma concentrations of docetaxel in the combination

[1] Janssen Research Foundation, data on file

with R101933 is consistent with the postulated concept that pharmacokinetic interference between Pgp modulators and anticancer drugs is the result of competition for (cytochrome P450) enzymes involved in drug metabolism (8). This is also in keeping with previous knowledge from data generated in Pgp knock-out mice (5,17-19) and sheds light on some important mechanistic aspects of drug-drug interactions. Most importantly, our data clearly indicate that inhibiting Pgp function in normal tissues by administration of an effective modulator, the physiological and pharmacological consequences of this treatment cannot be predicted based on plasma drug measurement alone.

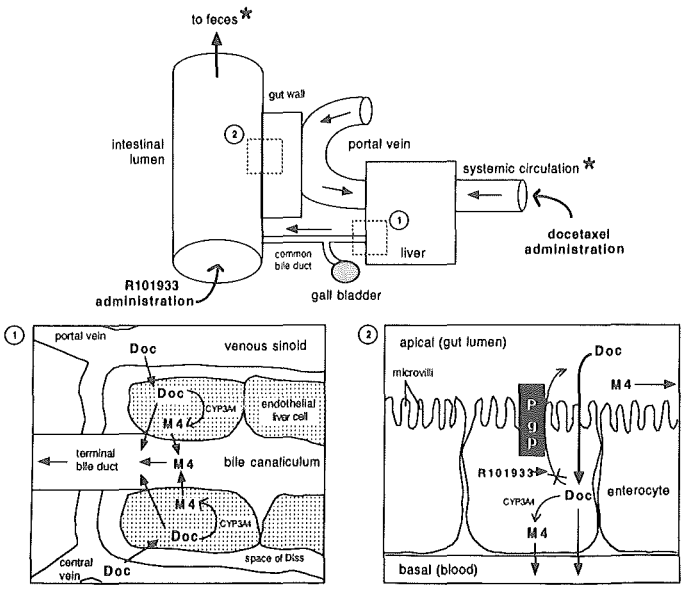

Figure 4 Schematic representation of the role of intestinal P-glycoprotein in docetaxel metabolism and elimination in humans.
①, model of the hepatic-cellular plate showing the bile collecting system with (*i*) hepatic metabolism of docetaxel from the systemic circuation or the portal vein after re-absorption following biliary secretion by cytochrome P450 3A4 isozymes into metabolite M4 and (*ii*) its subsequent secretion in the terminal bile duct.
②, model of the intestinal epithlium showing (*i*) re-uptake of docetaxel into the intestinal lumen, (*ii*) P-glycoprotein-mediated efflux of docetaxel and its inhibition by R101933 and (*iii*) intestinal metabolism by the cytochrome P450 3A4 enzyme system.
*, sites of drug measurement.
Abbreviations: **doc**, docetaxel; **M4**, 3'-de-*tert*-butoxy-carbonylamino-3'-[3-(5,5-dimethyl-2,4-dioxo-1,3-oxazolidinyl)]-docetaxel; CYP3A4, enzymes of the cytochrome P450 family, isoform 3A4.

Given the dominant pharmacological role of intestinal Pgp activity in the efficiency and pattern of fecal excretion of docetaxel, we expect that the possibility to inhibit its activity completely with an orally administered Pgp modulator as demonstrated in this study can have important pharmacological applications. One of these applications is to increase the oral bioavailability of taxane drugs, which are known to display poor absorption characteristics following oral drug administration (19). Indeed, recent experimental data have shown that co-administration of oral PSC833 or cyclosporin A (both substrates of cytochrome P450 3A4 isozymes) increased the AUC for paclitaxel in mice more than 10-fold (20), and preliminary findings from the same group indicate that the same applies to paclitaxel administered to cancer patients (21). Based upon our current data that show a very prominent and efficient detoxification of re-absorbed docetaxel into hydroxylated metabolites before it can reach the systemic circulation, we expect that this approach of increasing drug bioavailability is unlikely to succeed unless the modulator significantly interferes with docetaxel metabolism.

REFERENCES

1. Bradshaw DM, and Arceci RJ: Clinical relevance of transmembrane drug efflux as a mechanism of multidrug resistance. J Clin Oncol 16:3674-3690, 1998
2. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, and Willingham MC: Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci U.S.A. 84:7735-7738, 1987
3. Twentyman PR: Transport proteins in drug resistance: biology and approaches to circumvention. J Int Med 740:133-137, 1997
4. Randolph GJ, Beaulieu S, Pope M, Sugawara I, Hoffman L, Steinman RM, and Muller WA: A physiologic function for p-glycoprotein (MDR-1) during the migration of dendritic cells from skin via afferent lymphatic vessels. Proc Natl Acad Sci U.S.A.95:6924-6929, 1998
5. Van Asperen J, Mayer U, Van Tellingen O, and Beijnen JH: The pharmacological role of P-glycoprotein in the blood-brain barrier. J Pharm Sci 86:881-884, 1997
6. Verrelle P, Meissonnier F, Fonck Y, Feillel V, Dionet C, Kwiatkowski F, Plagne R, and Chassagne J: Clinical relevance of immunohistochemical detecton of multidrug resistance P-glycoprotein in breast carcinoma. J Natl Cancer Inst 83:111-116, 1991
7. Arceci RJ: Clinical significance of P-glycoprotein in multidrug resistance malignancies. Blood 81:2215-2222, 1993
8. Relling MV: Are the major effects of P-glycoprotein modulators due to altered pharmacokinetics of anticancer drugs? Ther Drug Monit 18:350-356, 1996

9. Wacher VJ, Wu C-Y, and Benet LZ: Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. Mol Carcinogen 13:129-134, 1995
10. Ringel I, and Horwitz SB: Studies with RP 56976 (taxotere): a semisynthetic analogue of taxol. J Natl Cancer Inst 83:288-291, 1991
11. Walle T, Walle UK, Kumar GN, and Bhalla KN: Taxol metabolism and disposition in cancer patients. Drug Metab Dispos 23:506-512, 1995
12. Sparreboom A, Van Tellingen O, Scherenburg EJ, Boesen JJB, Huizing MT, Nooijen WJ, Versluis C, and Beijnen JH: Isolation, purification and biological activity of major docetaxel metabolites from human feces. Drug Metab Dispos 24:655-661, 1996
13. Loos WJ, Verweij J, Nooter K, Stoter G, and Sparreboom A: Sensitive determination of docetaxel in human plasma by liquid-liquid extraction and reversed-phase high-performance liquid chromatography. J Chromatogr B 693:437-441, 1997
14. De Valeriola D, Brassine C, Gaillard C, Ketler JP, Tomiak E, Van Vreckem A, Fruhling J, Frydman A, Kerger J, Piccart M, Chapelle P, and Blanc C: Study of excretion balance, metabolism and protein binding of C^{14} radiolabelled Taxotere (TXT) (RP56976, NSC628503) in cancer patients. Proc Am Assoc Cancer Res 34:373, 1993
15. Monegier B, Gaillard C, Sable S, and Vuilhorgne M: Structures of the major human metabolites of docetaxel (RP 56976-Taxotere®). Tetrahedron Lett 35:3715-3718, 1994
16. Rosing H, Lustig V, Koopman FJ, Ten Bokkel Huinink WW, and Beijnen JH: Bioanalysis of docetaxel and hydroxylated metabolites in human plasma by high-performance liquid chromatpgraphy and automated solid-phase extraction. J Chromatogr B 696:89-98, 1997
17. Sparreboom A, Van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DKF, Borst P, Nooijen WJ, Beijnen JH, and Van Tellingen O: Limited oral bioavailability and active epithelial excretion of paclitaxel (taxol) caused by P-glycoprotein in the intestines. Proc Natl Acad Sci U.S.A. 94:2031-2035, 1997
18. Van Asperen J, Van Tellingen O, and Beijnen JH: The pharmacological role of P-glycoprotein in the intestinal epithelium. Pharmacol Res 37:429-435, 1998
19. Sparreboom A, Van Tellingen O, Nooijen WJ, and Beijnen JH: Preclinical pharmacokinetics of paclitaxel and docetaxel: a review. Anti-Cancer Drugs 9:1-16, 1998
20. Van Asperen J, Van Tellingen O, Van der Valk MA, Rozenhart M, and Beijnen JH: Enhanced oral absorption and decreased elimination of paclitaxel in mice cotreated with cyclosporin A. Clin Cancer Res 4:2293-2297, 1998
21. Meerum Terwogt JM, Beijnen JH, Ten Bokkel Huinink WW, Rosing H, and Schellens JHM: Co-administration of cyclosporin enables oral therapy with paclitaxel. Lancet 352:285, 1998

Chapter 4

Disposition of docetaxel in the presence of P-glycoprotein inhibition by intravenous administration of R101933

Lia van Zuylen[1], Alex Sparreboom[1], Ate van der Gaast[1], Ferry A.L.M. Eskens[1], Eric Brouwer[1], Cornelis J. Bol[2], Ronald de Vries[2], Peter A. Palmer[2], and Jaap Verweij[1]

[1]Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, The Netherlands and [2]Janssen Research Foundation, Beerse, Belgium

Submitted

ABSTRACT

Recently, a study of docetaxel in combination with the new orally administered P-glycoprotein (Pgp) inhibitor R101933 showed that this combination was feasible. However, due to low oral bioavailability of R101933 and high inter-patient variability, further attempts to increase the level of Pgp inhibition were canceled. Here, we assessed the feasibility of combining docetaxel with intravenously (i.v.) administered R101933, and determined the disposition of docetaxel with and without the Pgp inhibitor. Patients received R101933 i.v. alone at a dose escalated from 250 to 500 mg on day 1 (cycle 0), docetaxel 100 mg/m^2 as a 1-hour infusion on day 8 (cycle 1) and the combination every three weeks thereafter (cycle 2 and further). Twelve patients were entered of whom 9 received the combination treatment. Single treatment with R101933 i.v. was associated with minimal toxicity consisting of temporary drowsiness and somnolence. Dose-limiting toxicity consisting of neutropenic fever was seen in cycle 1 and cycle 2 or further at both dose levels. The plasma pharmacokinetics of docetaxel did not change by the R101933 regimen at any dose level tested, as indicated by plasma clearance values of 22.5 ± 6.2 L/h/m^2 and 24.2 ± 7.4 L/h/m^2 (P=0.38) in cycle 1 and 2, respectively. However, the fecal excretion of unchanged docetaxel decreased significantly after the combination treatment from 2.54 ± 2.05% to less than 1% of the administered dose of docetaxel, most likely due to inhibition of the intestinal P-glycoprotein by R101933. Plasma concentrations of R101933 were not different in cycle 0 or cycle 2 and the concentrations achieved in the first 12-hour period after i.v. infusion were capable of inhibiting Pgp in an *ex vivo* assay. It is concluded that the combination of docetaxel 100 mg/m^2 i.v. and R101933 500 mg i.v. is feasible, lacks pharmacokinetic interaction in plasma, and shows evidence of Pgp inhibition both in an *ex vivo* assay and *in vivo* by the indication of intestinal Pgp inhibition.

INTRODUCTION

One of the best described mechanisms of multidrug resistance (MDR), the *in vitro* phenomenon thought to be partly responsible for failure of cancer treatment with naturally occurring anticancer drugs such as taxanes, is associated with overproduction of the transmembrane transport

protein Pgp which acts as an ATP-dependent drug efflux pump and thus decreases the intracellular concentrations of these drugs (1,2). Abundant effort has been put in developing drugs which are able to inhibit Pgp and can be used in combination with anticancer drugs (3,4). However, it became evident that pharmacokinetic interactions occurred between the Pgp inhibitors and the co-administered anticancer drugs due to competitive inhibition of cytochrome P-450 enzymes resulting in significantly increased toxicity of the anticancer drugs (5,6). Recently, we described the results of a phase I and pharmacokinetic study with a new Pgp inhibitor, R101933, administered orally in combination with docetaxel i.v. (7). In this study the pharmacokinetic and clinical safety profiles of docetaxel were unchanged indicating a lack of pharmacokinetic interaction between the anticancer drug and the Pgp inhibitor. By means of a functional *in vitro* study it was shown that the achieved plasma concentrations of R101933 were able to inhibit Pgp (8). However, due to a low oral bioavailability of R101933 and significant inter-patient variability in exposure to R101933, further attempts to increase the level of Pgp inhibition by oral administration of R101933 were cancelled and a potential pharmacokinetic interaction with docetaxel at higher concentrations of R101933 could not be excluded (7). Here, we performed a new phase I and pharmacokinetic study with docetaxel in combination with R101933 given intravenously. Plasma samples as well as feces samples were collected. Earlier analysis of fecal excretion of docetaxel without and with oral R101933 revealed that the physiological and pharmacokinetic consequences of inhibition of Pgp by effective modulators can not be predicted based on plasma drug monitoring alone and collecting feces samples can be an important element of the pharmacokinetic studies of anticancer drugs given in combination with a Pgp inhibitor (8). To confirm that the achieved R101933 plasma levels after intravenous administration might be capable of inhibiting Pgp in tumor cells, we developed an *ex vivo* assay as a surrogate measure of Pgp antagonism.

PATIENTS AND METHODS

Eligibility

Patients with a histologically confirmed diagnosis of a solid tumor for whom docetaxel as monotherapy was a viable therapeutic option or for whom other treatment options were not available, were candidates for this

study. Additional eligibility criteria were: age ≥18 years; Eastern Cooperative Oncology Group performance status <3; life expectancy of at least 3 months; off previous anticancer therapy for at least 3 weeks; no previous treatment with taxanes or high dose chemotherapy requiring progenitor cell support; adequate bone marrow function (ANC >1.5 x 10^9/L, platelet count >100 x 10^9/L), renal function (serum creatinine ≤2 times upper limit of normal), and liver function (bilirubin level normal, aspartate/alanine aminotransferase ≤2.5 times upper limit of normal and alkaline phosphatase ≤2.5 times upper limit of normal); and symptomatic peripheral neuropathy <grade 2 (NCI criteria). Written informed consent was obtained from all patients, and the study was approved by the University Hospital Rotterdam Ethics Board.

Pretreatment and Follow-up

Pretreatment evaluation consisted of recording the history of the patient, physical examination, laboratory studies, electrocardiography, and chest-X-ray. Computer tomographic scans were performed for tumor measurements. Laboratory studies included a complete blood-cell count analysis, and measurement of WBC differential, electrolytes (including sodium, potassium, chloride, calcium, magnesium and inorganic phosphate), creatinine, urea, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, bilirubin, total plasma proteins, serum albumin, glucose, uric acid, and urinalysis. History, physical examination, and toxicity scoring (according to the NCI Expanded CTC) were repeated once a week. Complete blood cell counts including WBC differential were performed twice a week. During cylce 0 electrocardiographies were performed at baseline and approximately 1, 2, 6, 24 and 48 hours after start of the infusion with R101933. The electrocardiography was repeated at the end of cycle 2 and further on when clinically indicated. A final assessment was to be made after patients went off study. Formal tumor measurements were performed at 6-weeks intervals until documentation of progressive disease. Standard WHO response criteria were used.

Drug Administration

First, the patients received R101933 alone (cycle 0) followed by a 72-h wash-out period to allow assessment of the terminal half-life of

R101933. One week later, cycle 1 was initiated with docetaxel alone. Thereafter, the combination was given 3-weekly until progressive disease (PD) or dose limiting toxicity (DLT) occurred. Docetaxel was administered every 3 weeks as a 1-h infusion and in the combination was started immediately after the end of the i.v. administration of R101933. All patients received pre-medication with dexamethasone orally 8 mg b.i.d., starting one day prior to each infusion of docetaxel, and to be taken for a total of 3 days every course. R101933 (Janssen Research Foundation, Beerse, Belgium) was supplied as a 10-mg/mL solution in hydroxypropyl-beta-cyclodextrin, hydrochloric acid, citric acid monohydrate, sodium hydroxide and mannitol. The drug was administered by a syringe infusion pump as a single 1-h infusion. The dose of docetaxel was fixed at 100 mg/m^2 and the dose of R101933 was escalated. The first dose level of R101933 was set on 250 mg, a dose known to be capable of Pgp inhibition and to induce no toxicity in a simultaneous study with paclitaxel (9).

In each cohort 3 patients were treated unless DLT of R101933 alone or DLT of the combination treatment was observed. In that case, the accrual of 3 additional patients was required. The occurrence of DLT during the first cycle with docetaxel alone led to a replacement of the patient in the same cohort to which the patient was assigned. DLT of R101933 (DLTr) alone was defined as any non-hematological toxicity > grade 2 before chemotherapy was given. DLT of the combination treatment (DLTc) was defined as grade 3 non-hematological toxicity (with the exception of non-hematological toxicity that was still manageable in an out-patient setting, such as grade 2 alopecia or nausea/vomiting) or grade 4 neutropenia lasting more than 5 days, grade 4 thrombocytopenia or required delay > 2 weeks to a subsequent cycle due to toxicity with a reasonable possibility that the event could be attributed to the combined treatment with R101933 and docetaxel. Febrile neutropenia and neutropenia with severe infection (> grade 2 infection) were also considered as DLT of the combination treatment. For dose-escalation decisions only DLTs in cycles 0 and 2 were taken into account. The DLT of R101933 alone was reached when ≥ 1 out of 3 (or ≥ 2 out of 6) patients experienced DLTr. The DLT of the combination of R101933 with docetaxel was reached when ≥ 3 out of 6 patients experienced DLTc. The maximum tolerated dose (MTD) was defined as the dose level below DLT.

Sample Collection and Processing

Blood specimens were taken in all patients during cycle 0, 1 and 2. Blood volumes of 5-10 mL were drawn directly into Vacutainer tubes containing lyophilized sodium heparin (Becton Dickinson, Meylan, France) from a peripheral venous access device. In each patient, sufficient plasma was obtained before drug administration to evaluate possible interfering peaks in the chromatographic analysis. Samples for docetaxel analysis were collected immediately before infusion, and at 0.5, 1, 1.25, 1.5, 2, 4, 9, 24, and 48 h after start of infusion. For determination of R101933 concentrations, blood samples were collected immediately before infusion, and at 0.5, 1, 1.5, 2, 3, 5, 10, 24, 48, and 72 h after start of infusion. All blood samples were centrifuged immediately for 10 min at 1000 x *g* to yield plasma, which was stored frozen in polypropylene vials (Eppendorf, Hamburg, Germany) until the time of analysis.

Complete stool collections for docetaxel analysis were obtained in cycle 1 and 2 for the duration of the study (*i.e.*, up to 48 h after start of docetaxel administration). This interval was chosen based on previous fecal excretion studies with the structural related agent paclitaxel (10). Fecal specimens were collected in polystyrene containers and stored immediately at -20°C.

Drug Analysis

Plasma concentrations of docetaxel were determined by solid-phase extraction using Bond Elut nitrile microcolumns (Varian, Harobor City, CA) and a sensitive liquid chromatographic assay with tandem mass spectrometric detection, with a lower limit of quantitation of 1 ng/mL (200 μL-samples) (7). The analytical method for R101933 and its esterase-mediated metabolite R102207 was based on solid-phase extraction with Bond-Elut Certify microcolumns (Varian) and high-performance liquid chromatography with UV absorption measurements at 270 nm, with a lower limit of quantitation of 2 ng/mL (1-mL samples) (7). Concentrations of docetaxel and its major hydroxylated metabolites M2, M3, and M4 in feces homogenates were determined by reversed-phase high-performance liquid chromatography with UV detection at 230 nm as described in detail earlier (8,11). This method employs 0.5-mL samples of feces specimens [first homogenized in 0.01 M potassium phosphate buffer (1:3, w/v) and then

diluted (1:1, v/v) with human plasma], with a lower limit of quantitation of 2.0 μg/mL.

Pharmacologic Data Analysis

Plasma concentration-time profiles of docetaxel, R101933 and its metabolite R102207 were pharmacokinetically analyzed by compartimental and noncompartmental models using the software package WinNonlin (Pharsight, Mountain View, CA) (7). The drug disposition half-lives, AUC extrapolated to infinity, plasma clearance, steady-state volume of distribution and percentage of the absolute docetaxel dose excreted in feces as parent drug or metabolite were determined using methods and equations as defined elsewhere (7,8). Hematological pharmacodynamics were evaluated by analysis of the absolute nadir values of blood cell counts or the relative hematological toxicity, i.e. the percentage decrease in blood cell count, which was defined as:

$$\% \text{ decrease} = [(\text{pretherapy value} - \text{nadir value}) / (\text{pretherapy value})] \times 100\%$$

Within each patient, myelosuppression was described either using continuous variables, consisting of the percentage decrease in WBC, absolute neutrophil count and platelet count or as discrete variables in case of NCI-CTC myelotoxicity grade.

Ex Vivo Pharmacodynamics

The expression of high levels of Pgp in the anthracycline-resistant cell line $A2780_{T\text{-}100}$, obtained by transfection of Pgp, allowed us to use an *ex vivo* assay as a surrogate measure of Pgp antagonism in plasma samples of patients treated with R101933. The $A2780_{T\text{-}100}$ cell line was maintained in RPMI1640 medium without *l*-glutamine and phenol red (Gibco, Amsterdam, The Netherlands) supplemented with 10% bovine calf serum, penicillin/streptomycin 50U/50 μg/mL in a 5% CO_2/95% air atmosphere at 37°C. Cells were grown to 80 - 90% confluency and treated with trypsin-EDTA before subculturing. On the day of the experiment, the cells were centrifuged at room temperature and resuspended in protein-free RPMI1640 medium at a concentration of 80,000 cells/mL. Aliquots (500 μL) of cell suspension were transferred to polypropylene tubes containing 250 μL of incubation medium in the presence of daunorubicin and R101933. Extraction of R101933 was performed from 300-μL plasma samples

(patients' samples or spiked calibration samples) using 1500 µL acetonitrile-*n*-butyl chloride (1:4, v/v) in polypropylene microtubes (Eppendorf, Hamburg, Germany) by mixing for 30 seconds. After centrifugation for 5 min at 23,000 *g*, 1300 µL of the upper organic layer was transferred into a glass tube and evaporated to dryness at 50°C under nitrogen. R101933 was reconstituted in 300 µL RPMI1640 medium by agitation, and the sample was centrifuged again for 1 min at 3000 *g*. An aliquot of 250 µL clear supernatant was added to the cell suspension. Next, cells were incubated for 120 min to assure maximal retention of daunorubicin (8). Preliminary experiments had shown that daunorubicin used at a final concentration of 1 µg/mL provided reasonable fluorescence to distinguish differences between the various anticipated concentrations of R101933, thus making this level suitable for the Pgp inhibition studies. Fluorescence measurements of individual cells were performed with flow cytometry using a Becton-Dickinson FACS (San Jose, CA) equipped with an ultraviolet argon laser (excitation at 488 nm, emission at 530/30 and 570/30 nm band-pass filters). Analysis was gated to include single cells on the basis of forward and side light-scatter and was based on acquisition of data from 7500 cells. Log fluorescence was collected and displayed as single parameter histograms. Geometric mean fluorescence values of duplicate measurements were used for all patients' samples, and fluorescence data obtained from experiments done with the A2780 parental cell line (not expressing Pgp) were used as a positive control. Time-course studies were conducted with samples from the first treatment course in all patients using the fluorescence intensity in the resistant cells relative to a pretherapy plasma sample (i.e. in the absence of R101933), normalized to a value of 1.0 (arbitrary units). Previous studies have shown that inactivation (with R101933 in $A2780_{T\text{-}100}$ cells) or complete preclusion of Pgp active-efflux function (in A2780 cells) is associated with a normalized fluorescence value of 2.5 on this scale (8). Therefore, the experimental set-up provides a factor that normalizes the degree of inhibition by R101933 in each plasma sample relative to the total absence of Pgp function and allows for normalization of the results across sample sets both within and between patients. In each patient, the time to maximal inhibition of Pgp function following R101933 administration was determined in addition to the duration above K_i, defined as the concentration required for half-maximum inhibition of Pgp-mediated daunorubicin efflux.

Statistical Considerations

Parameters of all compounds are reported as mean values ± SD, except were indicated otherwise. The difference in pharmacokinetic parameters between the docetaxel administration days and between patient cohorts was evaluated statistically using a two-sided parametric matched-pairs Student's *t*-test (after testing for normality) *plus* the 95% confidence intervals. Probability values (two-sided) of less than 0.05 were considered statistically significant. All calculations were done on the Number Cruncher Statistical Systems v5.x software package (J.L. Hintze, East Kaysville, UT, 1992).

Table 1 Patient characteristics

Characteristic		No. of patients
Patients included		12
Sex		
	Male	6
	Female	6
Age, years		
	Median	52
	Range	34-66
Performance score (ECOG)		
	0	3
	1	8
	2	1
Primary tumor		
	Urogenital tract	3
	Gastrointestinal tract	1
	Respiratory tract	4
	Breast	3
	Sarcoma	1
Prior therapy		
	Surgery	7
	Radiotherapy	7
	Chemotherapy	8
	Hormonal therapy	3
	None	1

RESULTS

Twelve patients were entered into this study. Patient characteristics are listed in Table 1; all patients were eligible. Three patients were considered not evaluable for toxicity, response and pharmacokinetic analysis. In one patient the dose of docetaxel in cycle 2 and further was decreased to 75 mg/m^2 because of disturbed liver function tests after cycle 1. He received 7 cycles of the combination treatment without further deterioration of the liver function or other severe toxicities related to docetaxel or R101933. Two patients did not receive the combination treatment due to fatigue grade 3 and neutropenic fever related to chronic sinusitis, respectively, after treatment with docetaxel alone. The *ex vivo* assay was accomplished in all patients as all patients received cycle 0 with R101933 alone according to the protocol.

Toxicity and Hematological Pharmacodynamics

A total of 34 cycles docetaxel, including 25 cycles of combined docetaxel and R101933, were given. Table 2 lists the number of cycles at each dose level and the main toxicities at each dose level. At the first dose level of R101933, i.e. 250 mg, 3 patients were treated. Neutropenic fever in one patient after administration of docetaxel alone (cycle 1) led to the decision to provide prophylactic antibiotics (ciprofloxacine 500 mg b.i.d., day 4-15). Subsequently, this patient was treated with the combination therapy and experienced no further periods of neutropenic fever but ceased from treatment after cycle 3 due to neurotoxicity grade 3. Because of fatigue grade 4 and myalgia grade 3, another heavily pretreated patient at this dose level ended treatment after cycle 5. Since no DLTs were seen in cycle 0 and 2, the dose of R101933 was escalated to 500 mg. At this dose level all patients experienced fatigue and myalgia from cycle 1 on but these and other toxicities never exceeded grade 2. At both dose levels most patients experienced grade 1-2 drowsiness and somnolence after the administration of R101933. Considering a simultaneously performed study of R101933 i.v. combined with paclitaxel i.v. revealed a maximal tolerated dose of R101933 alone of 500 mg (9), no attempt was made to further increase the dose of R101933 in the present study despite the fact that formally DLT was not reached. All patients without severe toxicity were treated until progressive disease. Partial responses were not seen

Table 2 Main toxicities (worst per cycle) at the two dose levels expressed in number of cycles in which the toxicity occurred

R101933 (mg)	Docetaxel (mg/m^2)	Neutropenia CTC grade*					Somnolence CTC grade			Fatigue CTC grade					Myalgia CTC grade			
		Uk* *	1	2	3	4	0	1	2	0	1	2	3	4	0	1	2	3
250	100					10	5	2	3	2	2	5		1	6	2	1	1
500	100	2	3	2	5	12	21	1	2	15	1	8			11	10	3	

* NCI Common Toxicity Criteria

** uk, unknown

Statistical analysis of paired hematological data, available in all 9 evaluable patients, showed that R101933 co-administration with docetaxel was not associated with a greater percentage decrease in WBC and absolute neutrophil count or lower mean blood cell count nadir (Table 3). This suggests that the observed myelotoxicity in treatment cycles with docetaxel combined with R101933 is not attributable to inhibition of P-glycoprotein in bone marrow precursor cells, and is consistent with our previous observations in patients treated with docetaxel and oral R101933 (7).

Table 3 Summary of hematological pharmacodynamics *

Parameter	Cycle 1	Cycle 2	Difference	95% C.L.**	*P****
Leukocytes					
Nadir ($\times 10^9$/L)	1.63±1.01 (0.66-3.20)	1.97±1.47 (0.63-4.90)	-0.37±0.71	-2.05, 1.32	0.625
%decrease WBC	77.5±10.3 (62.5-88.2)	74.5±19.2 (45.2-94.2)	3.04±6.95	-13.3, 19.4	0.675
Neutrophils					
Nadir ($\times 10^9$/L)	0.62±0.71 (0.07-2.10)	1.00±1.38 (0.12-3.70)	-0.39±0.64	-1.95, 1.19	0.573
%decrease ANC	91.2±9.96 (72.5-98.3)	87.8±15.9 (58.4-97.8)	3.43±7.51	-14.9, 21.8	0.664

* Data were obtained from patients after treatment with a 1-h i.v. infusion of docetaxel at a dose level of 100 mg/m^2 given either alone (cycle 1) or the presence of i.v. R101933 at a dose level of 250 or 500 mg (cycle 2).The relative hematological toxicity (*i.e.*, the percentage decrease in blood cell count) was defined as: %decrease = [(pretherapy value – nadir value)/(pretherapy value)] × 100%. Data are presented as mean values ± SD, with the observed range shown in parenthesis.

** C.L., 95% confidence limits for the mean difference; ANC, absolute neutrophil count.

*** Paired Student's *t* test.

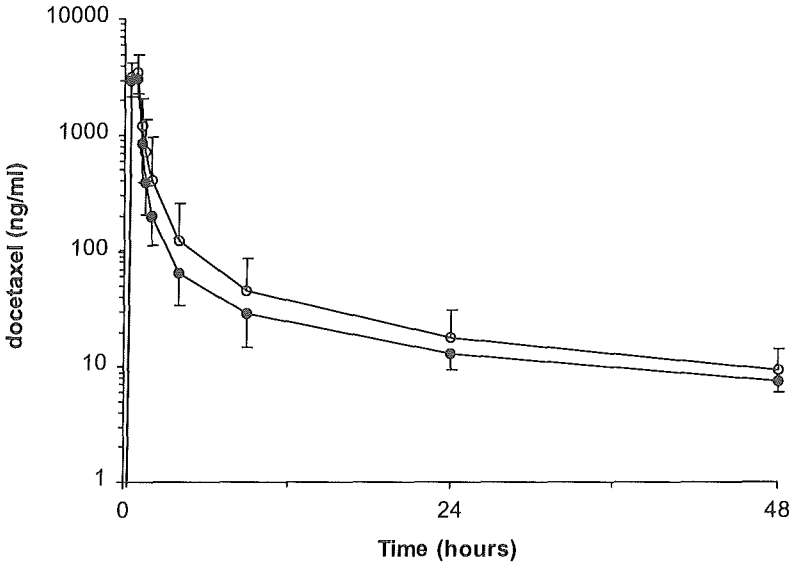

Figure 1 Plasma concentration-time profiles of docetaxel in patients treated with docetaxel at a dose level of 100 mg/m^2 given either alone (○) or in combination with R101933 given at 500 mg as a 1-h i.v. infusion (●). Data are presented as mean values (○, ●) ± SD (error bars).

Docetaxel Disposition

Paired plasma concentration-time profiles of docetaxel given alone or in combination with R101933 were available in all 9 patients (Fig. 1). There were no statistically significant differences in any of the studied parameters, suggesting that R101933 at the dose levels tested did not influence plasma pharmacokinetics of docetaxel (Table 4). At the final dose level tested, combining docetaxel at 100 mg/m^2 and R101933 at 500 mg (n = 6), docetaxel plasma clearance values averaged 22.4 ± 12.6 liters/h/m^2 and 23.8 ± 7.9 liters/h/m^2 in the absence and presence of R101933, respectively ($P = 0.69$). To test whether the pattern of drug excretion altered in the presence of R101933 administered as an i.v. infusion, we also studied the cumulative fecal excretion of docetaxel and its major hydroxylated metabolites over a period of 48 hours. In line with previous findings (8), a minor fraction of the administered docetaxel dose (2.54±2.05%) was recovered in the feces of patients as unchanged parent drug with single agent dosing (Fig. 2). In the presence of R101933, fecal excretion of unchanged drug was markedly reduced to less than 1% of the

Table 4 Plasma pharmacokinetic parameters of docetaxel in the absence or presence of R101933*

Cohort	AUC** (ng.h/mL)	C_{max} (μg/mL)	$T_{1/2(\alpha)}$ (h)	$t_{1/2(z)}$ (h)	V_{dss} (L/m^2)	MRT (h)
Docetaxel 100mg/m^2 ($n=3$)						
Without R	4047±487	2699±699	0.28±0.18	20.4±9.9	201±106	8.0±3.9
With R 250 mg	4277±1233	2628±819	0.17±0.02	13.1±0.9	117±57	4.6±1.2
Docetaxel 100mg/m^2($n=6$)						
Without R	5559±2660	3407±1102	0.27±0.20	14.6±1.5	144±77	6.6±0.8
With R 500 mg	4550±1317	3102±908	0.20±0.02	16.1±4.1	176±107	7.0±2.0

* Data were obtained from patients after the first (without R101933) and second treatment cycle (with R101933) of a 1-h infusion of docetaxel. The kinetic terms are mean values ± SD.

** AUC, area under the plasma concentration *versus* time curve; C_{max}, maximum plasma concentration of drug; $t_{1/2(i}$, half life of the *i*-th disposition phase; V_{dss}, volume of distribution at steady state; MRT, mean residence time; *n*, number of patients evaluated at both treatment courses; R, R101933.

dose ($P=0.01$, paired Student's t test). In contrast, however, the cumulative fecal excretion of the hydroxylated metabolites increased more than 2-fold ($P=0.01$).

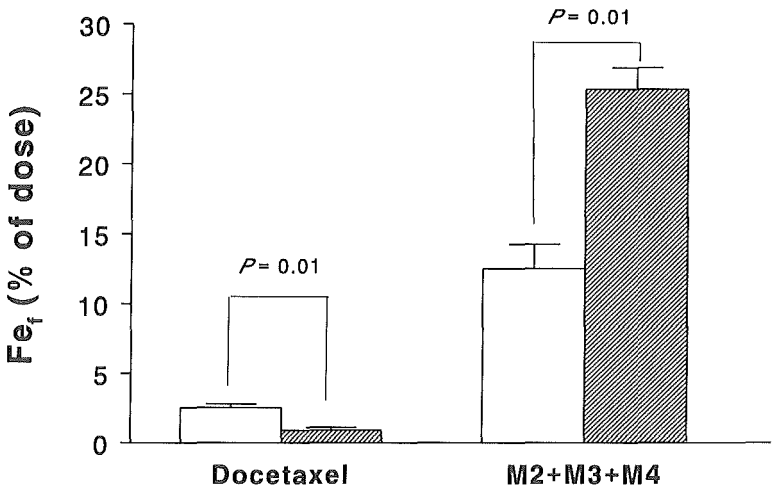

Figure 2 Percentage of the absolute docetaxel dose excreted in the feces within 31 hours after drug administration as unchanged drug or as the total of metabolites M2, M3, and M4. Data were obtained from patients treated with docetaxel at 100 mg/m^2 given either alone (white bars) or in combination with R101933 given at 250 or 500 mg as a 1-h i.v. infusion (hatched bars). Data are presented as mean values (bars) ± SEM (error bars).

R101933 Plasma Levels

No differences were observed in the pharmacokinetics of R101933 given either alone or in the presence of docetaxel (Fig. 3; Table 5). R101933 peak plasma concentrations after 500 mg i.v. administration ranged from 2580 to 5328 ng/mL. Peak plasma concentrations of its acid metabolite R102207 were about 4 to 5 times higher. Plasma concentrations of R101933 and R102207 declined distinctly biphasically with time, with terminal disposition half-lives ranging from 4 to 36 hour and from 9 to 37

hour, respectively. The determination of the terminal disposition half-life was highly dependent on the ability to quantify R101933 or R102207 in the terminal phase. The AUC of R101933 ranged from 2.94 to 19.6 μg.h/mL. The AUC of R102207 was about 20 to 25 times higher. Mean plasma clearance values of R101933 given alone were 52.7 ± 19.2 liters/h (n = 5) and 58.5 ± 19.7 liters/h (n = 7) at dose levels of 250 mg and 500 mg, respectively, suggesting a dose-proportional pharmacokinetic behavior. No differences were observed in R101933 plasma clearance given either alone or in the presence of docetaxel [mean values, 53.8 ± 18.4 liters/h (cycle 0) vs 57.2 ± 11.6 liters/h (cycle 2) ($P = 0.47$), indicating the absence of a pharmacokinetic interaction at these dose levels.

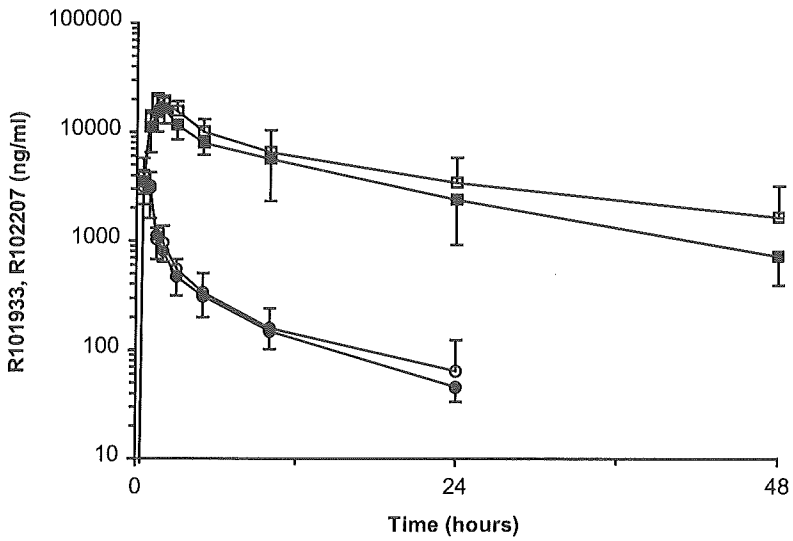

Figure 3 Plasma concentration-time profiles of R101933 (circles) and its acid metabolite R102207 (squares) in patients treated with R101933 given as a 1-h i.v. infusion at a dose level of 500 mg either given either alone (open symbols) or in combination with docetaxel given at 100 mg/m^2 (closed symbols). Data are presented as mean values (symbols) ± SD (error bars).

Table 5 Plasma pharmacokinetic parameters of R101933 and its metabolite R102207 in the absence or presence of docetaxel*

Parameter	250 mg R101933 ($n=3$) Without D	With D	500 mg R101933 ($n=6$) without D	with D
R101933				
C_{max} ** (µg/mL)	2.24 ±0.62	1.97 ±0.30	3.67±0.99	3.70 ±1.06
$t_{1/2(z)}$ (h)	12.9 ±1.4	24.2±12.6	10.5±8.4	7.5±1.8
AUC (µg.h/mL)	5.55±1.01	5.16±0.87	10.0±4.9	8.43±1.60
R102207				
C_{max} (µg/mL)	10.7 ±6.7	7.5±3.0	20.6 ±1.8	16.4±4.4
$t_{1/2(z)}$ (h)	15.6±5.3	13.0±4.1	21.3±8.5	15.5±2.8
AUC (µg.h/mL)	114±63	88.2±47.3	306±191	199±79

* Data were obtained from patients after cycle 0 (without docetaxel) and after cycle 2 (with docetaxel). The kinetic terms are mean values ± SD.

** AUC, area under the plasma concentration *versus* time curve; C_{max}, maximum plasma concentration of drug; $t_{1/2(z)}$, terminal disposition half-life; *n*, number of patients evaluated; D, docetaxel.

Pgp Antagonism Following R101933

To determine whether infusion of R101933 would be associated with systemic concentrations affecting Pgp activity in an *ex vivo* model based on daunorubicin retention in Pgp expressing $A2780_{T\text{-}100}$ cells, plasma samples were obtained before and after drug infusion in the first treatment course. In this assay, fluorescence values have previously been shown to reflect the degree of inhibition of Pgp-mediated efflux (8). Control experiments conducted with the A2780 parental cell line provided a maximum fluorescence value of 2.50, whereas the fluorescence measured in the $A2780_{T\text{-}100}$ will depend on the level of R101933 present in the patient's plasma. A total of 107 plasma samples from patients treated with R101933 (5 patients at 250 mg and 7 patients at 500 mg) were analyzed for pharmacologic activity in the assay. All pre-dose samples showed no measurable activity. Time-course studies with R101933 administered at the

2 tested dose levels showed that following a single i.v. dose, substantially decreased daunorubicin efflux was found in plasma samples of all patients collected within 30 min of treatment (Fig. 4).

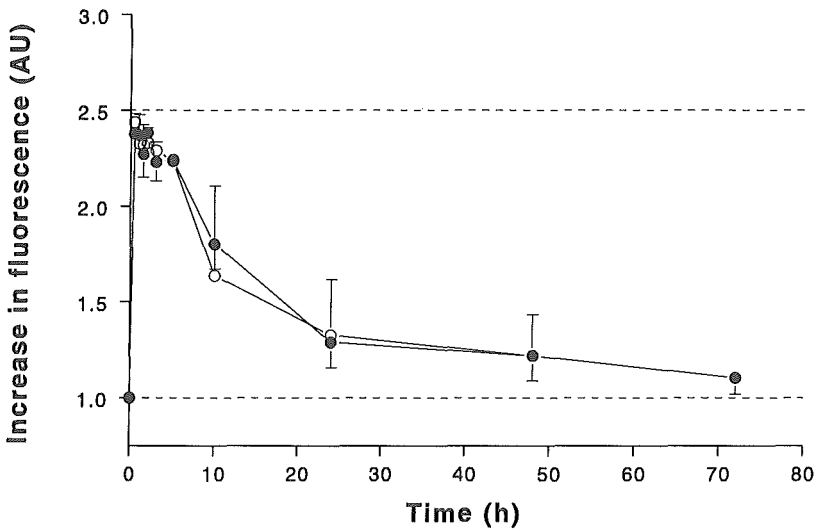

Figure 4 Time-course of R101933-mediated Pgp antagonism in patient samples obtained following R101933 administration as a 1-h i.v. infusion at dose levels of 250 mg (○) or 500 mg (●). Modulation of daunorubicin uptake in $A2780_{T\text{-}100}$ cells (expressing Pgp) by plasma samples containing R101933 were measured by a change in fluorescence intensity relative to that observed in the A2780 parental cell line in the absence of R101933. Data points are mean values ± SEM of duplicate measurements of plasma samples obtained from 3 patients (250-mg dose) or 7 patients (500-mg dose). The lower dotted line (fluorescence value of 1.0) indicates the base-line fluorescence (i.e. lack of influence on Pgp-mediated efflux), whereas the upper dotted-line (fluorescence value of 2.5) indicates the maximum fluorescence (complete inhibition of Pgp-mediated efflux).

Maximal inhibition of Pgp function was observed in all samples collected at times ranging from 30 to 60 min, with fluorescence increase values of 2.44±0.081 and 2.41±0.062 at R101933 dose levels of 250 and

500 mg, respectively, indicating near-complete reversal (i.e. >97%) in the *ex vivo* assay. The duration above K_i, defined as the concentration required for half-maximum inhibition of Pgp-mediated daunorubicin efflux, showed considerable interpatient variability (coefficient of variation, >50%). Mean values of the duration above K_i were 12.3±7.22 hours and 14.1±7.08 hours at dose levels of 250 mg and 500 mg, respectively ($P=0.709$, unpaired Student's t test). Overall, the data indicate that plasma concentrations achieved in patients within 48 hours after single 250 or 500 mg i.v. administration of R101933 inhibit Pgp-mediated drug efflux in tumor cells *ex vivo*. Correlation of Pgp antagonism with R101933 plasma concentrations, as measured by high-performance liquid chromatography, indicated the occurrence of a plateau in efflux antagonism (not shown), in line with previous findings (8). This suggests that the plasma levels of R101933 achievable in patients result in *ex vivo* concentrations that reach a maximum inhibition of Pgp-mediated efflux, which begins at approximately 300 ng/mL, a plasma concentration well below that achieved at the MTD of the current combination.

DISCUSSION

MDR is generally regarded to be one of the major stumbling blocks to the efficacy of chemotherapy and since the discovery of the MDR product Pgp, many efforts were made to convert Pgp-mediated drug resistance into drug sensitivity (6). However, a plethora of clinical studies involving a wide range of Pgp inhibitors and co-administered anticancer drugs have shown that these combination treatments almost always resulted in increased toxicity of the anticancer drugs solely due to pharmacokinetic interaction between the two agents related to competition on the level of cytochrome-P450 3A isozymes [reviewed in Ref. (5)]. Therefore, it has been proposed that the administration of Pgp inhibitors is unlikely to improve the therapeutic index of anticancer drugs such as docetaxel unless such agents lack a pharmacokinetic interaction (5,6). In a previous study with orally administered R101933 in combination with docetaxel i.v. we have shown that R101933 did not influence the plasma pharmacokinetic characteristics of docetaxel knowing that the major metabolic route of R101933 is cytochrome-P450-unrelated (7). Due to low oral bioavailability of R101933 and significant inter-patient variability in exposure to R101933, further

increase of the plasma levels of R101933, in an attempt to achieve higher local concentrations of R101933, can be expected to be better reached by intravenous administration. In the present study this resulted, as expected, in significantly higher plasma levels of R101933 and reduced in inter-patient variability. Mean peak concentrations of R101933 after 500 mg i.v. infusion of R101933 were about 3500 ng/mL, while only 150 ng/mL after 300 mg R101933 given as oral solution (7). Mean exposure (AUC) to R101933 was about 10-fold higher after i.v. infusion and a relatively lower exposure to the inactive metabolite R102207 was obtained. Furthermore, individual peak concentrations ranged only 2-fold after i.v. infusion compared to 10-fold after the oral solution, indicating a significantly reduced inter-patient variability. Notwithstanding the about 10-fold higher exposure to R101933 after i.v. infusion the plasma pharmacokinetics of docetaxel were still not influenced. The toxicologic profile of the combination treatment appeared to be very similar to that reported for docetaxel alone including neutropenic fever, neurotoxicity grade 3 and severe fatigue in combination with myalgia, all being reported in approximately 15%, 5%, and 6%, respectively, of cases treated with docetaxel alone (12). Treatment with R101933 i.v. at the tested dose levels was associated with minimal toxicity consisting of temporary drowsiness and somnolence beginning during the infusion and lasting for several hours.

As we have postulated earlier, the physiological and pharmacological consequences of treatment with an effective Pgp inhibitor cannot be predicted based on plasma drug monitoring alone (8). So, we collected feces up to 48 hours after administration of docetaxel in cycle 1 and 2 to determine an often overlooked and sometimes underestimated parameter of drug disposition, the fecal excretion of the anticancer agent. The excretion of unchanged docetaxel proved to be decreased after co-administration with R101933 indicating biological activity of the Pgp inhibitor most likely at the level of intestinal Pgp. Inhibition of intestinal Pgp leading to modification of the enterohepatic cycle was postulated after treatment with R101933 orally and now also seemed to be present after i.v. administration indicating sufficient concentrations of R101933 at the apical lumen side of the intestine. The re-absorption of docetaxel from the intestinal lumen did not result in increased plasma levels due to: *i.* the relative little fraction of docetaxel that is excreted via the feces (without R101933 only 2.5%), *ii.* a

virtually complete first-pass extraction and/or docetaxel metabolism in the liver and intestinal mucosa, which also has significant expression of cytochrome P450 3A isozymes (13).

In the kind of studies as the present one, designed to overcome Pgp-mediated MDR, measurement of the extent of *in vivo* inhibition of Pgp-mediated drug efflux is deemed essential. To that end, we presented the results of an *ex vivo* assay developed as a surrogate measure of Pgp antagonism in the plasma samples of all patients receiving R101933. We deliberately decided on another approach compared to the two most well-known surrogate assays to determine Pgp antagonism using the known Pgp substrates Tc-99m-sestamibi (sestamibi), a radionuclide imaging agent, and rhodamine 123 (rhodamine), a lipophilic dye. The first method determined the washout rates of sestamibi by scintigraphy indicating the presence of a functional efflux pump (14,15) and its efflux from tumor and liver has shown to be decreased in the presence of a Pgp inhibitor by enhanced tumor visualization (15-17). However, it is a time-consuming method in which patient compliance could be a restriction, it lacks a dose-response relationship between sestamibi uptake and the Pgp inhibitor levels (16), and establishment of the sensitivity of sestamibi scintigraphy as an adequate surrogate marker of Pgp inhibition is still needed. In this respect it is of importance to notice that a recent pilot study showed no correlation between sestamibi scintigraphy and the expression of Pgp determined in tumor tissue of patients with high-grade osteosarcoma (18). The second *ex vivo* method is based on the observation that hematopoietic cells, especially the CD56+ subset of peripheral blood lymphocytes, express functional Pgp (19). Plasma obtained from patients within 15 minutes after administration of the Pgp inhibitor SDZ PSC 833 showed *ex vivo* a decreased efflux of rhodamine from CD56+ cells with a clear dose-response relationship (20). However, accepting rhodamine-efflux from CD56+ cells as a surrogate marker requires the assumption that Pgp in CD56+ cells has identical substrate and antagonist specificities as Pgp in cancer cells. Moreover, both methods lack the possibility of interpatient comparison. An essentially different approach of evaluating the extent of Pgp inhibition has been the addition of plasma from patients treated with Pgp inhibitors and thus containing a certain concentration of this drug to an *in vitro* assay comprised of a known multidrug-resistant cell line and a detectable Pgp substrate such as radiolabeled daunorubicin or mitoxantrone (21). In the

present study we have favored this approach because this method allowed an interpatient and dose level comparison by the use of a cell line known to have a constant level of Pgp expression. Furthermore, this assay offered an easy possibility to confirm that pharmacologically and biologically relevant concentrations of R101933 were achieved over a distinguished period of time. Still, as with the other methods Pgp expression or the availability of the Pgp inhibitor in the tumor is not provided and the assay has to be performed in protein-free medium because it can be expected based on *in vitro* studies that protein binding of the inhibitor in the medium impaired the Pgp antagonism activity (21-24). Much concern has been raised from this experience because clinically Pgp inhibitors are exposed to high concentrations of drug-binding proteins and depending on the degree of serum protein binding (for R101933 more than 98%), this is thought to lead to significantly reduced drug availability of the (pharmacologically active) free drug fraction at the tumor site (24). However, this view does not justify the generally accepted physiologic concept of the extent of distribution of a compound within tissues, including a tumor, involving multiple equilibria, a situation never to be possible outside the body in *in vitro* as well as *ex vivo* experiments.

An important finding of the present *ex vivo* study is the observation that a plateau occurred in the inhibition of the Pgp substrate efflux, suggesting that the achieved levels of R101933 in the assay reached a maximum inhibition of Pgp-mediated efflux and complete reversal could not be attained. The plateau phase was reached at *ex vivo* concentrations that correspond to a plasma concentration of R101933 of approximately 300 ng/mL, a concentration well below that achieved at the maximal-tolerated dose level of R101933 i.v. This result is in line with calculations using experimental models with high levels of Pgp expression suggesting that a single Pgp modulator cannot completely inhibit Pgp-mediated efflux (25). However, the indicated substantial (near maximal) reversal may be sufficient to improve drug efficacy.

In conclusion, we have shown that the combination of R101933 i.v. directly followed by docetaxel infusion is safe lacking pharmacokinetic interaction in plasma and that at the plasma concentrations of R101933 achieved within 48 hours after infusion are capable of inhibiting Pgp in an *ex vivo* assay. These findings justify further development of this combination treatment in which the recommended dose of R101933 i.v.

will be 500 mg. A phase II study is possible for example in patients with metastatic breast cancer failing on treatment with taxanes alone, but as these kind of studies have been proven difficult in accrual we propose adequately powered phase III studies (6).

REFERENCES

1. Leighton JC, and Goldstein LJ: P-glycoprotein in adult solid tumors. Expression and prognostic significance. Hematology/oncology clinics of North America, 9 (2):251-271, 1995
2. Bradshaw DM, and Arceci RJ: Clinical relevance of transmembrane drug efflux as a mechanism of multidrug resistance. J Clin Oncol 16:3674-3690, 1998
3. Kaye SB: Reversal of multidrug resistance. Cancer Treat Rev 17(Suppl A):37-43, 1990
4. Raderer M, and Scheithauer W: Clinical trials of agents that reverse multidrug resistance. Cancer 73:3553-3563, 1993
5. Relling MV: Are the major effects of P-glycoprotein modulators due to altered pharmacokinetics of anticancer drugs? Ther Drug Monit, 18:350-356, 1996
6. Van Zuylen L, Nooter K, Sparreboom A, and Verweij J: Development of multidrug-resistance convertors: sense or nonsense? Invest New Drugs 18:205-220, 2000
7. Van Zuylen L, Sparreboom A, Van Der Gaast A, Van Der Burg MEL, Van Beurden V, Bol CJ, Woestenborghs R, Palmer PA, and Verweij J: The orally administered P-glycoprotein inhibitor R101933 does not alter the plasma pharmacokinetics of docetaxel. Clin Cancer Res 6:1365-1371, 2000
8. Van Zuylen L, Verweij J, Nooter K, Brouwer E, Stoter G, and Sparreboom A: Role of intestinal P-glycoprotein in the plasma and fecal disposition of docetaxel in humans. Clin Cancer Res 6:2598-2603, 2000
9. Awada A, Bourgeois P, Cornez N, Bleiberg H, McCabe S, Gil T, Lehmann F, de Valeriola D, Di Leo A, Van den Leemput E, Bol K, Palmer P, and Piccart M: A clinical pharmacodynamic (Pd) and pharmacokinetic (Pk) phase I study of I.V. R101933, an inhibitor of MDR1 P-glycoprotein (Pgp) given alone and in combination with Taxol in patients with solid tumors. Proc. AACR-NCI-EORTC #513, 1999.
10. Walle T, Walle UK, Kumar GN, and Bhalla KN: Taxol metabolism and disposition in cancer patients. Drug Metab Dispos, 23:506-512, 1995
11. Loos WJ, Verweij J, Nooter K, Stoter G, and Sparreboom A: Sensitive determination of docetaxel in human plasma by liquid-liquid extraction and reversed-phase high-performance liquid chromatography. J Chromatogr B 693:437-441, 1997
12. Trudeau ME: Docetaxel (Taxotere): An overview of first line monotherapy. Sem in Oncol 22(suppl 13):17-21, 1995
13. Wacher VJ, Wu CY, and Benet LZ: Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. Mol Carcinogen 13:129-134, 1995
14. Piwnica-Worms D, Chiu ML, Budding M, Kronauge JF, Kramer RA, and Croop JM: Functional imaging of multi-drug resistant P-glycoprotein with an organtechnetium complex. Cancer Res 53:977-984, 1993

15. De Moerloose B, Van de Wiele C, Dhooge C, Philippe J, Speleman F, Benoit Y, Laureys G, and Dierckx RA: Technetium-99m sestamibi imaging in paediatric neuroblastoma and ganglioneuroma and its relation to P-glycoprotein. Eur J Nucl Med 26:396-403,1998
16. Chen CC, Meadows B, Regis J, Kalafsky G, Fojo T, Carrasquillo JA, and Bates SE: Detection of *in vivo* P-glycoprotein inhibition by PSC 833 using Tc-99m Sestamibi. Clin Cancer Res 3:545-552, 1997
17. Piwnica-Worms D, Rao VV, Kronauge JF, and Croop JM: Characterization of multidrug resistance P-glycoprotein transport function with an organotechnetium cation. Biochemistry 26:12210-12220, 1995
18. Liao AC, Gorlick R, Antonescu C, Huvos AG, Healey J, Sowers R, Calleja E, Wexler L, Meyers PA, Yeh SD, and Larson SM: Functional scintigraphy with technetium-99m MIBI does not correlate with histologic response to induction chemotherapy or measures of P-glycoprotein expression in high grade osteosarcoma. Proc Amer Assoc Oncol, #2204, 2000
19. Whitherspoon SM, Emerson DL, Kerr BM, Lloyd TL, Dalton WS, and Wissel PS: Flow cytometric assay of modulation of P-glycoprotein function in whole blood by the multidrug resistance inhibitor GG918. Clin Cancer Res 2:7-12, 1996
20. Robey R, Bakke S, Stein W, Meadows B, Litman T, Patil S, Smith T, Fojo T, and Bates S: Efflux of rhodamine from CD56+ cells as a surrogate marker for reversal of P-glycoprotein-mediated drug efflux by PSC 833. Blood 93:306-314, 1999
21. Ayesh S, Lyubimov E, Algour N, and Stein WD: Reversal of P-glycoprotein is greatly reduced by the presence of plasma but can be monitored by an ex vivo clinical essay. Anti-Cancer Drugs 7:678-686, 1996
22. Wishart GC, Plumb JA, Morrison JG, Hamilton TG, and Kaye SB: Adequate tumour quinidine levels for multidrug resistance modulation can be achieved in vivo. Eur J Cancer 28:28-31, 1992
23. Ludescher C, Eisterer W, Hilbe W, Hofmann J, and Thaler J: Decreased potency of MDR-modulators under serum conditions determined by a functional assay. Br J Haematol 91:652-657,1995
24. Lehnert M, de Giuli R, Kunke K, Emerson S, Dalton WS, and Salmon SE: Serum can inhibit reversal of multidrug resistance by chemosensitizers. Eur J Cancer 32A:862-867, 1996
25. Stein WD: Kinetics of the multidrug transporter (P-glycoprotein) and its reversal. Physiol Rev 77:545-590, 1997

Pharmacologic modulation by Cremophor EL

Chapter 5

Role of formulation vehicles in taxane pharmacology

Lia van Zuijlen, Jaap Verweij, and Alex Sparreboom

Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, The Netherlands

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SUMMARY

The non-ionic surfactants Cremophor EL (CrEL) and Tween 80, both used as formulation vehicles of many (anticancer) agents including paclitaxel and docetaxel, are not physiological inert compounds. We describe their biological properties, especially the toxic side effects, and their pharmacological properties, such as modulation of P-glycoprotein activity. In detail, we discuss their influence on the disposition of the solubilized drugs, with focus on CrEL and paclitaxel, and of concomitantly administered drugs. The ability of the surfactants to form micelles in aqueous solution as well as biological fluids (e.g. plasma) appears to be of great importance with respect to the pharmacokinetic behavior of the formulated drugs. Due to drug entrapment in the micelles, plasma concentrations and clearance of free drug change significant leading to alteration in pharmacodynamic characteristics. We conclude with some perspectives related to further investigation and development of alternative methods of administration.

INTRODUCTION

After the identification of paclitaxel as the active ingredient in crude ethanolic extracts of the bark of the Western yew tree, *Taxus brevifolia*, against several murine tumors (1), the development of this promising drug was suspended for more than a decade due to problems in drug formulation. Paclitaxel is poorly soluble in water and in other common vehicles used for the parenteral administration of drugs. After investigation of a large variety of excipients to enable parenteral administration of the drug (reviewed in: Ref. 2), the formulation approach using 50% CrEL and 50% dehydrated ethanol USP represented the most viable option. Currently, paclitaxel is commercially available as vials containing 30 mg of drug dissolved in 5 mL of CrEL:dehydrated ethanol USP (1:1, v/v). CrEL is also being used as a vehicle for the solubilization of a wide range of other hydrophobic drugs, including anesthetics (e.g. propofol), photosensitizers, vitamins, immunosuppressive agents (e.g. cyclosporin A and tacrolimus), sedatives (e.g. diazepam), and (experimental) anticancer drugs (e.g. aplidine, clanfenur, didemnin B, halomon and teniposide). The amount of CrEL co-administered with these drugs varies up to about 5.5 mL/m^2,

although paclitaxel is an exception as the CrEL content of the formulated preparation is much higher per dose, about 12 mL/m^2 (3).

A good 10 years after the identification of paclitaxel, docetaxel was developed from 10-deacetyl baccatin-III, a noncytotoxic precursor isolated from the needles of the European yew tree, *Taxus baccata*. This semisynthetic taxane analogue is formulated for clinical use in polysorbate 80 (Tween 80), and the solution consists of 80 mg of docetaxel in 2 mL of Tween 80. Like CrEL, Tween 80 is used as a nonionic surfactant to solubilize several other anticancer drugs, including etoposide and the cyclopropylpyrroloindole compounds adozelesin, bizelesin and carzelesin.

Both formulation vehicles are known as biologically and pharmacologically active compounds. With a focus on CrEL and paclitaxel, we will describe in the present review the properties of CrEL and Tween 80 and the consequences of their intrinsic activity related to the formulated drugs and implications for other (anticancer) agents concomitantly administered.

PHYSICOCHEMICAL PROPERTIES

CrEL is a white to off-white viscous liquid with an approximate molecular weight of 3000 Da and a specific gravity (25°C/25°C) of 1.05-1.06, and is produced by the reaction of castor oil with ethylene oxide at a molar ratio of 1:35 (4). Castor oil is a colorless or pale yellow fixed oil obtained from the seeds of *Ricinus communis* (Fam. Euphorbiaceae), with an extremely high viscosity, and consists mainly of the glycerides of ricinoleic (12-hydroxyoleic acid), isoricinoleic, stearic and dihydroxystearic acids. The nonionic surfactant produced from castor oil is usually of highly variable composition, with the major component (about 87%) identified as oxyethylated triglycerides of ricinoleic acid (i.e. polyoxyethylene glycerol triricinoleate 35) (Fig. 1A). CrEL is known to leach plasticizers, e.g. di(2-ethylhexyl)-phthalate, from polyvinyl chloride (PVC) bags and polyethylene-lined tubing sets, which can cause severe hepatic toxicity (5), and it is recommended to use PVC-free equipment for drug administration.

In contrast to CrEL, Tween 80 is a relatively homogenous and reproducible, amber-colored, viscous liquid (270-430 centistokes) with a molecular weight of 1309.7 Da and a density of 1.064 g/mL. The base chemical name of the major component of Tween 80 is polyoxyethylene sorbitan monooleate (Fig. 1B), which is structurally similar to the

polyethylene glycols. Like most nonionic surfactants, CrEL and Tween 80 are capable of forming micelles in aqueous solution, with critical micellar concentrations of 0.009% (w/v) and 0.1% (w/v), respectively.

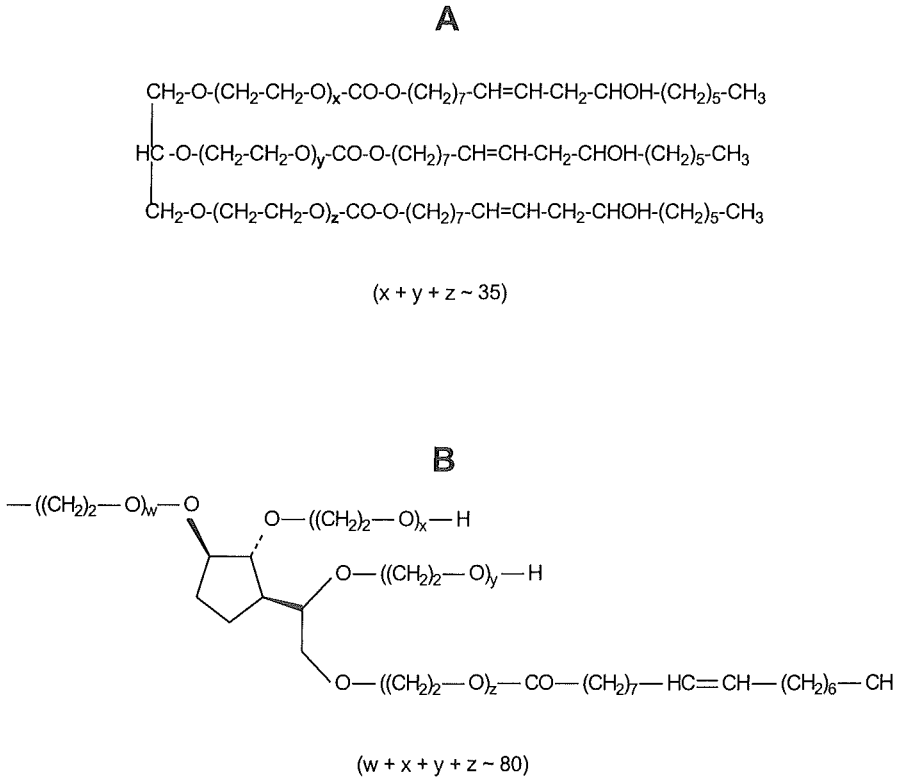

Figure 1 Chemical structures of the major components of CrEL, polyoxyethyleneglycerol triricinoleate 35 (**A**) and Tween 80, polyoxyethylenesorbitan monooleate (**B**)

TOXIC SIDE EFFECTS

Acute Hypersensitivity Reactions

The most well known toxicity of CrEL is an acute hypersensitivity reaction, which is characterized by dyspnea, flushing, rash, and generalized urticaria (6). It has been described in the 80's after the use of teniposide in pediatric patients treated for neuroblastoma and lymphoid malignancies (7) and after the infusion of cyclosporin A in posttransplant patients (8).

Nowadays, it is a well known side effect associated with the use of intravenous paclitaxel. The mechanism of this hypersensitivity to CrEL has not yet been fully elucidated. Mostly, the hypersensitivity reaction occurs within the first two courses of paclitaxel and can be prevented by reducing the infusion rate which are the reasons of the general belief that it resembles a non-immunological reaction, based on degranulation of mast cells or basophils (9,10). On this hypothesis, the rationale for the current pretreatment schedules of paclitaxel with H_2-receptor antagonists and high-dose corticosteroids is based (9,11). However, despite this extensive premedication, the overall frequency of minor reactions is estimated as high as 44%, with major reactions, necessitating discontinuation of paclitaxel therapy, still occurring in approximately 1.5 to 3% of patients (12,13). Already in the 70's, it was shown that Althesin and Stesolid MR, both compounds using CrEL as a vehicle, can activate C3 complement *in vivo* (14,15). Recently, it was postulated that complement activation is an important contributing mechanism to the hypersensitivity reaction from CrEL due to binding of naturally occurring anti-cholesterol antibodies to the hydroxyl-rich surface of CrEL micelles (16). Based on an elegant series of *in vitro* experiments, it was shown that CrEL-induced complement activation in human serum was clearly concentration dependent with a minimum activating CrEL level in the order of approximately 2 μL/mL, a concentration readily achieved clinically in plasma following standard doses of paclitaxel (17).

One of the constituents of CrEL, oleic acid, especially suspected for its histamine-releasing capability, is also present in Tween 80, and thus may be a cause of hypersensitivity reactions to docetaxel therapy (18). In addition, recent work performed has shown that allergens are present in Tween 80 that elicit hypersensitivity reactions in guinea pigs, and that new allergens, including formaldehyde, are formed in an oxidation process after air exposure (19).

Neurotoxicity

In contrast to the hypersensitivity reactions, it is less well known that CrEL can cause neurotoxicity, a toxicity known as one of the main side effects of paclitaxel. Electrophysiologic studies in patients with neurotoxicity after treatment with paclitaxel have shown evidence of both axonal degeneration and demyelination (11). One study performed with [^{3}H]paclitaxel in rats did not reveal detectable levels of paclitaxel in the

peripheral nervous system, but paclitaxel in this study was solubilised in dimethyl sulfoxide instead of CrEL (20). This finding may suggest that the neurotoxicity of paclitaxel is probably caused by CrEL.

Cyclosporin A (formulated in CrEL) administered intravenously has also been linked to nerve pathology, with approximately 25% of patients experiencing neurotoxicity (21). In contrast, this side effect rarely or never follows oral administration of cyclosporin A, which is consistent with recent observations that CrEL is not absorbed when given orally (22,23), presumably as a result of acid-induced hydrolysis in the stomach and duodenum (24). Moreover, CrEL levels in plasma achieved with therapeutic doses of paclitaxel and i.v. cyclosporin A, have been shown to produce axonal swelling, vesicular degeneration and demyelination in rat dorsal root ganglion neurons exposed to the formulation vehicle (25). These authors speculated that residual ethylene oxide, a known potent neurotoxin, may be responsible for this phenomenon. However, an earlier *in vitro* study using a neuronal model by the differentiating N1E.115 neuroblastoma cell line demonstrated that the neurotoxicity probably is unrelated to the residual ethylene oxide as toxic effects were unaffected after nitrogen-pretreated CrEL (26). Recent work indicated that the neurotoxic properties were induced by unsaturated fatty acids and preliminary evidence suggested that the changes were due to the appearance of peroxidation products (27). This suggests that the ethoxylated derivatives of castor oil, *i.e.* the primary constituents of CrEL, probably account for most of the neuronal damage observed (26).

Vesicular degeneration is also produced by other solubilizing agents such as Tween 80, a property which also depends on the polyoxyethylene substitutions produced by reaction of the parent compound with ethylene oxide. However, the incidence of neurotoxicity during treatment with docetaxel is much lower as compared to that with paclitaxel (28). Furthermore, the epipodophyllotoxin derivative etoposide which also contains considerable amounts of Tween 80, is not known to be neurotoxic.

Miscellaneous Side Effects

In animal models, it was shown that CrEL has acute but reversible cholestatic effects (29), although other data contradict this (30). Nephrotoxicity has also been linked to CrEL, as studies with rat kidney

models indicated that the addition of small quantities of CrEL resulted in marked renal vasoconstriction with decreased renal blood flow and deterioration in renal tubular function (31-33). Dermatitis is rarely observed after treatment with paclitaxel, but is a well known side effect of docetaxel, and Tween 80 has been known to cause this side effect (28,34). The rash primary involves pressure or trauma sites and resolves within two weeks, with skin biopsies revealing a non-specific perivascular lymphocytic infiltration. Cumulative fluid retention is another specific side-effect of docetaxel that can be dose-limiting. Tween 80 is known to alter membrane fluidity, leading to increased membrane permeability (35), and this may, at least in part, explain the observed reaction with docetaxel therapy.

PHARMACOKINETIC CHARACTERISTICS

Analytical Methods

Clinical pharmacokinetic studies with CrEL and Tween 80 had not been performed until very recently, and at present a variety of analytical procedures are available for this purpose. The first assay developed for measurement of CrEL concentrations in plasma was a bioassay based on the ability of CrEL to modulate daunorubicin efflux in multidrug-resistant T-cell leukemia VLB_{100} cells (36). Alternatively, CrEL levels have been determined by measurement of ricinoleic acid after saponification of CrEL followed by precolumn derivatization and reversed-phase high-performance liquid chromatography (37). A method has also become available based on a selective binding of CrEL to the Coomassie brilliant blue G-250 dye in protein-free extracts (38,39), that has also been used succesfully for measurement of Tween 80 concentrations in murine and human plasma (40). Most recently, a potentiometric titration method for CrEL was developed for quantitative analysis in urine and plasma based on a coated wire electrode as end-point indicator with sodium tetraphenylborate at 20° C and pH 10 (41). Each of the available assays has its drawbacks and limitations, and the methodologic differences between them probably contribute to the evident differences in the measured concentrations of CrEL and thus the calculated pharmacokinetic parameters.

In addition to the Coomassie brilliant blue G-250 colorimetric dye-binding assay, various other analytical procedures are available for Tween 80. Initially, attention was directed to measurement of the polyoxyethylated

portion of the molecule, since it would be impossible to distinguish the fatty acid moiety from endogenous compounds (42). The so-called polyol moiety is detectable by a wide variety of methods including a resorcinol-glucose precipitation, a colorimetric method using ammonium cobaltothiocyanate, turbidimetric or gravimetric procedures and complex formation with a barium phosphomolybdic reagent (43,44). The ammonium cobaltothiocyanate complexation has also been used in combination with high-performance liquid chromatography and UV detection for analysis of Tween 80 in urine and ascites fluid, using either post-column (45,46) or on-line complexation (47).

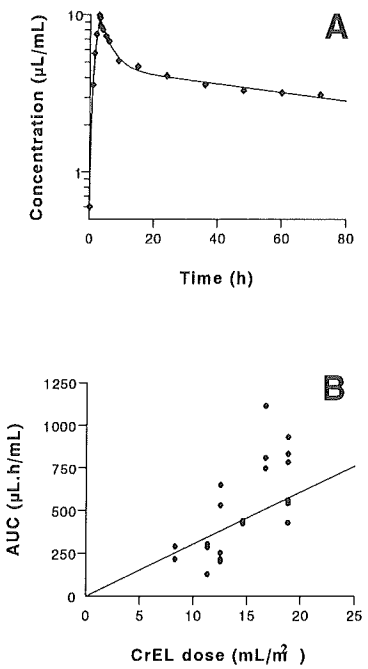

Figure 2 Representative plasma concentration *versus* time profile of CrEL in a single patient treated with a 3-h intravenous infusion of paclitaxel at a dose level of 175 mg/m^2 (**A**) and the relationship between the dose of CrEL and the measured AUC in 23 patients with a 3-h intravenous infusion of paclitaxel (**B**) (data from: Ref. 50).

Plasma Pharmacokinetics

Few studies have evaluated the pharmacokinetics of CrEL using the described various analytical methods, sometimes leading to differences in results and conclusions. Most of the available data indicate that CrEL delineates a linear pharmacokinetic behavior following 3-h paclitaxel administrations (Fig. 2), although with some other methods the kinetics were judged as non-linear with disproportional increases in systemic exposure with an increase in dose (48). With prolongation of the infusion duration from 1 to 3 and to 24 h, pharmacokinetic parameters have been found to change significantly; for example, after administration of paclitaxel (175 mg/m^2) applying a 1-, 3- or 24-h infusion schedule, the clearance increased from about 160 to 300 to 400 mL/h/m^2, respectively (17). It thus appears that CrEL shows dose-independent/schedule-dependent pharmacokinetics with disproportional increases in systemic exposure of CrEL being associated with shortening of the infusion duration.

The terminal elimination half-life of CrEL amounts to approximately 80 h with a large range from 10 - 140 h, depending on the method used for measurement. In addition, the limited sampling used in one study with the application of the bio-assay method may have led to underestimation of the terminal elimination half-life (49). However, a study using the colorimetric assay demonstrated extended persistence of CrEL with detectable levels of the compound even at 1 week after initial treatment (50). In all studies the observed volume of distribution of CrEL was extremely low and not (much) higher than the volume of the central blood compartment, implying that tumor and tissue delivery of CrEL is insignificant.

Not much is known about elimination routes of CrEL in humans. The results of a study in patients with hepatic dysfunction treated with paclitaxel suggested that hepatobiliary elimination of CrEL is not of primary importance, because severe impairment of the hepatic function did not reduce the clearance but, on the contrary, lead to increase of the clearance compared to historical controls (51). A possible explanation of this can be that the enzymes for which CrEL is a substrate (such as serum esterases) may be elevated in patients with severe hepatic dysfunction, leading to an accelerated rate of metabolism of CrEL within the systemic circulation. However, thus far there is no evidence that these mechanisms may play a role in the clinical situation (51). Despite its relatively high hydrophilic nature, the renal elimination of CrEL seems to be almost negligible

according to preliminary findings indicating that the cumulative urinary excretion of CrEL is very low, accounting for less than 0.1% of the administered dose (52). CrEL pharmacokinetics in a patient with predialysis renal impairment treated with paclitaxel were not significantly different from historic controls (52). CrEL may be largely degraded in the blood compartment although the pathway of its elimination is currently unknown. It is possible that elimination pathways for CrEL are mainly dictated by serum carboxylesterase-induced degradation causing release of ricinoleic acid. Given the long terminal half-life of CrEL, this metabolic route occurs at a low rate and the involved enzymes may be easily saturated, which may explain the peculiar schedule-dependent clearance of the compound.

The pharmacokinetic behavior of Tween 80 is very different from that of CrEL. In mice a rapid decline of the concentration was shown after bolus injection, with plasma concentrations decreasing to <0.05 μL/mL (i.e. the lower limit of quantitation of the analytical method) within 15 min after drug administration (40). Observations in 5 patients treated with docetaxel (100 mg/m^2) as a 1-h infusion showed peak plasma levels of Tween 80 of 0.16 ± 0.05 μL/mL, and in plasma samples collected after 1 h the levels had already fallen below the detection limit of the assay. *In vitro* experiments have shown that this rapid elimination is caused by a rapid carboxylesterase-mediated hydrolysis in the systemic circulation, catalyzing the cleavage of the oleic acid side chain from the molecule (40). Earlier studies with Tween 20 and Tween 40 performed in rats and humans have shown the same pattern of metabolism with ester bond cleavage and subsequent oxidation of the fatty acid moiety (53,54). After intravenous administration in adult rats, most of the polyoxyethylene moiety appeared in the urine (about 86% within the first 12 h), while some was present in feces, indicating a minor role of biliary secretion (55). Following oral administration, Tween 80 is known to be metabolized in the intestine by pancreatic lipase (56) into oleic acid and polyoxyethylene sorbitol (57), with only 3.2% of the administered dose being excreted unchanged into the urine (58).

MODULATION OF DRUG DISPOSITION

It is likely that CrEL in blood is capable of forming micelles with a highly hydrophobic interior, which can entrap the solubilised compound as

well as other co-administered compounds. As indicated, in aqueous solution the critical micellar concentration of CrEL is 0.009%, although the actual value in plasma may be higher due to the presence of proteins and interference by other macromolecules. It can be speculated that the pharmacokinetic behavior of the drugs using CrEL as a formulation vehicle will be influenced by CrEL, especially those which are highly hydrophobic as most of the newly developed anticancer drugs are. It can also be anticipated that CrEL may alter the distribution of compounds co-administered with the drugs using CrEL as a formulation vehicle.

Although Tween 80 will also be able to form micelles in the systemic circulation after intravenous administration, it is unlikely that it will play a role in the disposition of docetaxel or compounds co-administered with docetaxel based on its rapid decline in plasma.

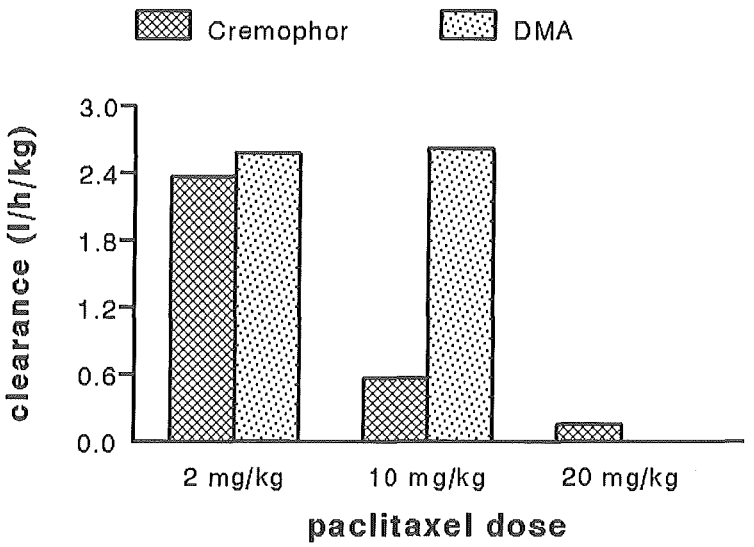

Figure 3 Effect of dose and formulation vehicle on plasma clearance of paclitaxel in female FVB mice. Bars represent mean values and error bars represent standard deviation (data from: Ref. 59).

Effects on Paclitaxel Pharmacokinetics

Initially, the effect of CrEL on the disposition of paclitaxel was studied in female FVB mice that received the drug by intravenous injection at dose levels of 2, 10 and 20 mg/kg by appropriate dilution of the commercially available drug formulation (59). The paclitaxel clearance of 2.4 L/h/kg at the lowest dose level was reduced to 0.33 and 0.15 L/h/kg at the 10 and 20 mg/kg dose levels, respectively. However, if administered at 10 mg/kg in Tween 80-ethanol or at 2 and 10 mg/kg in dimethylacetamide, the clearances were 2.66, 2.57 and 2.62 L/h/kg, respectively (Fig. 3), indicating that the nonlinearity of paclitaxel disposition resulted exclusively from CrEL. Despite that much higher plasma levels of paclitaxel are reached when given in the CrEL-containing formulation, the tissue levels were essentially similar with all tested drug preparations, suggesting that the profound influence of CrEL on paclitaxel pharmacokinetics is taking place in the central blood compartment. More recent *in vitro* work from our group has confirmed this hypothesis, by analyzing the effects of CrEL on cellular partitioning and the blood:plasma concentration ratio of paclitaxel in human samples (60). These experiments revealed a significant decrease in the blood:plasma concentration ratio of paclitaxel from 1.07±0.004 (mean±standard deviation) to 0.690±0.005 ($P<0.05$) up to concentrations corresponding to peak plasma levels of CrEL achieved after the administration of paclitaxel (175 mg/m^2 i.v. over a 3-h. period; *i.e.* 5.0 μL/mL). There was a clear concentration-dependent effect of CrEL on this ratio, with maximal inhibition of cellular paclitaxel uptake at a CrEL total blood concentration of 10 μL/mL, at which concentration the blood:plasma ratio averaged 0.625 ± 0.008, suggesting a distribution of paclitaxel merely outside blood cells (Fig. 4). These effects on the blood:plasma ratio were not seen with castor oil but were primarily caused by polyoxyethylene-glycerol triricinoleate along with fatty acid esters of polyoxyethylene glycol. Additional experiments were carried out with artificial binary mixtures relating washed erythrocytes to a buffer, figuring as a model excluding the influence of the plasma proteins, or plasma in which concentration ratios were determined after addition of paclitaxel in the absence or presence of CrEL. In both mixtures, paclitaxel in the presence of CrEL was only distributed in the water or plasma phase with ratios of 0.649 and 0.664, respectively. This shows that CrEL causes a profound alteration of paclitaxel accumulation in erythrocytes in a concentration-dependent

manner by reducing the free drug fraction available for cellular partitioning. Since this effect was also observed in the absence of plasma proteins, it was not caused by altered protein binding or an increased affinity of paclitaxel for protein dissociation products that are produced by the action of CrEL on native lipoproteins (61,62). Overall, the data suggested that drug trapping occurs in micelles composed primarily of polyoxyethylene-glycerol triricinoleate and that these micelles act as the principal carrier of paclitaxel in the systemic circulation. Not much later, our findings were indepently confirmed in two other publications (63,64).

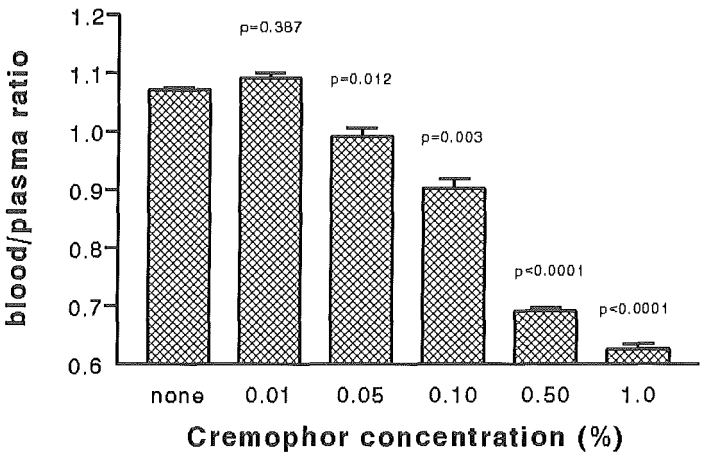

Figure 4 Effect of CrEL concentration on blood:plasma concentration ratios of paclitaxel (data from: Ref. 60)

The findings described above have serious implications for the interpretation of paclitaxel pharmacokinetics and for strategies attempting to find kinetic correlates that would assist in prediction of hematological toxicity. Previously, paclitaxel disposition in cancer patients has been reported to be nonlinear, with disproportional relationships between changes in the dose and the resulting plasma AUCs and peak plasma concentrations (65,66). This nonlinearity, particularly evident with the 3-h infusion schedule, has been speculated to result from two separate

saturable processes, one in distribution and one in elimination (65-68). By prospectively measuring whole blood and plasma concentrations of paclitaxel in a group of patients treated with consecutive dose levels of 135, 175 and 225 mg/m^2, we recently demonstrated the relevance *in vivo* and showed its importance related to the apparent nonlinear pharmacokinetics of paclitaxel (69). The plasma clearance of paclitaxel turned out to be dose-dependent decreasing from 16.7±2.53 L/h/m^2 at 135 mg/m^2 to 9.75±2.78 L/h/m^2 at 225 mg/m^2 (P=0.030). This was shown to be caused by a CrEL-concentration dependent decrease in uptake of paclitaxel in the erythrocytes, with blood:plasma concentration ratios altering significantly from 0.83±0.11 (135 mg/m^2) to 0.80±0.18 (175 mg/m^2) and 0.68±0.07 (225 mg/m^2), at which most of the drug was distributed outside the blood cells. Calculating the clearance of paclitaxel in whole blood samples showed that this was clearly dose-independent (P=0.063) and averaged 17.5±3.43 L/h/m^2. Like our *in vitro* findings, these *in vivo* results also indicate that the operation of Michaelis-Menten kinetics, the postulated cause of nonlinear paclitaxel disposition in plasma, is not related to saturable tissue binding or disproportional elimination kinetics, but appears to be an artifact caused by paclitaxel dose-related levels of CrEL in blood.

Based on the premise that paclitaxel can be included in micelles and thereby undergo a change in pharmacokinetic properties, a pharmacokinetic model was developed to predict the free fraction ratio of paclitaxel at any CrEL concentration (69). An example of the course of paclitaxel concentration *versus* time for the different forms of paclitaxel existing in blood based on this model is shown in Fig. 5. Pharmacokinetic/pharmaco-dynamic analysis of CrEL concentrations and corresponding blood distribution of paclitaxel showed that the disproportional accumulation of paclitaxel in plasma in a 3-h schedule is not seen in this manner with alternative infusion durations (17). This paradox can be understood in view of the fact that the high CrEL concentrations achieved early into the 1-h infusion produce only a low relative net change in the paclitaxel blood:plasma ratio (between 0.545 and 0.650), although the absolute quantitative effect is large already at low-dose input. Measurement of plasma concentrations of CrEL may, therefore, not be the exclusive indicator of the importance of the vehicle-drug interaction, but rather the combination of dose delivered and input-rate applied (17).

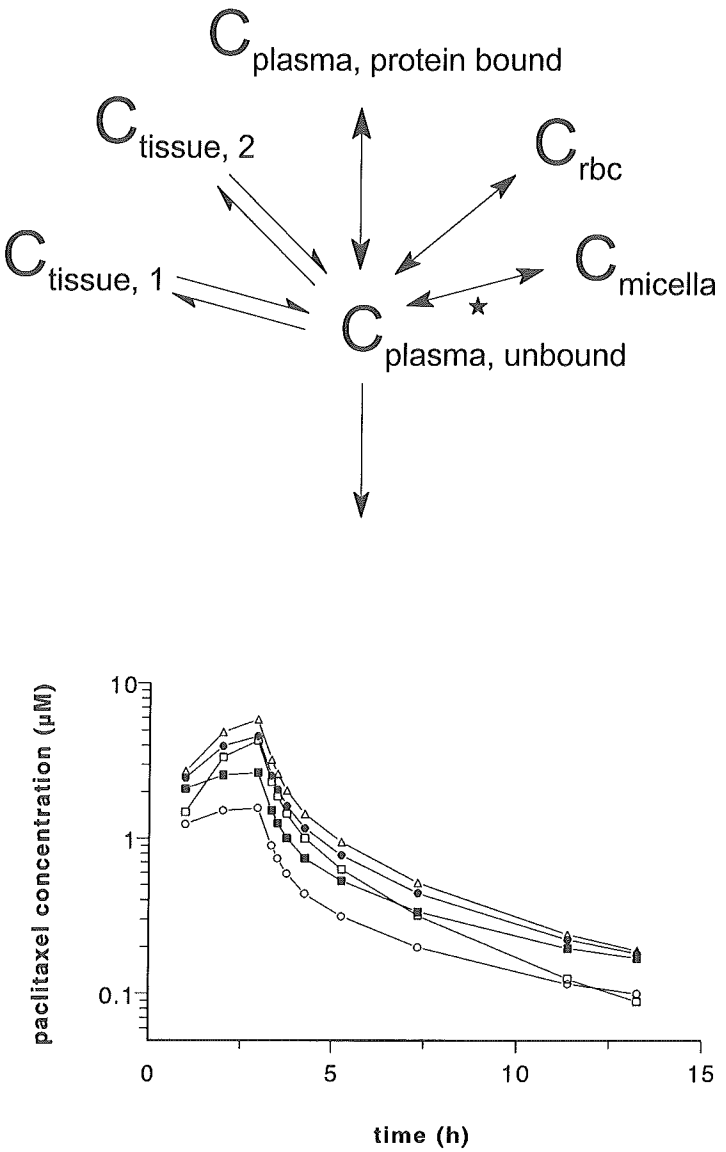

Figure 5 Paclitaxel concentration-time profiles using model-fit curves in a representative patient treated with paclitaxel at a dose level of 175 mg/m^2 [C_{plasma} [*i.e.* the sum of $C_{micellar}$ and $C_{non\text{-}micellar}$] (Δ), C_{blood} [*i.e.* the hematocrit-weighted average of C_{rbc} and C_{plasma}] (•), $C_{non\text{-}micellar}$ [*i.e.* the sum of $C_{unbound}$ and C_{bound}] (o), $C_{micellar}$ (□) and C_{rbc} (■)] (data from: Ref. 69).

The above mentioned results were in line with previous findings that in spite of nonlinear paclitaxel kinetics in plasma, tissue levels of the parent drug and its known metabolites in mice have a linear relationship with the dose administered (70). The paradox of disproportional increases in plasma concentrations and linear distribution processes in tissues thus seems to be caused by the fact that the plasma comprises a relatively small fraction of the total volume available for paclitaxel distribution and that, simultaneously, the CrEL-drug complex is not stable enough to substantially reduce the amount of drug that exists in the body in the active, diffusible, unbound form.

Recent work using the isolated perfused rat-liver model to investigate the influence of CrEL on the hepatobiliary elimination pathways of paclitaxel can also be explained by the principle of CrEL modulating the distribution of paclitaxel (71). It was found that CrEL caused a dose-dependent inhibition of paclitaxel elimination from the isolated perfused rat liver as can be explained by micellar entrapment of paclitaxel at the high dose of CrEL, thereby preventing paclitaxel from reaching sites of metabolism and excretion.

Effects on Pharmacokinetics of Other Agents

The existence of CrEL in blood as large polar micelles with a highly hydrophobic interior also raises the possibility of additional complexities in case of combination chemotherapy regimens with paclitaxel. For example, fluorescence studies on the interaction between anthracycline drugs and different surfactants indicated that daunorubicin, although relatively hydrophilic, is readily incorporated into CrEL micelles (72). Thus, in the systemic circulation, micellar incorporation of anthracyclines may result in altered cellular distribution and a concomitantly increased plasma concentration. In this respect, it is interesting to note that clinically significant pharmacokinetic interactions between paclitaxel and doxorubicin have been reported (73) as well as between CrEL alone and the anthracycline in both rodents and humans (74-76). By co-administration with CrEL, the apparent clearance of both doxorubicin and its metabolite doxorubicinol were significantly decreased (74). A murine pharmacokinetic study confirmed this finding, showing that both paclitaxel and docetaxel modified the distribution and metabolism of doxorubicin and that CrEL alone also showed this effect in contrast to Tween 80 alone (76). It can be

postulated that this pharmacokinetic interaction, at least partly attributable to CrEL, may contribute to the greater than expected toxicity of the combination treatment in which paclitaxel precedes doxorubicin with both agents given as prolonged infusion (77). Combining paclitaxel as a 3-h infusion with doxorubicin as a bolus infusion showed measurable pharmacokinetic differences between the sequences given although no pharmacodynamic differences were found (73). A more prolonged distribution and elimination phase of doxorubicin and its metabolite doxorubicinol was seen when the bolus infusion of doxorubicin was immediately followed by paclitaxel infusion leading to high concentrations of both agents at the same time. The overall pharmacokinetic results indicated that the dose of paclitaxel and doxorubicin, the duration of their infusion, and the interval between their administration were associated with nonlinear anthracycline disposition. A recent phase I study of paclitaxel and doxorubicin administered as concurrent 96-h infusions showed no change in the pharmacokinetics of both agents (78). Consequently, differences in the plasma concentration-time profile of CrEL, conditional to the paclitaxel administration schedule, account for the apparent sequence and schedule-dependent effect of paclitaxel on doxorubicin pharmacokinetics as is in line with our findings with paclitaxel, and it can be speculated that doxorubicin will also be encapsulated in CrEL micelles if administered in combination with 3-h infusions of the taxane.

Similarly, the elimination of etoposide is inhibited by CrEL in the isolated perfused rat-liver model through decreased total and biliary clearance from the liver (79). It can be postulated that the same phenomenon occurred as with paclitaxel in the same model, i.e. entrapment of etoposide in the micelles. Unpublished results in patients treated with the combination of paclitaxel (3-h infusion) and intravenous etoposide confirmed these observations (AS and JV). Similar pharmacokinetic interactions as described for CrEL and paclitaxel have also been reported for the photosensitizer C8KC (80), cyclosporin A (81) and the anesthetic induction agent propofol (82).

Tween 80 can also influence the pharmacokinetics of other drugs, but the reports in the literature on the effects of Tween 80 on drug distribution and elimination are conflicting. In the isolated perfused rat liver it decreases the clearance and volume of distribution of etoposide (79), but it increases the renal and biliary excretion of methotrexate (56). In mice, it

increases plasma levels of doxorubicin by decreasing the plasma volume as a result of the osmotic effect of high-dose Tween 80 when injected intraperitoneally (83). However, in patients who received the same amount of Tween 80 as is present in 100 mg/m^2 of etoposide, both the volume of distribution and the clearance of doxorubicin were increased due to reduced plasma concentrations of doxorubicin during the early phase of its kinetics (84). The effect of docetaxel on doxorubicin pharmacokinetics has not been reported, but initial results of a phase I trial of this combination did not indicate a greater than expected doxorubicin toxicity, suggesting that there is no major pharmacokinetic or pharmacodynamic interaction (85). Likewise, there is no apparent pharmacokinetic interaction between epirubicin and docetaxel in rats (86) and breast cancer patients (87), although AUC values of the 7-deoxydoxorubicinone metabolite were 1.9-fold higher in the presence of docetaxel as compared to single agent epirubicin treatment (87). Phase I clinical trials of combinations in which docetaxel was used together with vinorelbine, ifosfamide, 5-fluorouracil or cisplatin have shown also no evidence of relevant pharmacokinetic interactions (88).

There have been many reports highlighting the ability of Tween 80 to increase the absorption in animals and humans of various drugs, including steroids (89), phenobarbital (90), sulphonamides (91) and methotrexate (58), mainly by increasing biomembrane permeability. Furthermore, Tween 80 has been shown to induce significant increases in peak plasma levels and AUC values following intravesical instillation of thiotepa in patients with recurrent transitional cell carcinoma of the bladder (92), which contrasts recent work by Knemeyer et al (63) indicating that CrEL substantially reduces paclitaxel penetration into the bladder wall during intravesical treatment, most likely as a result of paclitaxel's affinity for the hydrophobic interior of CrEL micelles.

PHARMACODYNAMIC CHARACTERISTICS

Modulation of P-glycoprotein Activity

While examining *in vitro* the multidrug resistance-reversing potential of compounds dissolved with CrEL, it was observed that CrEL itself was able to modulate P-glycoprotein, the drug-transporting membrane protein elevated in tumor cells showing multidrug resistance *in vitro* (93-97). This effect of CrEL seems to be either due to a direct interaction with P-

glycoprotein (95,98), or the result of a general membrane perturbation affecting the function of the protein pump (99,100). CrEL at concentrations ≥ 0.1 μL/mL increased the sensitivity of multidrug resistance cells, with approximately 50% reversal at 1.0 μL/mL and complete reversal occurring at concentrations of 1.5-2.0 μL/mL *in vitro* (36,101). In solid tumors the significance of these *in vitro* findings is controversial, because the extremely low volume of distribution of CrEL suggests that concentrations necessary to affect reversal of the multidrug resistance phenotype (ranging from 0.3 to 1.0 μL/mL, depending on the cell lines tested) are probably not attained *in vivo* (50). Recent pharmacokinetic results from experiments conducted in *MDR1a* P-glycoprotein knock-out mice (102) and studies in tumor-bearing mouse models (103,104) support the conclusion that CrEL is not a very effective multidrug resistance modulator in solid tumors, because no enhancement of drug-induced antitumor activity was found in combination with CrEL, despite peak plasma levels of CrEL >40 μL/mL. In contrast, the pharmacokinetic selectivity of CrEL for the central blood/bone marrow compartment can be an advantage to hematological malignancies, in which the expression of P-glycoprotein is known as a factor contributing to resistance to chemotherapy (reviewed in: Ref. 105). Indeed, it was shown that CrEL functions independently as a modulator of the multidrug resistance phenotype displayed in blast cells obtained from previously untreated patients with acute myelogenous leukemia at concentrations as low as 0.12 μL/mL (106). The strategy of adding CrEL as a P-glycoprotein-modulating agent to chemotherapy in the treatment of hematological malignancies may also be of interest in view of recent findings that indicate that P-glycoprotein function in normal hematopoietic cells (i.e. CD3(+) lymphocytes) is less susceptible to CrEL than P-glycoprotein in leukemic myeloid cells (107). This selectivity in P-glycoprotein inhibition appears to be unique for CrEL, and suggest the possibility of targeted P-glycoprotein modulation in some malignancies with this agent.

Tween 80 has also shown to be a multidrug resistance modulator *in vitro*, even more potent than CrEL with approximately 50% reversal occurring already at levels between 0.2 and 0.3 μL/mL (94,95). Tween 80 has recently also been shown to increase etoposide cellular accumulation and potentiate cytotoxicity against various human lung adenocarcinoma cell lines by an unknown mechanism independent of P-glycoprotein (108). However, in cancer patients the plasma concentrations of Tween 80

measured following docetaxel infusion were only about 0.10 μL/mL, and thus too low to reverse the multidrug resistance phenotype or affect cellular accumulation at the tumor site (40,101). In addition, the very short terminal half life of Tween 80 does not contribute to the probability that these *in vitro* properties will have clinical ramifications.

Intrinsic Effects on Hematological Pharmacodynamics

Increase in circulating platelet levels was recorded following prolonged administration of high doses of CrEL in dogs (109). In contrast, in mice it was shown that peripheral blood cell parameters, including white blood cell count, reticulocyte and platelet counts were unaffected by CrEL administration (110; observation AS and JV). However, intravenous administration of CrEL in mice was associated with a decrease in bone marrow cellularity, an upregulation of B220 (B-cells) and 7/4 surface antigen expression (neutrophil and activated macrophage) in the marrow and an increase in the incidence of progenitors (110). Furthermore, CrEL protected mice from irradiation-induced death if administered prior to the dose (110). These intriguing effects are consistent with the interpretation that CrEL activates accessory cells, and modulates accessory factors regulating hematopoietic progenitor cells through the operation of cytokine cascades. The induction of histamine release may also play a role in the hematopoietic response to CrEL administration (18,111,112). In mice, no evidence was obtained of localized toxicity or marrow destruction as a result of CrEL injection (110), which is consistent with flow cytometric studies demonstrating that even very high concentrations of CrEL (>10%) did not lyse mammalian cell membranes (94).

Recently, in 5 patients with ovarian cancer treated with paclitaxel, rouleaux formation of red cells was found within 24 h after infusion (113). *In vitro* experiments with samples of whole blood revealed the same finding after addition of both paclitaxel and CrEL, which makes it likely that CrEL is responsible for the phenomenon. This experience suggests caution in the use of automated blood cell analyzers in patients receiving paclitaxel.

INFLUENCE ON ANTICANCER DRUG PHARMACODYNAMICS

Paclitaxel

Various hypotheses regarding the relationship between the plasma pharmacokinetics and the hematological toxicity of paclitaxel have been postulated, including those using a step function (threshold model) (114,115) and more general models that used a nonlinear continuous function for the time-dissociated component (116,117). However, since the nonlinear pharmacokinetics of paclitaxel in plasma can be explained by CrEL concentration-dependent changes in drug movement within the central compartment, the effects of entrapment of paclitaxel in the plasma compartment will be less with prolonged infusion schedules associated with lower concentrations of CrEL (17,69). Thus, total plasma levels of paclitaxel measured in these schedules will represent a higher fraction of free paclitaxel, and this may, in part, explain the increased incidence of severe hematological toxicity seen in the 24-h infusion schedules as compared with infusion of the same dose over 3 h (69).

Some studies suggested that CrEL and Tween 80 may enhance taxane cytotoxicity (118-120), and might have a cytotoxic effect of their own (121,122), although this could not be confirmed by others (123). Furthermore, some investigators observed that high levels of CrEL antagonized the *in vitro* cytotoxicity of paclitaxel at concentrations >1.35 μL/mL (124). This was shown to be related to a CrEL-mediated G1-phase arrest of the cell cycle, thereby preventing cells from entering mitosis in a phase where paclitaxel is most cytotoxic (125,126). This disruption of the cell cycle is likely to be one mode of action through which CrEL antagonizes paclitaxel cytotoxicity, in addition to the observation that high levels of CrEL reduced the rate of cellular accumulation of paclitaxel (126). This observation can also be related to micellar formation and subsequent drug encapsulation, and can be regarded as a confirmation of our above described findings on the clinical pharmacokinetic behavior of paclitaxel. Although CrEL (or Tween 80) concentrations have not yet been measured in tumor tissues in any study, it is unlikely that any potential difference in antitumor activity between various formulations is caused by direct cytotoxic effects or cell cycle arrest by the surfactants, because of their pharmacokinetic selectivity for the blood compartment and the undetectable CrEL levels in normal tissues of treated mice (59). Notwithstanding these

observations, it is of interest to note that in early studies conducted by the National Cancer Institute, paclitaxel was not effective in several tumor models when given intravenously as a solution in polyethyleneglycol 400 or 10 to 15% Tween 80-ethanol, suggesting that the CrEL-based vehicle is essential for *in vivo* antitumor activity (127). Furthermore, Fujimoto et al showed significantly higher *in vivo* antitumor activity of paclitaxel against P388 leukemia when formulated in CrEL as compared to drug dissolved in dimethylacetamide (128). Similar differences for a vehicle-dependent antitumor activity have been observed *in vitro* using clonogenic assays (129,130). Taken together, these data suggest a rather complex interaction between the vehicle and the drug that is not yet completely understood. A recent study on some cell lines exhibiting varying *MDR-1* mRNA expression levels with CrEL concentrations corresponding to those used in clinical formulation of paclitaxel revealed that there was not an independent effect on cell survival (131).

Anthracyclines

As discussed, CrEL affects the plasma pharmacokinetics of doxorubicin in both rodents and humans, and thus can be a critical determinant in the toxicity of this anticancer drug (74-76). This is of potential clinical interest in view of the fact that combinations of paclitaxel and anthracyclines were reported to be highly effective in the treatment of breast cancer (132-134). Several studies provided evidence that when paclitaxel as a 24-h infusion preceded doxorubicin greater toxicity was seen in the combination treatment (77,135). The dose-limiting toxicity, including stomatitis of the upper gastrointestinal tract, occurred at doses of both paclitaxel and doxorubicin that were approximately 30% less than when doxorubicin preceded paclitaxel. In contrast, no significant increase of hematological and gastro-intestinal toxicities were observed when paclitaxel as a 3-h infusion was given before doxorubicin, and it can be concluded that there exists no detectable sequence-dependent effect when both agents are administered as short infusions with the exception of cardiac toxicity (132). The risk of cardiac toxicity was found to be much higher than with other regimens including anthracyclines. A study in mice demonstrated that CrEL increased the tissue levels of doxorubicin due to an unknown mechanism, but it deserves attention that especially the most

vascularized tissues showed the most outspoken change of doxorubicin disposition (76).

Platinum Analogues

Clinical combination chemotherapy studies with paclitaxel or docetaxel in combination with cisplatin revealed important sequence-dependent differences in frequency and severity of toxicities, with less severe myelotoxicity when the taxane was given before cisplatin (136,137). In the sequence of docetaxel followed by cisplatin it was demonstrated that the reduced leukocytopenia was accompanied by a significant inhibition of cisplatin uptake and DNA-adduct formation in peripheral blood leukocytes as compared to the alternate sequence (138,139). The fundamental mechanism turned out to be not related to the taxanes themselves, but to their formulation vehicles (140). Both CrEL and Tween 80 selectively inhibit cisplatin uptake in human peripheral blood leukocytes and rat bone marrow cells. In case of CrEL, there was a clear concentration-dependent effect with maximal inhibition of approximately 50% achieved at an extracellular CrEL concentration of 10 μL/mL, but also a mere 25% decrease in cisplatin-uptake at concentrations of CrEL as low as 0.0001 μL/mL. However, the biochemical mechanism of the dose-dependent interaction of CrEL with the cellular uptake of cisplatin is not clear, but it has been speculated that inhibition of protein kinase C by CrEL (141) could play a role leading to decreased diffusion of cisplatin into the cells. It can be concluded that the extended exposure of normal hematopoietic cells to levels of CrEL achieved in patients after paclitaxel treatment might result in a selective protection against cisplatin accumulation in peripheral blood leukocytes (140). As such, the use of CrEL as a formulation vehicle may be an important determinant in the reduced cisplatin-associated myelotoxicity observed in clinical combination chemotherapy studies with paclitaxel (136,142). *In vitro*, the same studies revealed a lack of interaction between CrEL and the cisplatin analogue carboplatin, which is in line with previous clinical observations demonstrating a sequence-independent effect on the myelotoxicity of the combination paclitaxel and carboplatin in cancer patients (143,144), and a lack of effect of CrEL (equivalent to a paclitaxel dose of 175 mg/m^2) on the pharmacokinetics and hematologic toxicity of carboplatin (target AUC, 6.0 mg.min/mL) (145).

Miscellaneous

Sequential dependency was also seen in two phase I trials of paclitaxel administered as a 3-hour or prolonged intravenous infusion and cyclophosphamide in which the hematologic and gastro-intestinal toxicities were more severe when paclitaxel preceded cyclophosphamide (146-148). However, in these studies no alterations of the pharmacokinetics of both compounds were seen, and the mechanism underlying the pharmacodynamic interaction, and the role of CrEL therein, have not yet been elucidated.

CONCLUSIONS AND PERSPECTIVES

Paclitaxel and docetaxel are hydrophobic antineoplastic agents demonstrating significant antitumor activity against a broad range of human malignancies. The enthusiasm evoked by some exciting clinical results of these agents has also led to the publication of a substantial number of papers addressing the role of pharmaceutical vehicles in their pharmacologic behavior. The investigations have yielded fundamental insight into modes of action, pharmacokinetic profiles, and considerations of dosage and schedule and route of drug administration. The current paclitaxel formulation vehicle CrEL presents a number of serious concerns, including a wide variety of intrinsic toxic side effects that limit the amount of drug that can be safely administered. Modulation of the drug disposition profiles by alteration of the blood distribution resulting from entrapment of this compound in circulating micelles have been documented, and CrEL also seems to play a crucial role in changes of the pharmacokinetics and -dynamics of several other compounds co-administered or those using CrEL as a formulation vehicle. It can be anticipated that tremendous (therapeutic) advantage could be obtained from the use of a clinically useful formulation vehicle in which CrEL is absent. This is particularly evident from recent data of a dose-finding study with an albumin stabilized, CrEL-free, nanoparticle formulation of paclitaxel, which suggest a considerable increase in maximum tolerated dose of paclitaxel and a lack of hypersensitivity reactions in spite of the absence of premedication and a 30-min infusion (149). A dose-finding study with a new submicronic, Tween 80-free dispersion formulation of docetaxel, suggested a lower incidence and severity of hematological and non-hematological toxicity (including fluid retention and skin rashes) at

equimolar doses as a compared to the currently used preparation (150). Several other strategies are in progress to develop a CrEL-free and Tween 80-free formulation of paclitaxel and docetaxel, respectively, including the use of alternative formulation vehicles (e.g. co-solvents, emulsions, liposomes, cyclodextrins, nanocapsules and microspheres), development of more water-soluble derivatives or prodrugs, and alternative administration routes (e.g. local treatment and oral administration). Details of these strategies are discussed by Nuijen et al and Malingré et al, respectively, in the current issue of this journal. Continued investigations into the role of pharmaceutical vehicles in taxane-related drugs, coupled with new approaches in taxane drug design and formulation, should eventually lead to a more rational and selective chemotherapeutic treatment with these agents.

REFERENCES

1. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT: Plant antitumor agents. VI: The isolation and structure of Taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J Am Chem Soc 93:2325-2327, 1971
2. Adams JD, Flora KP, Goldspiel BR, Wilson JW, Arbuck SG, Finley R: Taxol: a history of pharmaceutical development and current pharmaceutical concerns. J Natl Cancer Inst Monogr 15:141-147, 1993
3. Dorr RT: Pharmacology and toxicology of Cremophor EL diluent. Ann Pharmaco-therapy 28:S11-S14, 1994
4. Hoffmann H. Polyoxyäthylenglycerol triricinoleat 35 DAC 1979. Pharm Zeit 129:1730-1733, 1984
5. Goldspiel BR: Guidelines for administration. In: McGuire WP, Rowinsky EK (eds) Paclitaxel in Cancer Treatment. Marcel Dekker Inc, New York, 1995, pp175-186
6. Onetto N, Dougan M, Hellmann S, Gustafson N, Burroughs J, Florczyk A, Canetta R, Rozenweig M: Safety profile. In: McGuire WP, Rowinsky EK (eds) Paclitaxel in Cancer Treatment. Marcel Dekker, New York, 1995, pp121-149
7. Hayes FA, Abromowitch, Green AA: Allergic reactions to teniposide in patients with neuroblastoma and lymphoid malignancies. Cancer Treat Rep 69:439-441, 1985
8. Howrie DL, Ptachcinski RJ, Griffith P, Hardesty RJ, Rosenthal JT, Burckart GJ, Venkataramanan R: Anaphylactoid reactions associated with parenteral cyclosporine use: possible role of Cremophor EL. Drug Intell Clin Pharm 19:425-427, 1985
9. Weiss RB, Donehower RC, Wiernik PH, Ohnuma T, Gralla R, Trump DL, Baker JR, Van Echo DA, Von Hoff DD, Leyland-Jones B: Hypersensitivity reactions from taxol. J Clin Oncol 8:1263-1268, 1990
10. Nannan Panday VR, Huizing MT, ten Bokkel Huinink WW, Vermorken JB, Beijnen JH: Hypersensitivity reactions to the taxanes paclitaxel and docetaxel. Clin Drug Invest 14:418-427, 1997

11. Onetto N, Canetta R, Winograd B, Catane R, Dougan M, Grechko J, Burroughs J, Rozencweig M: Overview of Taxol safety. J Natl Cancer Inst Monogr 15:131-139, 1993
12. Eisenhauer EA, Ten Bokkel Huinink WW, Swenerton KD, Gianni L, Myles J, Van Der Burg MEL, Kerr I, Vermorken JB, Buser K, Colombo N, Bacon M, Santabárbara P, Onetto N, Winograd B, Canetta R: European-Canadian randomized trial of paclitaxel in relapsed ovarian cancer: high-dose *versus* low-dose and long *versus* short infusion. J Clin Oncol 12:2654-2666,1994
13. Essayan DM, Kagey-Sobotka A, Colarusso PJ, Lichtenstein LM, Ozols RF, King ED: Successful parenteral desensitization to paclitaxel. J Allergy Clin Immunol 97:42-46, 1996
14. Watkins J, Ward AM, Appleyard TN: Adverse reactions to intravenous anaesthetic induction agents. Br Med J 2: 1084-1085, 1977
15. Hüttel MS, Schou Olesen A, Stoffersen E: complement-mediated reactions to diazepam with Cremophor as solvent (Stesolid MR). Br J Anaesth 52:77-79, 1980
16. Szebeni J, Muggia FM, Alving CR: Complement activation by Cremophor EL as a possible contributor to hypersensitivity to paclitaxel: an *in vitro* study. J Natl Cancer Inst 90:300-306, 1998
17. Sparreboom A, Van Zuijlen L, Verweij J, Mross K, Brouwer E, Gianni L. Interrelationships of paclitaxel disposition, infusion duration and Cremophor EL kinetics in cancer patients. ('Abstract') Proc AACR-NCI-EORTC Int Conf, 1:112, 1999
18. Lorenz WM, Reimann HJ, Schmal A, Dormann P, Schwarz B, Neugebauer E, Doenicke A: Histamine release in dogs by Cremophor EL and its derivatives: oxyethelated oleic acid is the most effective constituent. Agents Actions 7:63-67, 1977
19. Bergh M, Magnusson K, Nilsson JL, Karlberg AT: Contact allergenic activity of Tween 80 before and after air exposure. Contact Dermatitis 37:9-18, 1997
20. Lesser GJ, Grossman SA, Eller S, Rowinsky EK: The distribution of systemically administered [^{3}H]-paclitaxel in rats: a quantitiative autoradiographic study. Cancer Chemother Pharmacol 37:173-178, 1995
21. De Groen PC, Aksamit AJ, Rakela J, Forbes GS, Krom RAF: Central nervous system toxicity after liver transplantation: the role of cyclosporine and cholesterol. N Engl J Med 317:861-886, 1987
22. Sparreboom A, Van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DK, Borst P, Nooijen WJ, Beijnen JH, Van Tellingen O: Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc Natl Acad Science USA 4:2031-2035, 1997
23. Meerum-Terwogt JM, Malingré MM, Beijnen JH, Ten Bokkel Huinink WW, Rosing H, Koopman FJ, Van Tellingen O, Swart M, Schellens JHM: Coadministration of oral cyclosporin A enables oral therapy with paclitaxel. Clin Cancer Res 5:3379-3384, 1999
24. Evans WC: Drugs containing acids, alcohols and esters. In: Pharmacognosy, 13th ed. Baillière Tindall, London, 1989, p.333
25. Windebank AJ, Blexrud MD, De Groen PC: Potential neurotoxicity of the solvent vehicle for cyclosporine. J Pharmacol Exp Ther 268:1051-1056, 1994
26. Brat DJ, Windebank AJ, Brimijoin S: Emusifier for intravenous cyclosporin inhibits neurite outgrowth, causes deficits in rapid axonal transport and leads to structural abnormalities in differentiating N1E.115 neuroblastoma. J Pharmacol Exp Ther 261:803-810, 1992

27. Podratz JL, Windebank AJ: Dialyzer associated neurotoxicity due to a change in fatty acid structure. Trans Am Soc Neurochem 24:229, 1993
28. Verweij J, Clavel M, Chevalier B: Paclitaxel (Taxol) and docetaxel (Taxotere): not simply two of a kind. Ann Oncol 5:495-505, 1994
29. Roman ID, Monte MJ, Esteller A, Jimenez R: Cholestasis in the rat by means of intravenous administration of cyclosporine vehicle, CrEL. Transplantation 48:554-558, 1989
30. Chan FK, Shaffer EA: Cholestatic effects of cyclosporin in the rat. Transplant 63:1574-1578, 1997
31. Luke DR, Kasiske BL, Matzke GR, Awni WM, Keane WF: Effects of cyclosporine on the isolated perfused rat kidney. Transplantation 43:795-801, 1987
32. Besarab A, Jarrell BE, Hirsch S, Carabasi RA, Cressman MD, Green P: Use of the isolated perfused kidney model to assess the acute pharmacologic effets of cyclosporine and its vehicle, Cremophor EL. Transplant 44:195-201, 1987
33. Abraham JS, Bentley FR, Garrison RN, Cryer HM: The influence of the cyclosporine vehicle, Cremophor EL, on renal microvascular blood flow in the rat. Transplantation 52:101-105, 1991
34. Rothenberg ML: Taxol, Taxotere and other new taxanes. Curr Opin Invest Drugs 2:1269-1277, 1993
35. Drori S, Eytan GD, Assaraf YG: Potentiation of anticancer-drug cytotoxicity by multidrug-resistance chemosensitizers involves alterations in membrane fluidity leading to increased membrane permeability. Eur J Biochem 228:1020-1029, 1995
36. Webster L, Linsenmeyer M, Millward M, Morton C, Bishop J, Woodcock D: Measurement of CrEL following Taxol: plasma levels sufficient to reverse drug exclusion mediated by the multidrug-resistant phenotype. J Natl Cancer Inst 85:1685-1690, 1993
37. Sparreboom A, Van Tellingen O, Huizing MT, Nooijen WJ, Beijnen JH: Determination of polyoxyethyleneglycerol triricinoleate 35 (Cremophor EL) in plasma by pre-column derivatization and reversed-phase high-performance liquid chromatography. J Chromatogr B 681:355-362, 1996
38. Sparreboom A, Loos WJ, Verweij J, De Vos AI, Van der Burg MEL, Stoter G, Nooter K: Quantitation of Cremophor EL in human plasma samples using a colorimetric dye-binding microassay. Anal Biochem 255:171-175, 1998
39. Brouwer E, Verweij J, Hauns B, Loos WJ, Nooter K, Mross K, Stoter G, Sparreboom A: Linearized colorimetric assay for Cremophor EL: application to pharmacokinetics after 1-hour paclitaxel infusions. Anal Biochem 261:198-202, 1998
40. Van Tellingen O, Beijnen JH, Verweij J, Scherrenburg EJ, Nooijen WJ, Sparreboom A: Rapid esterase-sensitive breakdown of polysorbate 80 and its impact on the plasma pharmacokinetics of docetaxel and metabolites in mice. Clin Cancer Res 5:2918-2924, 1999
41. Kunkel M, Meyer T, Bohler J, Keller E, Frahm AW: Titrimetric determination of Cremophor EL in aqueous solutions and biofluids: part 2: ruggedness of the method with respect to biofluids. J Pharm Biomed Anal 21:911-922, 1999
42. Oszi Z, Petho G: Quantitative determination of polysorbate 20 in nasal pharmaceutical preparations by high-performance liquid chromatography. J Pharm Biomed Anal 18:715-720, 1998
43. Smullin CF, Wetterau FP, Olanski VL: The determination of polysorbate 60 in foods. J Am Oil Chem Soc 48:18-20, 1971

44. Smullin CF: Quantitative determination of polysorbate 60 in non-standard salad dressings. J Assoc Off Anal Chem 61:506-507, 1978
45. McKean DL, Pesce AJ: Determination of polysorbate in ascites fluid from a premature patient. J Anal Toxicol 9:174-176, 1985
46. Kato H, Nagai Y, Yamamoto K, Sakabe Y: Determination of polysorbates in foods by colorimetry with confirmation by infrared spectrophotometry, thin-layer chromatography, and gas-chromatography. J Assoc Off Anal Chem 72:27-29, 1989
47. McKean DL, Pesce AJ, Koo W: Analysis of polysorbate and its polyoxyethylated metabolite. Anal Biochem 161:348-351, 1987
48. Meerum Terwogt JM, Van Tellingen O, Nannan Panday VR, Huizing MT, Schellens JHM, Ten Bokkel Huinink WW, Boschma MUS, Giaccone G, Veenhof CHN, and Beijnen JH: Cremophor EL pharmacokinetics in a phase I study of paclitaxel (Taxol) and carboplatin in non-small cell lung cancer patients. In: Clinical Pharmacology of Anticancer Agents in Relation to Formulations and Administration Routes. Academic Thesis, Utrecht University, 1999, pp61-77
49. Rischin D, Webster LK, Millward MJ, Linahan BM, Toner GC, Woollett AM, Morton CG, Bishop JF: Cremophor pharmacokinetics in patients receiving 3-, 6-, and 24-hour infusions of paclitaxel. J Nat Cancer Inst 88:1297-1301, 1996
50. Sparreboom A, Verweij J, van der Burg MEL, Loos WJ, Brouwer E, Viganò L, Locatelli A, de Vos AI, Nooter K, Stoter G, Gianni L: Disposition of Cremophor EL in humans limits the potential for modulation of the multidrug resistance phenotype *in vivo*. Clin Cancer Res 4:1937-1942, 1998
51. Nannan Panday VR, Huizing MT, Van Tellingen O, Hakvoort RA, Willemse PHB, De Graeff A, Vermorken JB, Beijnen JH: Pharmacologic study of Cremophor EL in cancer patients with impaired hepatic function receiving taxol. In: Clinical Pharmacology of Paclitaxel and Platinum Compounds. Academic Thesis, Utrecht University, 1999, pp109-120
52. Gelderblom H, Verweij J, Brouwer E, Pillay M, de Bruijn P, Nooter K, Stoter G, Sparreboom A: Disposition of [*G*-^{3}H]paclitaxel and Cremophor EL in a patient severely impaired renal function. Drug Metab Dispos 27:1300-1305, 1999
53. Nelson MF, POulas TA, Gongwer LE, Kischman JC: Preparation of carbon-14-labeled polyoxyethylene (20) sorbitan monolaurate and their metabolitic fate in rats. J Food Sci 31:253-258, 1966
54. Culver PJ, Wilcox CS, Jones CM, Ross RS: Intermediary metabolism of certain polyoxyethylene derivatives in man. I. Recovery of the polyoxyethylene moiety from urine and feces following ingestion of polyoxyethylene (20) sorbitan monooletate and of polyoxyethylene (40) monostearate. J Pharmacol Exp Ther 103:377-381, 1951
55. Elworthy PH, Treon JF: Physiological activity of nonionic surfactants. In:Schick M (ed) Nonionic Surfactants, Surfactant Science Series, Vol 1. Marcel Dekker, New York, 1967, pp923-970
56. Azmin MN, Stuart JFB, Florence AT: The distribution and elimination of methotrexate in mouse blood and brain after concurrent administration of polysorbate 80. Cancer Chemother Pharmacol 14:238-242, 1985
57. Treon JF, Gongwer LE, Nelson MF, Kischman JC: Physiology and metabolic patterns of non-ionic surfactants. In: Paquot P (ed) Chemistry, Physics and Application of Surface-active Substances, vol 3. Gordon and Breach, London, 1967, p 381

58. Azmin MN, Stuart JFB, Calman KC, Florence AT: Effects of polysorbate 80 on the absorption and distribution of oral methotrexate (MTX) in mice. Cancer Chemother Pharmacol 9:161-164, 1982
59. Sparreboom A, Van Tellingen O, Nooijen WJ, Beijnen JH: Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle Cremophor EL. Cancer Res 56:2112-2115, 1996
60. Sparreboom A, Van Zuijlen L, Brouwer E, Loos WJ, de Bruijn P, Gelderblom H, Pillay M, Nooter K, Stoter G, Verweij J: Cremophor EL-mediated alteration of paclitaxel distribution in human blood: clinical pharmacokinetic implications. Cancer Res 59:1454-1457, 1999
61. Kessel D, Woodburn K, Decker D, Sykes E: Fractionation of Cremophor EL delineates components responsible for plasma lipoprotein alterations and multidrug resistance reversal. Oncol Res 7:207-212, 1995
62. Sykes E, Woodburn K, Decker D, Kessel D: Effect of Cremophor EL on distribution of Taxol to serum lipoproteins. Br J Cancer 70:401-404, 1994
63. Knemeyer I, Wientjes MG, Au JL-S: Cremophor reduces paclitaxel penetration into bladder wall during intravesical treatment. Cancer Chemother Pharmacol 44:241-248, 1999
64. Van Tellingen O, Huizing MT, Nannan Panday VR, Schellens JHM, Nooijen WJ, Beijnen JH: Cremophor EL causes (pseudo-) non-linear pharmacokinetics of paclitaxel in patients. Br J Cancer 81:330-335, 1999
65. Kearns CM: Pharmacokinetics of the taxanes. Pharmacotherapy 17:105S-109S, 1997
66. Gianni L, Kearns CM, Giani A, Capri G, Vigano L, Locatelli A, Bonadonna G, Egorin MJ: Nonlinear pharmcokinetics and metabolism of paclitaxel and its pharmacokinetic/pharmaco-dynamic relationships in humans. J Clin Oncol 13:180-190, 1995
67. Ohtsu T, Sasaki Y, Tamura T, Miyata Y, Nakanomyo H, Nishiwaki Y, and Saijo N: Clinical pharmacokinetics and pharmacodynamics of paclitaxel: a 3-hour infusion *versus* a 24-hour infusion. Clin Cancer Res 1:599-606, 1995
68. Wiernik PH, Schwartz EL, Einzig A, Strauman JJ, Lipton RB, and Dutcher JP: Phase I trial of Taxol given as a 24-hour infusion every 21 days: responses observed in metastatic melanoma. J Clin Oncol 5:1232-1239, 1987
69. Van Zuijlen L, Karlsson MO, Verweij J, Brouwer E, de Bruijn P., Nooter K, Stoter G, Sparreboom A : Pharmacokinetic modeling of paclitaxel encapsulation in Cremophor EL micelles. Cancer Chemother Pharmacol (in press)
70. Sparreboom A, Van Tellingen O, Nooijen WJ, Beijnen JH: Tissue distribution, metabolism and excretion of paclitaxel in mice. Anticancer Drugs 7:78-86, 1996
71. Ellis AG, Webster LK: Inhibiton of paclitaxel elimination in the isolated perfused rat liver by Cremophor EL. Cancer Chemother Pharmacol 43:13-18, 1999
72. Kessel D: Properties of Cremophor EL micelles probed by fluorescence. Photochem Photobiol 56:447-451, 1992
73. Gianni L, Vigano L, Locatelli A, Capri G, Giani A, Tarenzi E, Bonadonna G: Human pharmacokinetic characterization and *in vitro* study of the interaction between doxorubicin and paclitaxel in patients with breast cancer. J Clin Oncol 15:1906-1915, 1997
74. Webster LK, Cosson EJ, Stokes KH, Millward MJ: Effect of the paclitaxel vehicle, Cremophor EL, on the pharmacokinetics of doxorubicin and doxorubicinol in mice. Br J Cancer 73:522-524, 1996

75. Millward MJ, Webster LK, Rischin D, Stokes KH, Toner GC, Bishop JF, Olver IN, Linahan BM, Linsenmeyer ME, Woodcock DM: Phase I trial of Cremophor EL with bolus doxorubicin. Clin Cancer Res 4:2321-2329, 1998
76. Colombo T, Parisi I, Zucchetti M, Sessa C, Goldhirsch A, D'Incalci M: Pharmacokinetic interactions of paclitaxel, docetaxel and their vehicles with doxorubicin. Ann Oncol 10:391-395, 1999
77. Holmes FA, Madden T, Newman RA, Valero V, Theriault RL, Fraschini G, Walters RS, Booser DJ, Buzdar AU, Willey J, Hortobagyi GN: Sequence-dependent alteration of doxorubicin pharmacokinetics by paclitaxel in a phase I study of paclitaxel and doxorubicin in patients with metastatic breast cancer. J Clin Oncol 14:2713-2721, 1996
78. Duska LR, Penson R, Supko JG, Finkelstein DM, Makastorsis T, Gallagher J, Borden K, Goodman A, Fuller AF, Nikrui N, Seiden MV: A phase I study of continuous infusion doxorubicin and paclitaxel chemotherapy with granulocyte colony-stimulating factor for relapsed epithelial ovarian cancer. Clin Cancer Res 5:1299-1305, 1999
79. Ellis AG, Crinis NA, Webster LK: Inhibition of etoposide elimination in the isolated perfused rat liver by Cremophor EL and Tween 80. Cancer Chemother Pharmacol 38:81-87, 1996
80. Woodburn K, Chang CK, Lee S, Henderson B, Kessel D: Biodistribution and PDT efficacy of a ketochlorin photosensitizer as a function of the delivery vehicle. Photochem Photobiol 60:154-159, 1994
81. Kurlansky PA, Sadeghi AM, Michler RE, Coppey LJ, Re LP, Thomas WG, Smith CR, Reemtsma K, Rose EA: Role of the carrier solution in cyclosporine pharmacokinetics in the baboon. J Heart Transplant 5:312-316, 1986
82. Kay NH, Uppington J, Sear JW, Douglas EJ, Cockshott ID: Pharmacokinetics of propofol (Diprivan) as an induction agent. Postgrad Med J 61:55-57, 1985
83. Harrison SD, Cusic AM, McAfee SM: Tween 80 increases plasma adriamycin concentrations in mice by an apparent reduction of plasma volume. Eur J Cancer 17:387-389, 1981
84. Cummings J, Forrest GJ, Cunningham D, Gilchrist NL, Soukop M: Influence of polysorbate 80 (Tween 80) and etoposide (VP-16-213) on the pharmacokinetics and urinary excretion of Adriamycin and its metabolites in cancer patients. Cancer Chemother Pharmacol 17:80-84, 1986
85. Bourgeois H, Gruia G, Dieras V, Kalla S, Giaccetti S, Cvitkovic E, Aussel JP, Azli N, Riva A, Poullart P, Misser JL: Docetaxel in combination with doxorubicin as first line chemotherapy of metastatic breast cancer: a phase I dose finding study. ('Abstract') Proc Am Ass Clin Oncol 15:148, 1996
86. Sandstrom M, Simonsen LE, Freijs A, Karlsson MO: The pharmacokinetics of epirubicin and docetaxel in combination in rats. Cancer Chemother Pharmacol 44:469-474, 1999
87. Eposito M, Venturini M, Vannozzi MO, Tolino G, Lunardi G, Garrone O, Angiolini C, Viale M, Bergaglio M, Del Mastro L, Rosso R: Comparative effects of paclitaxel and docetaxel on the metabolism and pharmacokinetic of epirubicin in breast cancer patients. J Clin Oncol 17:1132-1138, 1999
88. D'Incalci M, Schuller J, Colombo T, Zucchetti M, Riva A: Taxoids in combination with anthracyclines and other agents: pharmacokinetic considerations. Sem Oncol 25:16-20, 1998
89. Gannt L, Crochman N, Dyniewicz JM: Effect of a detergent on gastro-intestinal absorption of a steroid. Lancet 1:486, 1961
90. Hikal AH: Effect of polysorbate 80 on the apparent partition coefficient of drugs on their intestinal absorption in the rat: II. Phenobarbital. Int J Pharm 7:205-210, 1981

91. Katzemi K, Arita T, Muranishi S: Absorption and excretion of drugs: XXVI. Effect of non-ionic surface-active agents on rectal absorption of sulphonamides. Chem Pharm Bull 13:976-980, 1965
92. Masters JR, McDermott BJ, Jenkins WE, Fenwick E, Shah PJ, Mundy AR, Loadman PM, Bibby MC: Thiotepa pharmacokinetics during intravesical chemotherapy and the influence of Tween 80. Cancer Chemother Pharmacol 25:267-273, 1990
93. Woodcock DM, Jefferson S, Linsenmeyer ME, Crowther PJ, Chojnowski GM, Williams B, Bertoncello I: Reversal of the multidrug resistance phenotype with Cremophor EL, a common vehicle for water-insoluble vitamins and drugs. Cancer Res 50:4199-4203, 1990
94. Woodcock DM, Linsenmeyer ME, Chojnowski G, Kriegler AB, Nink V, Webster LK, Sawyer WH: Reversal of multidrug resistance by surfactants. Br J Cancer 66:62-68, 1992
95. Friche E, Jensen PB, Sehested M, Demant EF, Nissen NN: The solvents Cremophor EL and Tween 80 modulate daunorubicin resistance in the multidrug resistant Ehrlich ascites tumor. Cancer Commun 2:297-303, 1990
96. Schuurhuis GJ, Broxterman HJ, Pinedo HM, Van Heijningen THM, Van Kalker CK, Vermorken JB, Spoelstra EC, Lankelma J: The polyoxyethylene castor oil Cremophor EL modifies multidrug resistance. Br J Cancer 62:591-594, 1990
97. Chervinsky DS, Brecher ML, Hoelcle MJ: Cremophor-EL enhances Taxol efficacy in a multi-drug resistant C1300 neuroblastoma cell line. Anticancer Res 13:93-96, 1993
98. Hwang M, Ahn C-H, Pine PS, Yin JJ, Hrycyna CA, Licht T, Aszalos A: Effect of combination of suboptimal concentrations of P-glycoprotein blockers on the proliferation of *MDR1* gene expressing cells. Int J Cancer 65:389-397, 1996
99. Sinicrope FA, Dudeja PK, Bissonnette BM, Safa AR, Brasitus TA: Modulation of P-glycoprotein-mediated drug transport by alterations in lipid fluidity of rat liver canalicular membrane vesicles. J Biol Chem 267:24995-25002, 1992
100. Dudeja PK, anderson KM, Harris JS, Buckingham L, Coon JS: Reversal of multidrug resistance phenotype by surfactants: relationship to membrane lipid fluidity. Arch Biochem Biophys 319:309-315, 1995
101. Webster LK, Linsenmeyer ME, Rischin D, Urch ME, Woodcock DM, Millward MJ: Plasma concentrations of polysorbate 80 measured in patients following administration of docetaxel or etoposide. Cancer Chemother Pharmacol 39:557-560, 1997
102. Sparreboom A, Van Asperen J, Van Tellingen O, Smit JW, Schinkel AH, Nooijen WJ, Beijnen JH: Pharmacokinetics of paclitaxel in *MDR1a* P-glycoprotein deficient mice after intravenous and oral administration. ('Abstract') Ann Oncol 7:91, 1996
103. Slater L, Sweet P, Wetzel M, Stupecky M, Osann K: Comparison of cyclosporin A, verapamil, PSC-833 and Cremophor EL as enhancing agents of VP-16 in murine lymphoid leukemias. Leuk Res 19:543-548, 1995
104. Watanabe T, Nakayama Y, Naito M, Oh-hara T, Itoh Y, Tsuruo T: Cremophor EL reversed multidrug resistance *in vitro* but not in tumor-bearing mouse models. Anticancer Drugs 7:825-832, 1996
105. Nooter K, Sonneveld P: Clinical relevance of P-glycoprotein expression in hematological malignancies. Leuk Res 18:233-243, 1994
106. Ross DD, Wooten PJ, Tong Y, Cornblatt B, Levy C, Sridhara R, Lee EJ, Schiffer CA: Synergistic reversal of multidrug-resistance phenotype in acute myeloid leukemia cells by cyclosporin A and Cremophor EL. Blood 83:1337-1347, 1995

107. Solomon MB, Meenem B, Heavey C, Paietta E: Differential modulation of P-glycoprotein, the multidrug resistance mediator, by Cremophor in normal *versus* leukemic hematopoietic cells. Blood 86:4385-4386, 1995
108. Tsujino I, Yamazaki T, Matsutani M, Sawada U, Horie T: Effect of Tween-80 on cell killing by etoposide in human lung adenocarcinoma cells. Cancer Chemother Pharmacol 43:29-34, 1999
109. Hacker M, Koeferl M, Hong CB: Cremophor and Emulphor induced alterations of serum lipids and lipoprotein electrophoretic patterns of dogs. Res Commun Chem Pathol Pharmacol 31:119-128, 1981
110. Bertoncello I, Kriegler AB, Woodcock DM, Williams B, Barber L, Nilsson SK: Haematopoietic radioprotection by Cremophor EL: a polyethoxylated castor oil. Int J Radiat Biol 67:57-64, 1995
111. Ennis M, Lorenz W, Gerland W, Heise J: Isolation of mast cells from rabbit lung and liver: comparison of histamine release induced by the hypnotics Althesin and propanidid. Agents Actions 20:219-222, 1987
112. Eschalier A, Lavarenne J, Burtin C, Renoux M, Chapuy E, Rodriguez M: Study of histamine release induced by acute administration of antitumor agents in dogs. Cancer Chemother Pharmacol 21:246-250, 1988
113. Shimomura T, Fujiwara H, Ikawa S, Kigawa J, Terakawa N: Effects of Taxol on blood cells. Lancet 352:541-542, 1998
114. Kearns CM, Gianni L, Egorin MJ: Paclitaxel pharmacokinetics and pharmacodynamics. Sem Oncol 22:16-23, 1995
115. Huizing MT, Keung AC, Rosing H, Van der Kuij V, Ten Bokkel Huinink WW, Mandjes IM, Dubbelman AC, Pinedo HM, Beijnen JH: Pharmacokinetics of paclitaxel and metabolites in a randomized comparative study in platinum-pretreated ovarian cancer patients. J Clin Oncol 11:2127-2135, 1993
116. Karlsson MO, Molnar V, Bergh J, Freijs A, Larsson R: A general model for time-dissociated pharmacokinetic-pharmacodynamic relationship exemplified by paclitaxel myelosuppression. Clin Pharmacol Ther 63:11-25, 1998
117. Minami H, Sasaki Y, Saijo N, Ohtsu T, Fujii H, Igarashi T, Itoh K: Indirect-response model for the time course of leukopenia with anticancer drugs. Clin Pharmacol Ther 64:511-521, 1998
118. Nygren P, Csóka K, Jonsson B, Fridborg H, Bergh J, Hagberg H, Glimelius B, Brodin O, Tholander B, Kreuger A, Lönnerholm G, Jakobsson Å, Olsen L, Kristensen J, Larsson R: The cytotoxic activity of Taxol in primary cultures of tumour cells from patients is partly mediated by Cremophor EL. Br J Cancer 71:478-481, 1995
119. Csóka K, Dhar S, Fridborg H, Larsson R, Nygren P: Differential activity of Cremophor EL and paclitaxel in patients' tumor cells and human carcinoma cell lines in vitro. Cancer 15:1225-1233, 1997
120. Fjällskog ML, Frii L, Bergh J: Paclitaxel induced cytotoxicity – the effects of Cremophor EL (castor oil) on two human breast cancer cell lines with acquired multidrug resistant phenotype and induced expression of the permeability glycoprotein. Eur J Cancer 30A:687-690, 1994
121. Fjällskog ML, Frii L, Bergh J: Is Cremophor EL, solvent for paclitaxel, cytotoxic? Lancet 342:873, 1993
122. Crispens CG, Sorenson JRJ: Treatment of reticulum cell carcinoma in SJL/J mice with Tween 80. Anticancer Res 8:1341-1344, 1988
123. Terzis AJ, Thorsen F, Heese O, Visted T, Bjerkvig R, Dahl O, Arnold H, Gundersen G: Proliferation, migration and invasion of human glioma cells exposed to paclitaxel (Taxol) in vitro. Br J Cancer 75:1744-1752, 1997

124. Liebmann JE, Cook JA, Lipschultz C, Teague D, Fisher J, Mitchell JB: Cytotoxic studies of paclitaxel (Taxol) in human tumour cell lines. Br J Cancer 68:1104-1109, 1993
125. Liebmann J, Cook JA, Mitchell JB: Cremophor EL, solvent for paclitaxel, and toxicity. Lancet 342:1428, 1993
126. Liebmann J, Cook JA, Lipschultz C, Teague D, Fisher J, Mitchell JB: The influence of Cremophor EL on the cell cycle effects of paclitaxel (Taxol) in human tumor cell lines. Cancer Chemother Pharmacol 33:331-339, 1994
127. Rose WC: Taxol: a review of its preclinical *in vivo* antitumor activity. Anticancer Drugs 3:311-321, 1992
128. Fujimoto S: Study for modifying activity of solvents on antitumor activity of paclitaxel. Gan To Kagaku Ryoho 21:665-670, 1994
129. Adler LM, Herzog TJ, Williams S, Rader JS, Mutch DG: Analysis of exposure times and dose escalation of paclitaxel in ovarian cancer cell lines. Cancer 74:1891-1898, 1994
130. Reinecke P, Corvin J, Gabbert HE, Gerharz CD: Antiproliferative effects of paclitaxel (Taxol) on human renal clear cell carcinomas in vitro. Eur J Cancer 33:1122-1129, 1997
131. Engblom P, Pulkkinen JO, Rantanen V, Hirvonen H, Kulmala J, Grènman R, Grènman S: Effects of paclitaxel with or without Cremophor EL on cellular clonogenic survival and apoptosis. Eur J Cancer 35:284-288, 1999
132. Gianni L, Munzone E, Capri A, Fulfaro F, Tarenzi E, Villani F, Spreafico C, Laffranchi A, Caraceni A, Martini C, Stefanelli M, Valagussa P, Bonadonna G: Paclitaxel by 3-hour infusion in combination with bolus doxorubicin in women with untreated metastatic breast cancer: high antitumor efficacy and cardiac effects in a dose-finding and sequence-finding study. J Clin Oncol 13:2688-2699, 1995
133. Gehl J, Boesgaard M, Paake T: Combined doxorubicin and paclitaxel in advanced breast cancer: Effective and cardiotoxic. Ann Oncol 7:687-693, 1996
134. Hortobagyi GN, Willey J, Rahman Z, Holmes FA, Theriault RL, Buzdar AU: Prospective assessment of cardiac toxicity during a randomized phase II trial of doxorubicin and paclitaxel in metastatic breast cancer. Semin Oncol 24:S17-65-S17-68, 1997
135. Sledge GW: Doxorubicin/paclitaxel combination chemotherapy for metastatic breast cancer: the Eastern Cooperative Oncology Group experience. Semin Oncol 22:123-125, 1995
136. Rowinsky EK, Gilbert MR, McGuire WP, Noe DA, Grochow LB, Forastiere AA, Clark B, Sartorius SE, Cornblath DR, Hendricks CB, Donehower RC: Sequences of taxol and cisplatin: a phase I and pharmacologic study. J Clin Oncol 9:1692-1703, 1991
137. Pronk LC, Schellens JHM, Planting AST, Van den Bent MJ, Hilkens PH, Van der Burg MEL, De Boer-Dennert M, Ma J, Blanc, C, Harteveld M, Bruno R, Stoter G, Verweij J: Phase I and pharmacologic study of docetaxel and cisplatin in patients with advanced solid tumors. J Clin Oncol 15:1071-1079, 1997
138. Schellens JHM, Ma J, Bruno R, Paccaly D, Vergniol JC, Planting AST, De Boer-Dennert M, Van der Burg MEL, Stoter G, Verweij J: Pharmacokinetics of cisplatin and Taxotere (docetaxel) and PBL DNA-adduct formation of cisplatin in the sequence Taxotere/cisplatin and cisplatin/Taxotere in a phase I/II study in solid tumor patients. ('Abstract') Proc Am Soc Clin Oncol 13:132, 1994

139. Ma J, Verweij J, Planting AST, De Boer-Dennert M, Van Ingen HE, Van der Burg MEL, Stoter G, Schellens JHM: Current sample handling methods for measurement of cisplatin-DNA-adducts in leukocytes in man lead to discrepant results in DNA-adduct levels and DNA-repair. Br J Cancer 71:512-517, 1995
140. De Vos AI, Nooter K, Verweij J, Loos WJ, Brouwer E, De Bruijn P, Ruijgrok EJ, Van der Burg MEL, Stoter G, Sparreboom A: Differential modulation of cisplatin accumulation in leukocytes and tumor cell lines by the paclitaxel vehicle Cremophor EL. Ann Oncol 8:1145-1150, 1997
141. Zhao F-K, Chuang LF, Israel M, Chuang RY: Cremophor EL, a widely used parenteral vehicle, is a potent inhibitor of protein kinase C. Biochem Biophys Commun 159:1359-1367, 1989
142. Van der Burg MEL, De Wit R, Logmans A, Van Lent M, Stoter G, Verweij J: Phase I study of weekly cisplatin and weekly or four weekly taxol in patients with advanced ovarian cancer. Eur J Cancer 33:S245, 1997
143. Huizing MT, Giaccone G, van Warmerdam LJC, et al: Pharmacokinetics of paclitaxel and carboplatin in a dose escalating and sequencing study in patients with non-small cell lung cancer: A European Cancer Centre (ECC) trial. J Clin Oncol 15:317-329, 1997
144. Van Warmerdam LCJ, Huizing MT, Giaccone G, Postmus PE, Ten Bokkel Huinink WW, Van Zandwijk N, Koolen MG, Helmerhorst TJ, Van der Vijgh WJ, Veenhof CH, Beijnen JH: Clinical pharmacology of carboplatin administered in combination with paclitaxel. Semin Oncol 24(2):97-104, 1997
145. Millward MJ, Webster LK, Rischin D, Ellis AG, Linahan BM, Maisano R, Toner GC, Stokes KH: Randomised, cross-over trial to determine the effect of Cremophor on the pharmacokinetics and pharmacodynamics or carboplatin-based therapy. ('Abstract') Ann Oncol 9(2):130, 1998
146. Kennedy MJ, Zahurak ML, Donehower R, Noe DA, Sartorius S, Chen TL, Bowling K, Rowinsky EK: Phase I and pharmacologic study of sequences of paclitaxel and cyclophosphamide supported by granulocyte colony-stimulating factor in women with previously treated metastatic breast cancer. J Clin Oncol 14:783-791, 1996
147. Kennedy MJ, Zahurak ML, Donehower RC, Noe DA, Grochow LB, Sartorius S, Chen TL, Bowling K, Duerr M, Rowinsky EK: Sequence-dependent hematological toxicity associated with 3-hour paclitaxel/cyclophosphamide doublet. Clin Cancer Res 4:349-356, 1998
148. Tolcher AW, Cowan KH, Noone MH, Denicoff AM, Kohler DR, Goldspiel BR, Barnes CS, McCabe M, Gossard MR, Zujewski J, O'Shaughnessy JA: Phase I study of paclitaxel in combination with cyclophosphamide and granulocyte colony-stimulating factor in metastatic breast cancer patients. J Clin Oncol 14:95-102, 1996
149. Ibrahim NK, Ellerhorst JA, Theriault RL, Rivera E, Esmaeli B, Legha SS, Ring SE, Soon-Shiong P, Benjamin RS, Hortobagyi GN: Phase I study of Cremophor-free, protein-stabilized, nanoparticle formulation of paclitaxel (Abi-007) in solid tumors. ('Abstract') Proc Am Ass Clin Oncol 609F, 2000
150. Fumoleau P, Tubiana-Hulin M, Soulié P, Delecroix V, Sistac F, Mefti F, Soulas F, Appia F, Lebecq A, Vergniol JC, Vernillet L, Riva A: A dose finding and pharmacokinetic (PK) phase I study of a new formulation of docetaxel (D) in advanced solid tumors. ('Abstract') Ann Oncol 9(Suppl 2):101, 1998

Chapter 6

Cremophor EL-mediated alteration of paclitaxel distribution in human blood: Clinical pharmacokinetic implications

Alex Sparreboom, Lia van Zuijlen, Eric Brouwer, Walter J Loos, Peter de Bruijn, Hans Gelderblom, Marrimuthoo Pillay, Kees Nooter, Gerrit Stoter, and Jaap Verweij

Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, The Netherlands

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ABSTRACT

We have determined the *in vitro* and *in vivo* cellular distribution of the antineoplastic agent paclitaxel (Taxol) in human blood and the influence of Cremophor EL (CrEL), the vehicle used for i.v. drug administration. In the absence of CrEL, the blood/plasma concentration ratio was 1.07±0.004 (mean±SD). The addition of CrEL, at concentrations corresponding to peak levels in plasma achieved after administration of paclitaxel (175 mg/m^2 i.v. over 3 h; i.e. 0.50%), resulted in a significant decrease in the concentration ratio to 0.690±0.005 ($P<0.05$). Kinetic experiments revealed that this effect was caused by reduced erythrocyte uptake of paclitaxel by polyoxyethyleneglycerol triricinoleate, the major compound present in CrEL. Using equilibrium dialysis, it was shown that the affinity of paclitaxel for tested matrices was in decreasing order CrEL > plasma > human serum albumin, with CrEL present at or above the critical micellar concentration (approx. 0.01%). Our findings in the present study demonstrate a profound alteration of paclitaxel accumulation in erythrocytes caused by a trapping of the compound in CrEL micelles, thereby reducing the free drug fraction available for cellular partitioning. It is proposed that the nonlinearity of paclitaxel plasma disposition in patients reported previously should be re-evaluated prospectively by measuring free drug fractions and whole blood/plasma concentration ratios.

INTRODUCTION

A substantial number of clinical studies with the antineoplastic agent paclitaxel (Taxol) have been performed to date, and have revealed a nonlinear disposition of the drug, which may have significant implications in that greater than expected increases in systemic exposure may result from a given increase in dose (1). For example, 3-h infusions of paclitaxel at 135 mg/m^2 resulted in a mean C_{max} of 3.3 μM and a mean AUC of 10.4 μM.h, while at 175 mg/m^2 the mean C_{max} and AUC values were 5.9 μM and 18.0 μM.h, respectively (2). Thus, a 30% increase in dose results in an 80% increase in C_{max} and a 75% increase in AUC. In line with results from dose escalation studies in humans, paclitaxel disposition in mice also increased disproportionally with doses increasing from 2 to 10 or 20 mg/kg (3). Subsequent studies have demonstrated that both distribution and

elimination appeared to be linear processes in the absence of CrEL, the formulation vehicle used for i.v. drug administration (4). Because plasma concentrations of CrEL in mice and humans are within the same range, it is very likely that CrEL also plays an important role in the nonlinear paclitaxel disposition in humans (5,6). At present, the biochemical mechanisms responsible for the dose-dependent interaction of CrEL with the pharmacokinetics of paclitaxel remain unclear. It has been suggested that CrEL might interfere with P-glycoprotein-mediated biliary drug secretion, thereby influencing paclitaxel elimination (7). Recent studies, however, indicate that drug-transporting P-glycoproteins are not essential *per se* for normal hepatobiliary secretion of paclitaxel (8,9), and that the disposition of CrEL itself limits the potential to modulate P-glycoprotein activity *in vivo* (10,11). In the present study, we have determined the *in vitro* and *in vivo* cellular distribution of paclitaxel in human blood in an effort to extend our insight into the role of CrEL in the pharmacokinetics of paclitaxel.

MATERIALS AND METHODS

Chemicals and Reagents

Paclitaxel powder and a sterile solution of paclitaxel formulated in a mixture of CrEL and dehydrated ethanol USP (1:1, v/v) at 6 mg/ml were obtained from the Bristol-Myers Squibb Company (Wallingford, CT). Stock solutions of paclitaxel at 1 mg/ml in dimethylsulfoxide were stored at -80°C, and diluted further in methanol-dimethylsulfoxide (1:1, v/v) prior to use. CrEL, pure castor oil and human serum albumin (fraction V) standards were obtained from Sigma Chemical Co. (St. Louis, MO), and Coomassie brilliant blue G-250 from Bio-Rad Laboratories (Munich, Germany) as a concentrated solution in 85% (w/v) phosphoric acid-ethanol (2:1, v/v). Five reversed-phase HPLC fractions of crude CrEL, each with progressively increased hydrophobicity were kindly donated by Dr. David Kessel (Wayne State University, Detroit, MI) (12). Miscellaneous chemicals and reagents were of the highest grade available and originated from Rathburn (Walkerburn, United Kingdom). Purified water was obtained by filtration and deionization using a Milli-Q-UF system (Millipore, Bedford, MA), and was used throughout.

Experimental Techniques

Samples of human blood were obtained from four healthy volunteers in glass vials containing lyophilized sodium heparin as anticoagulant, and were used within 1 hour after collection. Aliquots of the blood were centrifuged for 5 min at 3000 x *g* (4°C) to separate plasma, which was transferred to a clean polypropylene tube and then stored frozen at -20°C until used. Erythrocyte suspensions were prepared with freshly donated heparinized blood from which plasma and buffy coat were removed by aspiration. The cells were washed twice, each time with 3 ml ice-cold phosphate-buffer (containing 0.01 *M* potassium phosphate, 0.137 *M* sodium chloride and 2.7 m*M* potassium chloride) in the presence of 0.05% (w/v) glucose at pH 7.4, and resuspended in the same buffer to give the desired hematocrit. For preparation of platelet-rich plasma, heparinized blood was centrifuged at 200 x *g* for 20 min at room temperature to pack down erythrocytes and leukocytes. The resulting supernatant, with very low contamination from red cells and leukocytes, was used as platelet-rich plasma.

Blood/plasma ratio experiments were conducted using aliquots (2 ml) of the various matrices, which were placed in a 37°C-shaking water bath for 5 min prior to addition of paclitaxel in the presence or absence of CrEL. At the time required for maximal cellular paclitaxel uptake at equilibrium, duplicate aliquots of 250 μl were withdrawn from the incubation tubes (Eppendorf, Hamburg, Germany), and kept at -80°C for 5 min to achieve complete hemolysis. The remaining blood fraction was centrifuged, and the supernatant diluted 3-fold with drug-free human plasma to determine plasma concentrations as described above.

Equilibrium dialysis was accomplished at 37°C in a humidified atmosphere of 5% CO_2 using test cells made from 1.5-ml polypropylene microtubes (Eppendorf) carrying a 250-μl inside recess in the lids. The experiments were carried out with 250-μl aliquots of paclitaxel-containing (1 μg/ml) plasma or a 40 mg/ml solution of human serum albumin in 0.01 *M* phosphate buffer (pH 7.4) against an equal volume of the same buffer. Spectra/Por 3 dialysis tubing with a 12,500 molecular weight cut-off (Spectrum Medical, Kitchener, Canada) were soaked in 0.9% (w/v) sodium chloride solution prior to use. The time to reach equilibrium was determined in preliminary experiments and ranged between 5 and 24 h, depending on the composition of the fluid in the receptor compartment. The ratio of drug

concentrations measured by HPLC in the buffer and plasma or serum albumin solution after dialysis was taken as an estimate of the unbound (free) fraction of paclitaxel. Because the volume shift during dialysis was negligible (<10%), the results were used directly without applying a correction factor. In both the blood/plasma ratio and equilibrium dialysis experiments, it was confirmed that the total drug recovery from all the fractions was equal to the amount of paclitaxel added to blood, plasma or buffer mixtures.

Clinical Pharmacokinetics

The patient studied was a 65-year old female suffering from recurrent ovarian cancer after earlier cisplatin-containing chemotherapy. She received paclitaxel formulated in CrEL-ethanol at an absolute dose of 256 mg supplemented with 100 μCi of [*G*-^{3}H]paclitaxel (specific activity: 2.4 Ci/mmol; radiochemical purity: 99.7% by HPLC; Moravek Biochemicals, Inc., Brea, CA). The majority of the tritium is in the *m*- and *p*-positions of the aromatic rings, with minor amounts in the 10-, 3'-, and 2-position of the taxane ring system. The dose was administered as a 3-h i.v. infusion in 500 ml of sterile and isotonic sodium chloride, after a standard premedication comprising dexamethasone (10 mg i.v.), clemastine (2 mg i.v.) and ranitidine (50 mg i.v.). Blood samples (approx. 5 ml) were obtained in glass tubes with lyophilized sodium heparin at the following time points: immediately before dosing; at 0.5, 1, 1.5, 2, 2.5, and 3 h after start of infusion; and at 5, 15, 30, and 45 min, and 1, 2, 4, 6, 8, 12, and 24 h after end of infusion. One-ml aliquots of whole blood were stored frozen immediately at -20°C, and the remaining blood was centrifuged at 4000 x *g* for 5 min to separate plasma. The clinical protocol was approved by the Rotterdam Cancer Institute Ethics Board, and the patient signed informed consent prior to study entry. Concentrations of paclitaxel were measured using an isocratic reversed-phase HPLC method with UV detection at 230 nm as described previously (13). The analytical procedure for CrEL was based on a colorimetric dye-binding assay using Coomassie brilliant blue G-250 (14), with modifications as described (6).

RESULTS

In Vitro Partition in Blood Fractions

The time to equilibrium of paclitaxel between whole blood and plasma, determined by measuring the concentration on blood cells at several time points at 37°C, and calculating the rate of migration, was reached within 15 min of pooling cells and plasma. At this time point, a blood/plasma ratio of 1.07±0.004 (mean±SD) was observed, which was independent of the paclitaxel concentration, indicating that the cellular concentration is proportional to the concentration in plasma water. This shows that the rate of exchange between blood cells and plasma water is effectively instantaneous on the time scale of paclitaxel disposition, and that the steady-state load on cells represents approximately half of the total blood concentration. The addition of CrEL, at concentrations corresponding to peak levels in plasma achieved after administration of paclitaxel (175 mg/m^2 i.v. over 3 h; i.e. 0.50%) (10), resulted in a significant decrease in the blood/plasma concentration ratio to 0.690±0.005 ($P<0.05$).

Further experiments revealed a clear concentration-dependent effect of CrEL on this ratio, with maximal inhibition of cellular paclitaxel uptake at a CrEL total blood concentration of 1.0% (Table 1). At this concentration, the blood/plasma ratio averaged 0.625±0.008, suggesting a distribution of paclitaxel merely outside blood cells, with approximately 88% of total paclitaxel associated with the plasma fraction, assuming a mean hematocrit of 0.45. Incubation of paclitaxel in whole blood with castor oil, the major component in the CrEL vehicle prior to polyoxyethylation, showed no evidence of significant alterations in cellular uptake with a mean blood/plasma ratio of 1.23±0.17 ($P>0.05$). Experiments with reversed-phase HPLC fractions of CrEL indicated that the effect of the unfractionated vehicle was not observed with the hydrophilic components present in the first fractions, mainly containing polyethylene glycol and oxyethylated glycerol (Table 1), but are primarily caused by compounds from fraction 4, e.g. polyoxyethyleneglycerol triricinoleate along with fatty acid esters of polyethyleneglycol.

Table 1 Blood/plasma concentration ratio of paclitaxel in the absence and presence of various concentrations of CrEL, castor oil and HPLC fractions of CrEL*

Compound added (%)	Blood/plasma ratio	% change *vs.* Control	*P***
None	1.07±0.004		
CrEL (0.01)	1.09±0.009	+1.83	0.387
CrEL (0.05)	0.990±0.015	-.35	0.012
CrEL (0.10)	0.901±0.017	-15.8	0.003
CrEL (0.50)	0.690±0.005	-35.5	<0.0001
CrEL (1.0)	0.625±0.008	-41.6	<0.0001
Castor oil (0.50)	1.23±0.171	+13.0	0.061
CrEL fraction 1 (0.50)	1.06±0.008	-0.94	0.520
CrEL fraction 2 (0.50)	0.926±0.018	-13.5	0.043
CrEL fraction 3 (0.50)	0.763±0.055	-28.7	0.010
CrEL fraction 4 (0.50)	0.645±0.051	-39.7	0.003
CrEL fraction 5 (0.50)	0.943±0.039	-11.9	0.103

* Paclitaxel was used at an initial concentration of 1 μg/ml, and incubated in whole blood for 15 min at 37°C prior to fractionation and HPLC analysis. Ratio data are presented as mean values ± SD of (at least) triplicate measurements.

** Probability value *vs.* control (unpaired two-sided Student's *t* test).

The distribution of paclitaxel within an artificial mixture of washed erythrocytes, occupying 45% of the total volume, and buffer indicated red cell/buffer concentration ratios of 3.70±0.061 and 0.649±0.014 in the absence and presence of CrEL, respectively, further indicating that, in the latter case, paclitaxel is only distributed in the water phase (Table 2). The apparent contradiction between the low blood/plasma ratio and high accumulation into erythrocytes was shown to be due to the compensating effect of plasma protein binding, which has been observed previously for a diversity of other drugs (for an overview, see: Ref. 15), including paclitaxel (16). This is borne out by the finding that in the presence of plasma proteins, cellular partitioning of paclitaxel in erythrocytes was markedly impaired (Table 2). Surprisingly, paclitaxel accumulation in platelets was less affected by CrEL than erythrocyte uptake (Table 2), with a platelet/plasma ratio in the order of approximately 300, taking into account

the low total platelet volume in blood (approx. 4 µl/ml blood). As suggested recently by Wild and co-workers, this high degree of platelet uptake likely reflects a tight binding of paclitaxel to the intracellular tubulin/microtubule system, and has previously been shown to occur with other tubulin-interactive drugs, such as the vinca alkaloids (17).

Table 2 Distribution of paclitaxel in artificial binary mixture systems of the main blood fractions in the absence and presence of CrEL*

Binary system	CrEL (%)	Total/extracellular ratio	% change *vs.* control	*P*** **
*Erythrocytes/buffer***				
	None	3.70±0.061		
	0.50	0.649±0.014	-82.5%	<0.001
*Erythrocytes/plasma***				
	None	1.03±0.021		
	0.50	0.664±0.007	-35.5%	0.002
*Platelets/plasma****				
	None	1.33±0.037		
	0.50	1.26±0.024	-5.26%	0.018

* Paclitaxel was used at an initial concentration of 1 µg/ml, and incubated in whole blood for 15 min at 37°C prior to fractionation and HPLC analysis. Ratio data are presented as mean values ± SD of (at least) triplicate measurements.

** Hematocrit, approx. 0.45

*** Platelet count, approx. 750 x 10^9/L

**** Probability value *vs.* control (unpaired two-sided Student's *t* test).

Binding Interactions

In order to gain insight into the mechanism underlying this phenomenon, equilibrium dialysis was used to determine the effects of CrEL on the fraction unbound paclitaxel. Preliminary experiments verified that CrEL could not cross the semipermeable dialysis membrane, as was indicated by the absence of detectable levels in the buffer compartment after dialysis. As predicted by earlier experiments (5), paclitaxel was found to bind extensively to human plasma (89.1±0.595%, mean±SD; $n=3$) in

the absence of CrEL, with human serum albumin accounting for approximately 50% of the total binding. In the presence of CrEL (0.50%), however, a clear and statistically significant decrease in the free (unbound) drug fraction of up to 66% ($P=0.013$) and 92% ($P=0.002$) was seen for plasma and human serum albumin, respectively. Interestingly, equilibrium dialysis experiments performed with CrEL in the absence of any plasma proteins revealed that at a spiked concentration of 0.50%, a free drug fraction of only 0.044 was observed, suggesting that paclitaxel affinity for CrEL is substantially greater than for either plasma or human serum albumin. This binding affinity for CrEL was distinctly concentration-dependent, with no change in the free fraction of paclitaxel at CrEL concentrations below 0.01% [i.e., the amount of surfactant corresponding to the critical micellar concentration in aqueous solutions (18) and maximal binding (at a free drug fraction of approx. 0.017) in the presence of CrEL at a concentration of 1.0%. Equilibrium dialysis of paclitaxel-containing plasma against a buffer with CrEL resulted in a virtually quantitative shift of drug (90.1±1.09%) to the receptor fluid, further pointing to a preferential binding to the surfactant.

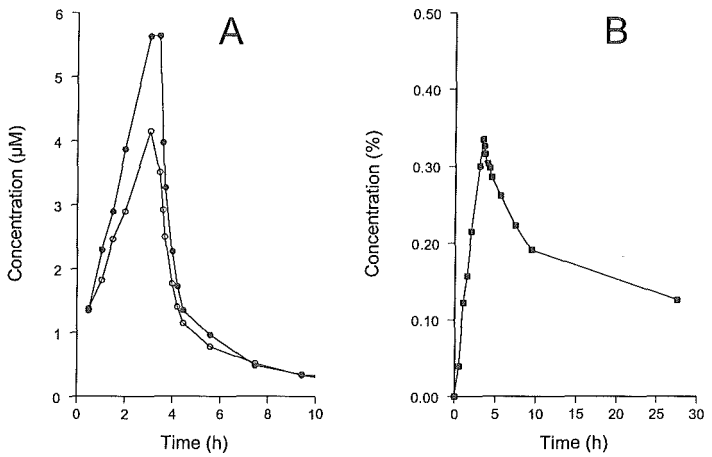

Figure 1 Concentration *vs.* time curves of paclitaxel in whole blood (○) and plasma (●) [*panel A*] and of CrEL (■) in plasma [*panel B*]. Pharmacokinetic data were obtained from a female patient receiving the drug formulated at 6 mg/ml in a mixture of CrEL-dehydrated ethanol USP (1:1, v/v) at an absolute dose of 256 mg.

In Vivo Pharmacokinetics

Figure 1 shows the logarithmic concentration-time curves of paclitaxel in whole blood and plasma (i.e. the unbound ultrafiltrate fraction *plus* the protein/CrEL bound blood fraction) [*panel A*], and of CrEL in plasma [*panel B*], after an i.v. dose of 256 mg to a female patient with advanced ovarian cancer. Similar to our *in vitro* partition experiments, a distinct CrEL-concentration dependency was noted for the whole blood/plasma concentration ratio and the unbound drug fraction (Table 3). This suggests that erythrocytes form a secondary transport system in whole blood, which becomes less significant as the CrEL concentration increases, and hence, the unbound drug concentration decreases. In all, these data appear to indicate that CrEL micelles act as the principal carrier of paclitaxel in the systemic circulation.

Table 3 CrEL concentration-dependent blood/plasma ratio and unbound paclitaxel fraction in plasma of a patient*

Time (h)**	CrEL (%)	Blood/plasma ratio	Unbound fraction
0.50	0.039	1.02	0.076
1.0	0.122	0.795	0.057
1.5	0.157	0.851	0.038
2.0	0.215	0.749	0.028
2.5	0.300	0.737	0.022
3.0	0.335	0.623	0.016

* Paclitaxel was administered as an i.v. infusion over a 3-h period at an absolute dose of 256 mg to a single female with advanced ovarian cancer.

** Sample collection time point after start of a 3-h i.v. infusion of paclitaxel.

DISCUSSION

In the present study we have shown that CrEL, the formulation vehicle used for i.v. drug administration, causes a profound alteration of paclitaxel accumulation in erythrocytes, by reducing the free drug fraction available for cellular partitioning. This effect was also observed in the

absence of plasma proteins, indicating that it was not caused by altered protein binding, or an increased affinity of paclitaxel for protein dissociation products which are produced by the action of CrEL on native lipoproteins (12,19). The data indicate that erythrocytes form a secondary transport system in whole blood, which becomes less significant as the CrEL concentration increases, and hence, the free drug fraction decreases. Current data suggest that drug trapping occurs in micelles composed primarily of polyoxyethyleneglycerol triricinoleate, and that these micelles act as the principal carrier of paclitaxel in the systemic circulation.

The findings of extensive red cell uptake of paclitaxel at low CrEL levels, and the high binding to plasma proteins and CrEL micelles may have a substantial impact on the interpretation of the drug's pharmacokinetic behavior that has not been fully appreciated. Previously, paclitaxel disposition in animals and humans has been reported to be nonlinear, with disproportional relationships between changes in the dose and the resulting plasma AUCs and peak plasma concentrations. This nonlinearity has been speculated to result from two separate saturable processes, one in distribution and one in elimination and both described by Michaelis-Menten kinetics, based on the usefulness of complex mathematical pharmacokinetic models to accurately describe plasma profiles of paclitaxel over a wide dosage range (1,2). The authors have generated estimates of the maximal process rate (i.e., V_{max}) and the paclitaxel concentration associated with $0.5xV_{max}$ (i.e., K_m), and observed that the K_m estimate for the distribution process is much smaller than that for elimination. This suggested that distribution should be the first process to exhibit saturation. Our present data indicate, that the resulting disproportional accumulation of paclitaxel in plasma, which is most pronounced with the drug administered by the 3-h infusion schedule (1), is related to a CrEL-mediated alteration of drug distribution within the circulation during paclitaxel infusion. This is exactly what is observed clinically in our patient, and is supported by our previous finding that, in spite of nonlinear paclitaxel kinetics in plasma, tissue levels of the parent drug and its known mono- and dihydroxylated metabolites in mice are linear with the dose administered (3). The paradox of disproportional increases in plasma concentrations and linear distribution processes in tissues is likely caused by the fact that the plasma comprises a relatively small fraction of the total volume available for paclitaxel distribution and that, simultaneously, the CrEL-drug complex is not stable

enough to substantially reduce the amount of drug that exists in the body in the active, diffusible, unbound form. In addition, it is also possible that the equilibrium between blood and tissues is not solely based on paclitaxel dissolved in the plasma water phase, but also on direct drug transport from loaded erythrocytes to tissues (15). This hypothetical transport may be of great importance for paclitaxel in cases of low concomitant CrEL levels that are associated with low dose schedules. Regardless of the tissues uptake processes, our current findings indicate that the operation of Michaelis-Menten kinetics, the postulated cause of nonlinear paclitaxel disposition in plasma, is not related to saturable tissue-binding or disproportional elimination kinetics, but appears to be an artifact caused by paclitaxel-dose related levels of CrEL in the blood. We are currently prospectively re-evaluating the linearity of paclitaxel pharmacokinetics in humans using different infusion schedules by measuring free drug fractions and blood/plasma ratios, based on the expectation that if the fraction of drug which is bound changes appreciably with concentration in the concentration range of interest, then the AUC of free (unbound) paclitaxel should be a linear function of the dose administered.

The existence of CrEL in blood as large polar micelles with a highly hydrophobic interior also raises the possibility of additional complexities in case of combination chemotherapy regimens with paclitaxel. For example, fluorescence studies on the interaction between anthracycline drugs and different surfactants indicated that daunorubicin, although relatively hydrophilic with an octanol-water partition ratio of approximately 3.5, is readily incorporated into CrEL micelles (18). Thus, in the systemic circulation, micellar incorporation of anthracyclines may result in altered cellular distribution and a concomitantly increased plasma concentration. In this regard, it is interesting to note that both paclitaxel (in the clinical formulation) and CrEL alone strongly affect the plasma pharmacokinetics of another anthracycline antineoplastic agent, doxorubicin, in both rodents and humans (7,20). Similarly, CrEL is known to decrease etoposide clearance in rats (21), and preliminary findings in humans treated with the combination of paclitaxel (≥175 mg/m^2 over 3 h) and i.v. etoposide confirmed these observations[1]. At present, we are investigating the effects of micellar incorporation in the biodistribution and pharmacokinetics of doxorubicin and etoposide, and the role of CrEL as a determinant in the increased incidence

[1] Sparreboom A and Verweij J, unpublished observations

and severity of hematological toxicity in clinical trials (7) with the combination of paclitaxel and doxorubicin[2].

REFERENCES

1. Kearns CM: Pharmacokinetics of the taxanes. Pharmacother 17:105S-109S, 1997
2. Gianni L, Kearns CM, Giani A, Capri G, Vigano L, Locatelli A, Bonadonna G, and Egorin MJ: Nonlinear pharmacokinetics and metabolism of paclitaxel and its pharmacokinetic/pharmacodynamic relationships in humans. J Clin Oncol 13:180-190, 1995
3. Sparreboom A, Van Tellingen O, Nooijen WJ, and Beijnen JH: Tissue distribution, metabolism and excretion of paclitaxel in mice. Anti-Cancer Drugs 9:78-87, 1996
4. Sparreboom A, Van Tellingen O, Nooijen WJ, and Beijnen JH: Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle Cremophor EL. Cancer Res 56:2112-2115, 1996
5. Sparreboom A, Van Tellingen O, Nooijen WJ, and Beijnen JH: Preclinical pharmacokinetics of paclitaxel and docetaxel. Anti-Cancer Drugs 9:1-17, 1998
6. Brouwer E, Verweij J, Hauns B, Loos WJ, Nooter K, Mross K, Stoter G, and Sparreboom A: Linearized colorimetric assay for Cremophor EL: Application to pharma-cokinetics after 1-hour paclitaxel infusions. Anal Biochem, 261:198-202, 1998
7. Gianni L, Vigano L, Locatelli A, Capri G, Giani A, Tarenzi E, and Bonadonna G: Human pharmacokinetic characterization and *in vitro* study of the interaction between doxorubicin and paclitaxel in patients with breast cancer. J Clin Oncol 15:1906-1915, 1997
8. Sparreboom A, Van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DKF, Borst P, Nooijen WJ, Beijnen JH, and Van Tellingen O: Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc Natl Acad Sci USA 94:2031-2035, 1997
9. Schinkel AH, Mayer U, Wagenaar E, Mol CA, Van Deemter L, Smit JJ, Van der Valk MA, Voordouw AC, Spits H, Van Tellingen O, Zijlmans JM, Fibbe WE, and Borst P: Normal viability and altered pharmacokinetics in mice lacking MDR1-type (drug-transporting) P-glycoproteins. Proc Natl Acad Sci USA 94:4028-4033, 1997
10. Sparreboom A, Verweij J, Van der Burg MEL, Loos WJ, Brouwer E, Vigano L, Locatelli A, De Vos AI, Nooter K, Stoter G, and Gianni L: Disposition of Cremophor EL in humans limits the potential for modulation of the multidrug resistance phenotype *in vivo*. Clin Cancer Res 4:1937-1942, 1998
11. Van Asperen J, Sparreboom A, Van Tellingen O, and Beijnen JH: Cremophor EL masks the effect of MDR1a P-glycoprotein on the plasma pharmacokinetics of paclitaxel in mice. *In:* J. van Asperen, The pharmacological role of drug-transporting P-glycoproteins in mice, PhD thesis, Ch. 3.2, pp.109-124 Utrecht University, 1998

[2] Brouwer E, Nooter K, Verweij J, Loos WJ, Stoter G and Sparreboom A: Modulation of doxorubicin uptake in peripheral blood leukocytes by Cremophor EL, manuscript in preparation

12. Kessel D, Woodburn K, Decker D, and Sykes E: Fractionation of Cremophor EL delineates components responsible for plasma lipoprotein alterations and multidrug resistance reversal. Oncol Res 7:207-212, 1995
13. Sparreboom A, De Bruijn P, Nooter K, Loos WJ, Stoter G, and Verweij J: Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. J Chromatogr 705:159-164, 1998
14. Sparreboom A, Loos WJ, Verweij J, De Vos AI, Van der Burg MEL, Stoter G, and Nooter K: Quantitation of Cremophor EL in human plasma samples using a colorimetric dye-binding microassay. Anal Biochem 255:171-175, 1998
15. Highley MS, and De Bruijn EA: Erythrocytes and the transport of drugs and endogenous compounds. Pharmaceut Res 13:186-195, 1996
16. Kumar GN, Walle UK, Bhalla KN, and Walle T: Binding of taxol to human plasma, albumin and α_1-acid glycoprotein. Res Commun Chem Pathol Pharmacol 80:337-342, 1993
17. Wild MD, Walle UK, and Walle T: Extensive and saturable accumulation of paclitaxel by the human platelet. Cancer Chemother Pharmacol 36:41-44, 1995
18. Kessel D: Properties of Cremophor EL micelles probed by fluorescence. Photochem. Photobiol 56:447-451, 1992
19. Sykes E, Woodburn K, Decker D, and Kessel D: Effects of Cremophor EL on distribution of Taxol to serum lipoproteins. Br J Cancer 70:401-404, 1994
20. Webster LK, Cosson EJ, Stokes KH, and Millward MJ: Effect of the paclitaxel vehicle, Cremophor EL, on the pharmacokinetics of doxorubicin and doxorubicinol in mice. Br J Cancer 73:522-524, 1996
21. Ellis AG, Crinis NA, and Webster LK: Inhibition of etoposide elimination in the isolated perfused rat liver by Cremophor EL and Tween 80. Cancer Chemother Pharmacol 38:81-87, 1996

Chapter 7

Interrelationships of paclitaxel disposition, infusion duration and Cremophor EL kinetics in cancer patients

Lia van Zuijlen[1], Luca Gianni[2], Jaap Verweij[1], Klaus Mross[3], Eric Brouwer[1], Walter J Loos[1] and Alex Sparreboom[1]

[1]Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, The Netherlands. [2]Division of Medical Oncology, Istituto Nazionale Tumori, Milan, Italy. [3]Department of Medical Oncology, Tumor Biology Center, Albert Ludwigs University, Freiburg, Germany.

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Cremophor EL (CrEL) is a castor oil surfactant used as a vehicle for formulation of a variety of poorly water-soluble agents, including paclitaxel. Recently, we found that CrEL can influence the *in vitro* blood distribution of paclitaxel by reducing the free-drug fraction, thereby altering drug accumulation in erythrocytes. The purpose of this study was to investigate the clinical pharmacokinetics of CrEL, and to examine interrelationships of paclitaxel disposition, infusion duration and CrEL kinetics. The CrEL plasma clearance, studied in 17 patients for a total of 28 courses, was time-dependent and increased significantly with prolongation of the infusion duration from 1 to 3 to 24 h ($P<0.03$). An indirect response model, applied based on use of a Hill function for CrEL concentration-dependent alteration of *in vivo* blood distribution of paclitaxel, was used to fit experimental data of the 3-h infusion ($r^2=0.733$; $P=0.00001$). Simulations for 1- and 24-h infusions using predicted parameters and CrEL kinetic data revealed that both short and prolonged administration schedules induce a low relative net change in paclitaxel blood distribution. Our pharmacokinetic/pharmacodynamic model demonstrates that CrEL causes disproportional accumulation of paclitaxel in plasma in a 3-h schedule, but is unlikely to affect drug pharmacokinetics in this manner with alternative infusion durations.

INTRODUCTION

Paclitaxel, a natural product first isolated from the Western Yew tree, *Taxus brevifolia*, acts by stabilizing the microtubule cytoskeleton, and possesses antitumor activity against a relatively broad spectrum of malignancies (reviewed in 1). The variability in clinically tested treatment schedules of this drug is enormous, ranging from short i.v. infusions of less than 1 h to 10-day or even 7-week continuous-infusion administrations (2-4), with large differences in experienced toxicity profiles and with fortuitous implications for the pharmacokinetics of paclitaxel. The latter refers to the experience of nonlinear plasma disposition, which appears to be most pronounced with the drug administered as a 3-h infusion, the most frequently used schedule nowadays (5-7). The mechanisms underlying the nonlinearity of paclitaxel disposition are not yet completely understood, although it has been shown that distribution and elimination appear to be linear in the absence of CrEL, the nonionic castor oil vehicle used in the clinical formulation (8,9). We have shown recently, that *in vitro* CrEL

causes a profound alteration of paclitaxel accumulation in erythrocytes in a concentration-dependent manner, by reducing the free drug fraction available for cellular partitioning (10). In this report, we (*i*) evaluated the importance of this *in vitro* observation for cancer patients treated with i.v. administered paclitaxel, (*ii*) describe the clinical pharmacokinetics of CrEL administered with a 1-h, 3-h and 24-h i.v. paclitaxel infusion, and (*iii*) propose a pharmacokinetic/pharmacodynamic model to describe the pharmacokinetic profiles of CrEL for these three treatment groups and the relationship between CrEL plasma concentrations and the paclitaxel blood distribution.

MATERIALS AND METHODS

Patients and Treatment

A total of 17 patients enrolled in Phase I trials performed in Rotterdam (the Netherlands; $n=5$), Milan (Italy; $n=6$) or Freiburg im Breisgau (Germany; $n=6$) was studied during treatment with paclitaxel as a single agent for a variety of malignant solid tumors refractory to standard therapy. They represented either newly recruited patients (Rotterdam) or those for which plasma samples had been previously collected and detailed clinical profiles documented elsewhere (5,11). Paclitaxel was administered as a 1-h (6 courses), 3-h (13 courses) or 24-h (9 courses) i.v. infusion every 3 weeks at doses ranging from 135-225 mg/m^2. The drug was formulated in a mixture of CrEL and dehydrated ethanol USP (1:1, v/v) (Taxol; provided by Bristol-Myers Squibb) and diluted further into isotonic sodium chloride or dextrose prior to dosing.

Sample Collection and Drug Analysis

For CrEL, pharmacokinetic evaluation was performed in all patients for a total of 28 courses. Heparinized blood samples of 5 mL were obtained at the following time points: before dosing and at 0.5, 1, 4, and 24 h after infusion (1-h schedule) or at 1, 2, 3, 3.08, 3.25, 3.5, 3.75, 4, 5, 7, 9, 11, 15, and 24 h after infusion (3-h schedule) or at 1, 22, 23, 23.92, 24, 24.08, 24.15, 24.5, 25, 26, 27, 30, 36, and 45 h after infusion (24-h schedule). One-mL aliquots of whole blood were snap-frozen at -20°C (3-h schedule only at 1, 2, and 3 h after infusion), and plasma was separated by centrifugation for 5 min at 4000 × *g* (all schedules).

Concentrations of paclitaxel in whole blood and plasma were determined using a validated isocratic reversed-phase high-performance liquid chromatography (HPLC) procedure with UV detection ($\lambda = 230$ nm), as reported (12). The analytical method for CrEL was based on binding of the surfactant to Coomassie Brilliant Blue G-250 in protein-free plasma extracts by measuring the change in ratio of absorbances at 595 nm over 450 nm (13,14).

Pharmacokinetics

The CrEL plasma concentration-time curves were analyzed using the software package Siphar v4 (SIMED, Créteil, France) by determination of slopes and intercepts of the plotted curves with a bi-exponential function (15). Initial parameter estimates were obtained by an automated peeling-algorithm procedure, with an integrated numerical algorithm based on the Powell method and a weighted-least squares operation. The area under the plasma concentration-time curve of CrEL was determined on the basis of the best fitted curves from time zero up to the last sampling point with detectable levels (AUC_{0-t}) (lower limit of quantitation: 0.5 μL/mL). Extrapolation to calculate $AUC_{0-\infty}$ was not performed because based on the limited sampling procedure, half-life estimates of the terminal disposition phase [reported previously to be >80 h with 3-h paclitaxel infusions] (15) and subsequent determinations of total clearance and volume of distribution could not be done with sufficient precision [i.e. the extrapolated part represents a significant amount (>20%) of the $AUC_{0-\infty}$]. Therefore, the apparent plasma clearance of CrEL was estimated by dividing the delivered volume (expressed in mL per m^2 body-surface area) by the observed AUC_{0-t}, to allow evaluation of the impact of infusion duration on the pharmacokinetic behavior. Peak plasma concentrations (C_{max}) of CrEL and paclitaxel were determined graphically by visual inspection from scattered plots of concentration-time profiles.

For pharmacodynamic analysis, paclitaxel concentrations were considered as 'total paclitaxel', because an acetonitrile/*n*-butyl chloride extraction was applied to separate paclitaxel from its binding proteins and CrEL micelles for the assay (12). Based on our previous *in vitro* partitioning experiments (10), we assumed that CrEL can decrease the unbound fraction in serum *in vivo* with subsequently decreased erythrocyte binding in a concentration-dependent manner, whereas the elimination rate of paclitaxel

from the central compartment remains unchanged (16). An indirect response model based on the premise that inhibition of paclitaxel uptake by erythrocytes will change the blood to plasma [(B/P)] concentration ratio was applied as follows:

$$d\,(B/P)\ \text{ratio} / d\,t = (B/P)_0 + (B/P)_{min} \bullet \{[C_{CrEL}]^{\gamma} / ([C_{CrEL}]^{\gamma} + [C_{CrEL}]_{50}{}^{\gamma})\} \quad \text{(eq. 1)}$$

where $(B/P)_0$ is the paclitaxel blood:plasma concentration ratio in the absence of CrEL, $(B/P)_{min}$ is the minimum paclitaxel blood:plasma ratio that can be achieved, $[C_{CrEL}]$ is the CrEL plasma concentration, which is a forcing function defined by the kinetic analysis, $[C_{CrEL}]_{50}$ is the CrEL plasma concentration producing 50% of maximum decrease of the paclitaxel blood to plasma ratio, and γ is the slope coefficient for the Hill function describing the sigmoidity of the fit.

Statistics

All pharmacokinetic parameters are reported as mean values ± SD. Intrapatient differences between the first and second course in dose-normalized parameters were assessed by the coefficient of variation, expressed as the ratio of the SD and the observed mean. Pharmacokinetic variability between the various dose levels and infusion duration was evaluated by the Kruskal-Wallis statistic and Dunn's multiple comparison test, if necessary. Pharmacodynamic parameters [$(B/P)_0$, $(B/P)_{min}$, $[C_{CrEL}]_{50}$ and γ] were estimated by the maximum likelihood method using the Siphar program, and the model evaluated for goodness of fit by minimization of sums of squared residuals and by reduction of the estimated coefficient for fitted parameters. Significance of the relationship was assessed by construction of contingency tables with subsequent χ^2 analysis. Statistical analysis was performed using the NCSS software (v5.X; Dr. Jerry Hintze, Kaysville, UT). All test were two-tailed, and the level of significance was set at $\alpha = 0.05$.

RESULTS

CrEL Kinetics

Mean pharmacokinetic parameters of CrEL at each of the dose levels tested are listed in Table 1 as a function of the infusion duration. In all cases,

CrEL plasma concentrations increased progressively and mono-exponentially throughout the infusion in a first-order fashion (Fig. 1), most likely because the fractional term associated with the terminal phase is approximately 0.97 (9), causing spontaneous CrEL distribution in the circulation relative to elimination. Both C_{max}, observed immediately after cessation of the infusion, and AUC_{0-t} were significantly higher at an increased dose with all three schedules ($P<0.04$). The plasma clearance was independent of the dose within each infusion duration ($P>0.44$), although with the 24-h infusion there was a trend toward slower clearance with an increase in dose (Table 1).

Table 1 Effect of dose and infusion duration on CrEL pharmacokinetics*

T_{inf}** (h)	Paclitaxel dose (mg/m^2)	CrEL dose (mL/m^2)	*n*	C_{max} (μL/mL)	AUC_{0-t} (μL.h/mL)	CL (mL/h/m^2)
1	150	12.5	1	4.30	48.4	258
	175	14.6	2	5.30, 5.70	81.6, 89.9	162, 179
	225	18.8	3	5.37±0.96	84.6±4.38	223±11.8
3	135	11.3	3	2.32±0.34	28.9±2.40	392±32.5
	175	14.6	5	3.43±0.47	46.3±7.55	321±55.7
	225	18.8	5	4.46±0.75	59.4±10.0	322±49.2
24	135	11.3	3	0.69±0.14	22.4±4.63	520±121
	175	14.6	6	1.43±0.39	43.1±12.3	364±115

* Data were obtained from cancer patients after treatment courses of 1-, 3-, or 24-h i.v. infusion of paclitaxel at dose levels ranging from 135 to 225 mg/m^2. The parameters were calculated by non-compartmental analysis, and data represent mean values ± SD.

** T_{inf}, infusion duration of drug administration; *n*, number of treatment courses studied; C_{max}, peak plasma concentrations; AUC_{0-t}, area under the plasma concentration-time curve up to the last time point with detectable levels; CL, apparent plasma clearance.

However, a clear time-dependency was noted in CrEL pharmacokinetics with the plasma clearance increasing significantly with an increase in infusion time [*e.g.* $CL_{1h}=223\pm11.8$ *vs* $CL_{3h}=322\pm49.2$ mL/h/m^2; $P=0.02$ (at 225

mg/m^2) or CL_{3h} = 392±32.5 *vs* CL_{24h} = 520±121 mL/h/m^2; P = 0.03 (at 135 mg/m^2)]. Accumulation effects of multiple dosing were not observed in any patient that had blood samples collected on two or three separate consecutive occasions (3-weekly schedules of 3-h or 24-h i.v. infusion), and the coefficient of variation in observed AUC values was typically below 10%.

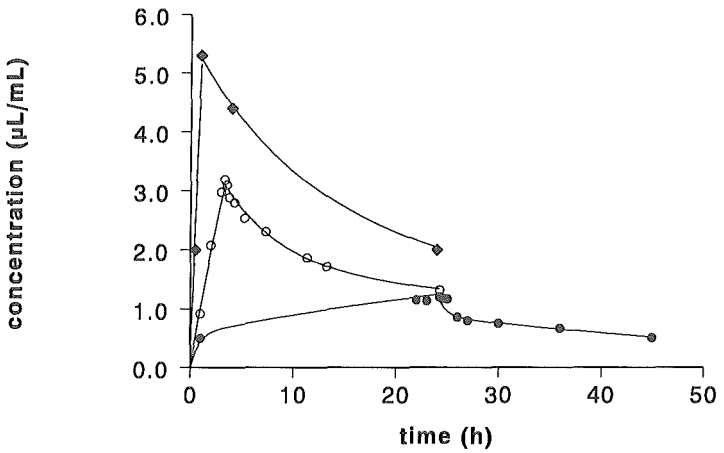

Figure 1 Representative plasma concentration-time curves of CrEL in 3 patients treated with paclitaxel at a dose level of 175 mg/m^2 given as a 1-h (◆), 3-h (○) or 24-h i.v. infusion (●). In all cases, paclitaxel was formulated at 6 mg/mL in a mixture of CrEL-dehydrated ethanol USP (1:1, v/v), and diluted in 5% dextrose or 0.9% sodium chloride solution within 3 h prior to dosing. Pharmacokinetic curves were fitted to a bi-exponential equation assuming a 2-compartmental model for distribution and elimination phases using the Siphar v4 software package.

Paclitaxel Blood Partitioning

The concentration of paclitaxel in plasma and blood after a 3-h i.v. infusion is shown in Fig. 2 as a function of the dose administered. At the lowest dose tested (i.e. 135 mg/m^2), the blood:plasma ratio was independent of the concentration (0.99±0.12 to 0.93±0.13; P>0.15). Increasing the dose to 175 and 225 mg/m^2, however, resulted in significant decreases in blood:plasma ratios toward the end of infusion to 0.68±0.08, at which approximately 90% of drug is distributed outside blood cells. To demonstrate

the comparative relationship between CrEL concentrations and alterations in the paclitaxel blood:plasma ratio, a simulation based on the proposed pharmaco-kinetic/pharmacodynamic model (eq. 1) is presented in Fig. 3.

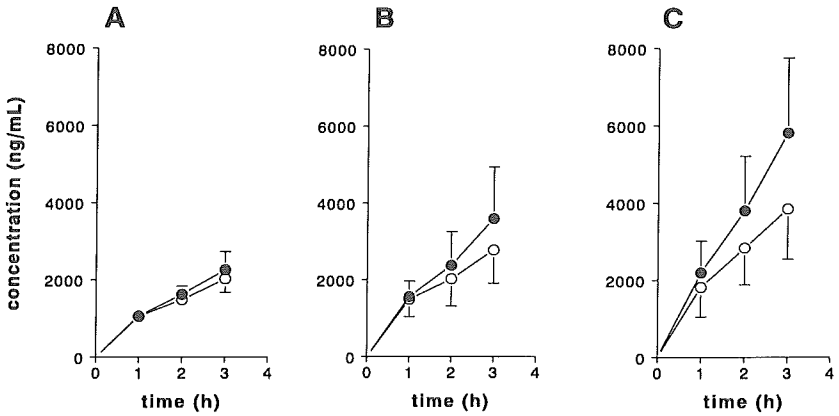

Figure 2 Plasma (●) and blood (○) concentration-time plots of paclitaxel in patients treated with a 3-h i.v. infusion of paclitaxel at dose levels of 135 (*panel A*), 175 (*panel B*) or 225 mg/m^2 (*panel C*). Data represent mean values (symbols) ± SD (error bars).

Using the computer fitting (r^2=0.733; P=0.00001), the paclitaxel blood:plasma ratio in the absence of CrEL was estimated as 1.09, which is in excellent agreement with our previous *in vitro* finding of 1.07±0.004 at a paclitaxel concentration of 1 μg/mL (10). After increasing CrEL levels, the blood:plasma ratio gradually decreased to a value in which paclitaxel is distributed only in the water phase (corresponding to an erythrocyte:plasma water ratio of approx. 0.65), with an estimated $[C_{CrEL}]_{50}$ value of 2.297 μL/mL and Hill coefficient γ of 1.35. Simulations for the patients receiving 1-h or 24-h i.v. infusions using the predicted parameter values and superposition of the pharmacokinetic data of CrEL are shown in Fig. 3. Based on the proposed model, a change in blood:plasma ratio during 24-h infusions is predicted between 0.911 and 1.01 (up to 16.5% decrease of initial value). The 1-h infusions have the lowest relative net change in the ratio (between 0.545 and 0.650), despite having the highest CrEL concentrations. These results show that only with 3-h i.v. infusions substantial disproportional accumulation of

paclitaxel in plasma, resulting from CrEL-mediated alteration of blood distribution, is likely to occur.

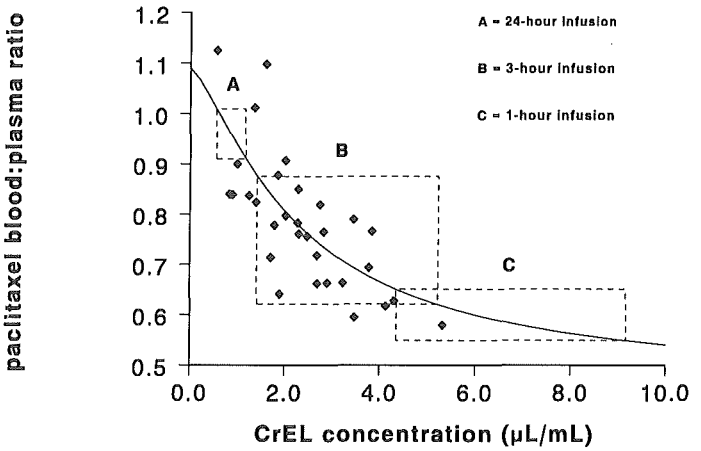

Figure 3 Relationship between the blood:plasma concentration ratio of paclitaxel and the observed plasma concentration of CrEL during 3-h i.v. infusions of paclitaxel. Data were obtained from 5 patients (13 courses) treated at 135, 175, or 225 mg/m^2. The solid line represents a fit of the data with a sigmoidal minimum-effect model using 1/(CrEL concentration) weighting and an integrated numerical algorithm based on the Powell method. Boxes with dotted lines indicate the range of CrEL peak levels associated with i.v. paclitaxel administration over 1, 3, or 24 h at dose levels ranging between 135 and 225 mg/m^2 (see Table 1), and the predicted paclitaxel blood: plasma concentration ratio obtained by interpolation of the fitted model.

DISCUSSION

The present study was performed to investigate the clinical pharmacokinetics of CrEL administered over 1-, 3-, and 24-h i.v. infusions of paclitaxel and to examine the interrelationships of paclitaxel disposition, infusion duration and CrEL kinetics. The CrEL plasma clearance was found to be time-dependent and increased significantly with prolongation of the infusion duration from 1 to 3 to 24 h. This observation is at odds with most literature concerning CrEL pharmacokinetics following 3-h paclitaxel

administration, showing linear increases in both C_{max} and AUC with increasing dose (15,17), although some other authors have reported slower CrEL clearances at the lower end of paclitaxel dose ranges when using body-surface area-based dose-calculation methods (18). Our results are in line with those found using an HPLC assay for CrEL described recently (19). The processes involved in CrEL elimination are not yet fully discerned, although preliminary findings indicate that the cumulative urinary excretion is very low, accounting for less than 0.1% of the administered dose, despite its relatively high hydrophilic nature (20). Given the limited volume of distribution of CrEL (15) and the prominent role of serum carboxylesterase-mediated metabolic transformation in the elimination of the related surfactant polysorbate 80 (21), it is possible that the underlying time-dependent mechanism is that of capacity-limited CrEL metabolism within the systemic circulation. Regardless of the processes affecting the pharmacokinetic profile of CrEL, our findings indicate that CrEL disposition is highly dependent on infusion duration, with disproportional increases in systemic exposure being associated with shortening of infusion. This finding may be of particular importance in view of the potential role of CrEL in the frequent presence of acute hypersensitivity reactions associated with clinical use of paclitaxel, which are characterized by dyspnea, flushing, rash, and generalized urticaria (22). Despite extensive premedication with high-dose corticosteroids and H_1/H_2-receptor antagonists, the overall frequency of minor reactions is estimated as high as 44%, with major reactions, necessitating discontinuation of paclitaxel therapy, still occurring in approximately 1.5 to 3% of patients (23,24). Consistent with the pharmacokinetic selectivity of CrEL for the plasma water phase (13), Szebeni et al recently postulated a novel concept suggesting that an important contributing mechanism to hypersensitivity reactions from paclitaxel is complement activation due to binding of naturally occurring anti-cholesterol antibodies to the hydroxyl-rich surface of CrEL micelles (25). Based on an elegant series of *in vitro* experiments, it was shown that CrEL-induced complement activation in human serum was clearly concentration dependent with a minimum activating CrEL level in the order of 2 μL/mL (25), concentrations readily achieved clinically in plasma (Fig. 1). It should be mentioned in this context that the current trend to pursue shorter schedules of paclitaxel administration in an attempt to simplify complex multidrug regimens, can have a much greater impact on complement-activating CrEL levels at the site of infusion and on total CrEL exposure than would be

expected in case of time-independent pharmacokinetics. Indeed, a recent clinical pilot experience in attempting to administer paclitaxel at 175 mg/m^2 by 30- or 45-min i.v. infusion indicated symptoms and signs of significant adverse hypersensitivity reactions in all patients treated within 15 min after start of drug administration, even in the presence of standard antiallergenic premedication (2).

Consistent with our previous *in vitro* observations (10), we found in the present study a distinct CrEL-concentration dependency of the paclitaxel blood:plasma ratio during 3-h paclitaxel infusions (Fig. 2). This finding demonstrates for the first time that CrEL affects the clinical pharmacokinetics of paclitaxel by a disproportional accumulation process in plasma, and lends further support to our prior supposition that the ability of CrEL to modulate the murine disposition of various compounds, including paclitaxel and doxorubicin (9,26,27) may have important clinical ramifications. Mathematical models able to accurately and completely describe paclitaxel concentration-time profiles have shown that the nonlinear drug disposition is most likely dictated initially by saturable distribution kinetics (5,6), and is particularly evident with the 3-h infusion schedule. This is also in keeping with results obtained in mice showing that disproportional plasma levels with an increase in dose did not reflect higher tissue levels (8), and with evidence obtained from trials with prolonged (≥24-h) i.v. infusions indicating that the nonlinearity of paclitaxel pharmacokinetics was less evident (5,28,29). Using an indirect response model applied in the pharmacokinetic/pharmacodynamic analysis of CrEL concentrations and corresponding blood distribution of paclitaxel (Fig. 3), a change in blood:plasma ratio during 24-h infusions between 0.911 and 1.01 (up to 16.5% decrease of initial value) was predicted, indicating a minor effect of CrEL on paclitaxel distribution. The model also provided additional information on the disposition characteristics of shorter schedules, and shed light on some important mechanistic aspect of the paclitaxel-CrEL interaction. Since the estimated Michaelis-Menten constant (K_m) for paclitaxel distribution was previously found to be in the order of paclitaxel peak plasma concentrations following 24-h infusions (135 to 175 mg/m^2) (6), dose and infusion schedules associated with high drug concentrations relative to K_m (approx. 270 ng/mL) were expected to display the most distinct signs of nonlinearity. In contrast, however, no evidence of nonlinear paclitaxel disposition has been described in any trial using 1-h infusion schedules at dose levels associated with distinct deviation of linearity using 3-h schedules

(i.e. between 135 and 225 mg/m^2) (11,30-34). This paradox can be understood in view of the fact that the high CrEL concentrations achieved early into the infusion produce only a low relative net change in the paclitaxel blood:plasma ratio (between 0.545 and 0.650), although the absolute quantitative effect is large already at low-dose input. Measurement of plasma concentrations of CrEL may, therefore, not be the exclusive indicator of the importance of the vehicle-drug interaction, but rather the combination of dose delivered and input-rate applied.

CONCLUSION

Our current study demonstrates that the pharmacokinetic behavior of the paclitaxel vehicle CrEL is subject to considerable variability depending on the duration of i.v. infusion for drug administration. We have identified CrEL-mediated alterations of the paclitaxel blood:plasma ratio as a major contributing mechanism of the nonlinear paclitaxel disposition observed in patients with 3-h infusion schedules. In addition, a pharmacokinetic/-pharmacodynamic model could accurately describe the biological events for CrEL kinetics and subsequent change in blood:plasma ratio of paclitaxel and predict the interrelationships between the parameters under diverse dosing conditions. Since CrEL is increasingly used as a formulation vehicle in pharmaceutical preparations of novel agents, recognition of the complex interplay between kinetic profiles of CrEL and paclitaxel is of particular significance. The present data should therefore be of importance as a guide to better understand the role of this drug vehicle in the future.

REFERENCES

1. Verweij J, Clavel M, Chevalier B: Paclitaxel (Taxol) and docetaxel (Taxotere): not simply two of a kind. Ann Oncol 5:495-505, 1994
2. Tsavaris NB, Kosmas C: Risk of severe acute hypersensitivity reactions after rapid paclitaxel infusion of less than 1-h duration. Cancer Chemother Pharmacol 42:509-11, 1998
3. Schade RJ, Pisters KM, Huber MH, *et al.*: Phase I study of paclitaxel administered by ten-day continuous infusion. Invest New Drugs 16:237-43, 1999
4. Dowell JE, Sinard R, Yardley DA, et al: Seven-week continuous-infusion paclitaxel concurrent with radiation for locally advanced non-small cell lung and head and neck cancers. Semin Radiat Oncol 2(S1):97-101, 1999

5. Gianni L, Kearns CM, Giani A, *et al.*: Nonlinear pharmacokinetics and metabolism of paclitaxel and its pharmacokinetic/pharmacodynamic relationships in humans. J Clin Oncol 13:180-190, 1995
6. Kearns CM, Gianni L, Egorin MJ: Paclitaxel pharmacokinetics and pharmacodynamics. Sem Oncol 22 (S6):16-23, 1995
7. Bhalla KN, Kumar GN, Walle UK, *et al.*: Phase I and pharmacologic study of a 3-hour infusion of paclitaxel followed by cisplatinum and 5-fluorouracil in patients with advanced solid tumors. Clin Cancer Res 5:1723-1730, 1999
8. Sparreboom A, Van Tellingen O, Nooijen WJ, Beijnen JH: Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle Cremophor EL. Cancer Res 56:2112-15, 1996
9. Cabanes A, Briggs KE, Gokhale PC, Treat JA, Rahman A: Comparative *in vivo* studies with paclitaxel and liposome-encapsulated paclitaxel. Int J Oncol 12:1035-49, 1998
10. Sparreboom A, Van Zuijlen L, Brouwer E, *et al.*: Cremophor EL-mediated alteration paclitaxel distribution in human blood: clinical pharmacokinetic implications. Cancer Res 59:1454-57, 1999
11. Mross K, Hauns B, Häring B, *et al.*: Clinical phase I study with one-hour paclitaxel infusion. Ann Oncol 9:569-72, 1998
12. Sparreboom A, De Bruijn P, Nooter K, Loos WJ, Stoter G, Verweij J.: Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. J Chromatogr B 705:159-64, 1998
13. Sparreboom A, Loos WJ, Verweij J, *et al.*: Quantitation of Cremophor EL in human plasma samples using a colorimetric dye-binding microassay. Anal Biochem 255:171-75, 1998
14. Brouwer E, Verweij J, Hauns B, *et al.*: Linearized colorimetric assay for Cremophor EL: application to pharmacokinetics after 1-hour paclitaxel infusions. Anal Biochem 261:198-202, 1998
15. Sparreboom A, Verweij J, Van der Burg MEL, *et al.*: Disposition of Cremophor EL in humans limits the potential for modulation of the multidrug resistance phenotype *in vivo*. Clin Cancer Res 4:1937-42, 1998
16. Sparreboom A, Van Tellingen O, Nooijen WJ, Beijnen JH: Tissue distribution, metabolism and excretion of paclitaxel in mice. Anticancer Drugs 9:78-87, 1996
17. Rischin D, Webster LK, Millward MJ, *et al.*: Cremophor EL pharmacokinetics in patients receiving 3- 6-, and 24-hour infusions of paclitaxel. J Natl Cancer Inst 85:1685-90, 1996
18. Meerum Terwogt JM, Van Tellingen O, Nannan Panday VR, *et al.*: Cremophor EL pharmacokinetics in a phase I study of paclitaxel (Taxol) and carboplatin in non-small cell lung cancer patients. In: JM Meerum Terwogt. *Clinical Pharmacology of Anticancer Agents in Relation to Formulations and Administration Routes*. Academic Thesis, Utrecht University 1998
19. Van Tellingen O, Huizing MT, Nannan Panday VR, Schellens JHM, Nooijen WJ, Beijnen JH: Cremophor EL causes (pseudo-)non-linear pharmacokinetics of paclitaxel in patients. Br J Cancer 81:330-35, 1999
20. Gelderblom H, Verweij J, Brouwer E, *et al.*: Disposition of [*G*-^{3}H]paclitaxel and Cremophor EL in a patient with severely impaired renal function. Drug Metab Dispos 27: 1300-1305, 1999
21. Van Tellingen O, Beijnen JH, Verweij J, Scherrenburg EJ, Nooijen WJ, Sparreboom A: Rapid esterase-sensitive breakdown of polysorbate 80 and its impact on the plasma pharmacokinetics of docetaxel and metabolites in mice. Clin Cancer Res 5:2918-24, 1999

22. Onetto N, Dougan M, Hellmann S, *et al.*: Safety profile. In: McGuire WP, Rowinsky EK, eds. Paclitaxel in Cancer Treatment. New York: Marcel Dekker Inc 121-49, 1995
23. Eisenhauer EA, Ten Bokkel Huinink WW, Swenerton KD, *et al.*: European-Canadian randomized trial of paclitaxel in relapsed ovarian cancer: high-dose *versus* low-dose and long *versus* short infusion. J Clin Oncol 12:2654-66, 1994
24. Essayan DM, Kagey-Sobotka A, Colarusso PJ, Lichtenstein LM, Ozols RF, and King ED: Successful parenteral desensitization to paclitaxel. J Allergy lin Immunol 97:42-46, 1996
25. Szebeni J, Muggia FM, Alving CR: Complement activation by Cremophor EL as a possible contributor to hypersensitivity to paclitaxel: an *in vitro* study. J Natl Cancer Inst 90:300-6, 1998
26. Webster LK, Cosson EJ, Stokes KH, Millward MJ: Effect of the paclitaxel vehicle, Cremophor EL, on the pharmacokinetics of doxorubicin and doxorubicinol in mice. Br J Cancer 73:522-24, 1996
27. Colombo T, Parisi I, Zucchetti M, Sessa C, Goldhirsch A, D'Incalci M. Pharmacokinetic interactions of paclitaxel, docetaxel and their vehicles with doxorubi-cin. Ann Oncol 10:391-95, 1999
28. Ohtsu T, Sasaki Y, Tamura T, *et al.*: Clinical pharmacokinetics and pharmacodynamics of paclitaxel: a 3-hour infusion *versus* a 24-hour infusion. Clin Cancer Res 1:599-606, 1995
29. Wiernik PH, Schwartz EL, Einzig A, Strauman JJ, Lipton RB, Dutcher JP. Phase I trial of Taxol given as a 24-hour infusion every 21 days: responses observed in metastatic melanoma. J Clin Oncol 5:1232-39, 1987
30. Longnecker SM, Donehower RC, Cates AE, *et al.*: High-performanceliquid chromatographic assay for taxol in human plasma and urine and pharmacokinetics in a phase I trial. Cancer Treat Rep 71:53-59, 1987
31. Grem JL, Tutsch KD, Simon KJ, *et al.*: Phase I study of taxol administered as a short i.v. infusion daily for 5 days. Cancer Treat Rep 71:1179-84, 1987
32. Maier-Lenz H, Hauns B, Häring B, *et al.*: Phase I study of paclitaxel administered as a 1-hour infusion: toxicity and pharmacokinetics. Semin Oncol 6 (S19):S19-16-S19-19, 1997
33. Fenelly D, Aghajanian C, Shapiro F, *et al.*: Phase I and pharmacologic study of paclitaxel administered weekly in patients with relapsed ovarian cancer. J Clin Oncol 15:187-92, 1997
34. Greco FA, Thomas M, Hainsworth JD: One-hour paclitaxel infusions: review of safety and efficacy. Cancer J Sci Am 5:179-91, 1999

Chapter 8

Pharmacokinetic modeling of paclitaxel encapsulation in Cremophor EL micelles

Lia van Zuijlen[1], Mats O Karlsson[2], Jaap Verweij[1], Eric Brouwer[1], Peter de Bruijn[1], Kees Nooter[1], Gerrit Stoter[1], and Alex Sparreboom[1]

[1]Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, The Netherlands, [2]Division of Biopharmaceutics and Pharmacokinetics, Department of Pharmacy, Uppsala University, Sweden

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ABSTRACT

Nonlinear disposition of paclitaxel (Taxol) in cancer patients has been described in several studies, but the underlying mechanism is still speculative. Previously, we have shown *in vitro* that the paclitaxel formulation vehicle, Cremophor EL (CrEL), alters the blood distribution of paclitaxel as a result of entrapment of the compound in circulating CrEL micelles, thereby reducing the free drug fraction available for cellular partitioning. Based on these findings, we have prospectively re-evaluated the linearity of paclitaxel disposition in patients using whole blood and plasma analysis, and we took effort to define a new pharmacokinetic model to describe the data. Seven patients with solid tumors were treated with paclitaxel infused over 3 h, each at consecutive 3-weekly dose levels of 225, 175 and 135 mg/m^2 (CrEL dose level, 18.8, 14.6, and 11.3 mL/m^2, respectively). Patient samples were collected up to 24 h after start of infusion, and analyzed by high-performance liquid chromatography. Paclitaxel peak levels and areas under the curve in whole blood increased linearly with dose, whereas plasma levels showed substantial deviation from linearity. This was shown to be caused by a CrEL-concentration dependent decrease in paclitaxel uptake in blood cells, as reflected by the blood:plasma concentration ratios which altered significantly from 0.83±0.11 (at 135 mg/m^2) to 0.68±0.07 (at 225 mg/m^2). It is concluded that the nonlinear disposition of paclitaxel is related to paclitaxel-dose related levels of the formulation vehicle CrEL, leading to disproportional drug accumulation in the plasma fraction. The developed pharmacokinetic model could accurately describe the data, and will help guide future development and refinement of clinical protocols, especially in defining the exposure measure best linked to paclitaxel effects and toxicities.

INTRODUCTION

Nonlinear disposition of drugs is a pharmacokinetic characteristic which implies that a given increase in dose may lead to a disproportional increase in systemic drug exposure. After i.v. drug administration, the phenomenon is most commonly caused by saturation of enzyme, binding or transporter capacity (36). Although there are many drugs that display nonlinear elimination kinetics, either resulting from saturable metabolic

pathways (e.g. phenytoin (1)) or saturable excretion pathways (e.g. cyclophosphamide (3)), capacity-limited distribution in humans is rare, although concentration dependence has been described for the binding of some drugs to plasma proteins, blood cells and extravascular tissue or binding sites (20). The pharmacokinetic behavior of the anticancer agent paclitaxel (Taxol), for example, was shown to be distinctly nonlinear in several studies, and seemed a result of saturable distribution in combination with saturable elimination processes (9,15,24). Interestingly, studies in mice have demonstrated that in the absence of CrEL, a polyoxyethylated castor oil derivative used as formulation vehicle for i.v. drug administration, both distribution and elimination of paclitaxel appeared to be linear processes (26). Because plasma concentrations of CrEL in mice and humans are within the same range, it is likely that CrEL also plays a pivotal role in the nonlinear paclitaxel disposition in humans (27). The cause of the observed nonlinearity, however, has not yet been discovered, but possible explanations include CrEL-mediated inhibition of endogenous P-glycoprotein activity causing altered hepatobiliary secretion (10,7) and altered protein binding resulting from CrEL-induced lipoprotein dissociation (32). Recently, we have found *in vitro* that CrEL causes profound concentration-dependent alterations of paclitaxel distribution in human blood, possibly because the highly hydrophobic paclitaxel favors partitioning in CrEL micelles (31). Based on these findings, we speculated that the nonlinear kinetics of paclitaxel is not related to saturable tissue binding or Michaelis-Menten elimination kinetics, but is caused by dose and time-varying CrEL concentrations in the central compartment. In the present study, we carried out a comprehensive pharmacokinetic analysis of paclitaxel and CrEL in patients by measuring paired whole blood and plasma levels to discover the cause of the saturable kinetic processes, and present explicit modeling of the disposition of the compounds to permit a formal explanation of the observed phenomena.

MATERIAL AND METHODS

Patients and Treatment

Patients with a histologically confirmed diagnosis of a malignant solid tumor for whom paclitaxel as monotherapy was a viable therapeutic option or for whom other treatment options were not available, were candidates for this study. Additional eligibility criteria were: age ≥18 years; World

Health Organization performance status <3; life expectancy of at least 3 months; no previous treatment with taxanes; adequate bone marrow function (WBC count >3.0x10^9/L, platelet count >100x 10^9/L), renal function (serum creatinine ≤140 μM and creatinine clearance ≥60 mL/min), and hepatic function (serum bilirubin, alkaline phosphatase, aspartate aminotransfer-ase and alanine aminotransferase concentrations within normal limits); no pre-existing peripheral neuropathy graded >1 according to NCI Common Toxicity Criteria.

The study drug was formulated in a mixture of CrEL and dehydrated ethanol USP (1:1, v/v) (Taxol; provided by Bristol-Myers Squibb, Woerden, the Netherlands) and diluted further into 500 mL of isotonic sodium chloride prior to dosing. Paclitaxel was administered every 3 weeks as a 3-h i.v. infusion at consecutive dose levels of 225, 175 and 135 mg/m^2 (CrEL dose level, 18.8, 14.6, and 11.3 mL/m^2, respectively). Patients continuing treatment thereafter were given paclitaxel at a dose of 175 mg/m^2 out of protocol. Premedication was uniform for all patients and consisted of (*i*) dexamethasone (10 mg i.v.), (*ii*) clemastine (2 mg i.v.) and (*iii*) ranitidine (50 mg i.v.), all given 30 min before start of paclitaxel administration. During chemotherapy, the patients did not use any other co-medication that might have interfered with paclitaxel disposition. The clinical protocol was approved by the Rotterdam Cancer Institute Review Board, and all patients signed informed consent before entering the study.

Sample Collection

Blood samples (approx. 5 mL) were obtained from all patients during all courses of treatment and collected in Vacutainer glass tubes containing 143 USP units of lithium heparin as anticoagulant. Samples were obtained at the following time points: before dosing and at 1, 2, 3, 3.08, 3.25, 3.5, 3.75, 4, 5, 7, 9, 11, 15, and 24 h after start of the infusion. After agitation, 2-mL aliquots of whole blood were snap-frozen at -20°C at the patient site, and plasma was separated by centrifugation for 5 min at 4000x*g* (4°C) and then stored at -80°C. Complete stool collections were obtained from each patient up to 24 h after drug administration in polystyrene containers and stored immediately at -80°C. Weighted feces samples were homogenized individually in 4 volumes of deionized Milli-Q-UF water (Millipore, Milford, MA) using five 1-min bursts of an Ultra-Turrax T25 homogenizer (IKA-Labortechnik, Dottingen, Germany) operating at 20,500 rpm. Aliquots of

feces homogenates (200 μL) were diluted 5-fold with drug-free human plasma prior to further sample processing.

Drug Analysis

Paclitaxel (batch: 494034, purity: 98.3% by reversed-phase high-performance liquid chromatography [HPLC]) and docetaxel, used as internal standard, were provided by Bristol-Myers Squibb and Rhône-Poulenc Rorer (Vitry-sur-Seine, France), respectively. Reference stocks of pure 6α-hydroxypaclitaxel, 3′-p-hydroxypaclitaxel and 6α,3′-p-dihydroxypaclitaxel were obtained by isolation and purification of a patient feces sample, as described (25). Stock solutions of each of the taxanes were prepared at 1.0 mg/mL in dimethyl sulfoxide. Concentrations of paclitaxel in whole blood and plasma were determined using isocratic reversed-phase HPLC with UV detection ($\lambda = 230$ nm) with a lower limit of quantitation of 10 ng/mL, as reported (28). The same assay methodology was also used for determination of paclitaxel in whole blood, and was validated as described (28) using spiked quality control samples containing 40, 200, 400, and 15,000 ng of paclitaxel/mL. The within-run precision and between-run precision ranged from 2.23-8.74% and 1.81-8.07% ($n = 14$ at each of the concentrations), respectively, with a mean percentage deviation from nominal values less than ±3.30%. A potential CrEL-concentration dependency in extraction recovery of paclitaxel from whole blood was tested by repeated analysis of spiked whole blood (nominal concentration: 1000 ng/mL) containing 0, 0.1, 0.5, 1.0, 5.0 or 10 μL of CrEL/mL. The mean (±SD) recovered concentrations in these samples were 1037±28.4, 1067±33.7, 1064±7.91, 1074±1.90, 1041±35.1 and 1057±6.71, respectively, with no trend in significant deviations from the control value ($P = 0.194$; $n = 16$ at each CrEL concentration). The detector response of the internal standard docetaxel, as measured by the peak height, was also not significantly different during analysis of either plasma or whole blood samples with mean values of 44,019±4739 μV and 43,728±5410 μV, respectively ($P = 0.525$; $n = 164$ for each matrix). These data were considered acceptable for analysis of paclitaxel in whole blood samples obtained from patients for the conduct of the present study.

For determination of paclitaxel and its hydroxylated metabolites in feces homogenates, this HPLC methodology was further modified as described (8). In brief, standard curves were prepared in drug-free human

plasma and were expanded to encompass concentrations between 0.1 and 5 μg/mL. A 1-mL aliquot of standard or plasma-diluted sample was mixed for 5 min with 100 μL of 10 μg/mL docetaxel in 50% (v/v) methanol in water and 5 mL of 20% (v/v) acetonitrile in *n*-butyl chloride. After centrifugation for 5 min at 4000x*g* (4°C), the entire upper organic phase was separated and dried under nitrogen at 60°C. The dried residue was reconstituted in 125 μL of 50% (v/v) methanol in water by vortex-mixing, and following a brief centrifugation step, 100 μL of the clear supernatant was injected into the HPLC system from low-volume glass inserts by a temperature-controlled Waters 717Plus autosampling device (Milford, MA). Chromatographic separations were performed at 60°C on an Inertsil ODS-80A column (150x4.6 mm internal diameter, 5 μm particle size; GL Science, Tokyo, Japan) and a guard column (4.0x4.0 mm, 5 μm) packed with LiChrospher 100 RP-18 material (Merck, Darmstadt, Germany). The mobile phase consisted of methanol:tetrahydrofuran:0.2 M aqueous ammonium hydroxide (60:2.5:37.5, v/v/v) with the pH adjusted to 6.0 (formic acid), and was degassed by ultrasonication prior to use. The flow-rate was set at 1.0 mL/min, and the eluent was monitored at an absorption wavelength of 230 nm with a SpectraPhysics UV-200 detector (San Jose, CA). Integration of chromatographic data and calculation of calibration graphs was performed as described (28). A formal method validation was performed by repeat analysis of quality control samples spiked to contain 0.50, 2.5, 10, and 20 μg of paclitaxel/mL on 4 consecutive days along with a 7-point calibration curve processed in duplicate. There were no endogenous compounds in any of the studied patient specimens that could have interfered with the peaks of interest in the system. Standard curves were fitted by an equation with proportional weighting, and were strictly linear in the tested range, with an R^2 value greater than 0.995 in all cases. The within-run precision and between-run precision ranged from 2.49–7.82% and 1.68–10.6% ($n = 20$ at each of the concentrations), respectively, with a mean percentage deviation from nominal values less than ±8.23%.

The analytical method for determination of CrEL concentrations in plasma was based on binding of the surfactant to the Coomassie Brilliant Blue G-250 dye in protein-free plasma extracts by measuring the change in ratio of absorbances at 595 nm over 450 nm (2,29). For this purpose, 50-μL samples of plasma were deproteinized by addition of 500 μL of acetonitrile in Teflon-capped 12-mL tubes followed by vortex-mixing for 1 min. Next, 2 mL

of *n*-butyl chloride were added followed by vigorous mixing for 5 min. The organic layer was then separated by centrifugation for 5 min at 4000 × *g*, transferred to a clean 10-mL glass tube, and dried under nitrogen at 60°C for 30 min. The residue was reconsituted in 50 μL of water by vortex-mixing, and a 25-μL volume was pipetted into a 96-well flat-bottom cluster (Costar Corp., Cambridge, MA). Finally, 250 μL of water-diluted (1:4, v/v) Coomassie Brilliant Blue G-250 reagent were added, and the absorbance maximum of the dye at 595 nm after binding to CrEL, and the simultaneous decrease in absorbance at 450 nm were measured within 24 h against a reagent blank using a Bio-Rad Model 550 automated microplate reader (Bio-Rad Laboratories, Hercules, CA). The lower limit of this procedure is 0.50 μL/mL, and over the entire range studied (up to 10.0 μL/mL), the method has excellent specificity, accuracy (≤6.33% relative error), and precision (<10%) (2).

Pharmacokinetic Analysis

The pharmacokinetics of paclitaxel were initially evaluated by a non-compartmental method using the Siphar package (version 4.0; SIMED, Créteil, France). The actual times of drug intake and blood sampling were taken into account. Peak drug levels (C_{max}) were determined by visual inspection of the concentration-time data. The area under the concentration-time curve (AUC) from zero to the last sampling time point with a detectable concentration (C_{last}) was calculated by the linear trapezoidal rule. The apparent half-life of the terminal disposition phase ($T_{1/2}$) was defined as $\ln 2/\lambda$, in which λ was the elimination rate constant of the terminal phase, estimated by a least-squares regression analysis of the final 3 data points of the concentration-time profiles. Total body clearance (CL) was calculated as the quotient of dose (expressed in mg/m^2) and AUC extrapolated to infinity by dividing C_{last} by λ. The relationships between paclitaxel dose and C_{max} or AUC in plasma and blood were evaluated in a scattered plot with linear and nonlinear fitting using Graphstatics Plus (Manugistics Inc., Rockville, MA). The AUC of CrEL in plasma was also calculated using the Siphar package as described (30).

Statistical Considerations

All pharmacokinetic parameters are reported as mean values ± SD. Since multiple measurements were performed at different times on the

same patients, comparisons between the sets of observations were based on *within* subject differences. Therefore, variation *between* subjects, which is usually considerable, does not affect our ability to distinguish differences between the sets of observations, which here relate to the 3 paclitaxel dose levels. The effect of drug dose on the apparent paclitaxel clearance in plasma and blood was evaluated using a Friedman's two-way analysis of variance test, with the level of significance set at $P<0.05$. Statistical analysis was performed using the Number Cruncher Statistical System (NCSS) software package (version 5.X; J.L. Hintze, Kaysville, UT, 1992).

Model Development

Paclitaxel in blood was assumed to exist in the following pharmacokinetically distinguishable forms: unbound ($C_{unbound}$), bound to plasma protein (C_{bound}), in micellar form together with CrEL ($C_{micellar}$), and bound to or distributed into red blood cells (C_{rbc}). Further, $C_{unbound}$ was assumed to be in equilibrium with tissues. For some tissues and organs, the rate to attain this equilibrium may be fast and therefore part of the central volume of distribution. For other tissues and organs it may be slow, where the number of peripheral compartments will reflect the heterogeneity of the rate of equilibration between tissues of importance for the distribution of paclitaxel. As neither $C_{unbound}$ nor C_{bound} was measured separately and since the relationship between the two was assumed linear and instantaneous, there was no possibility to separately characterize these based on pharmacokinetic data. However, no approximation of the model was necessary because of this, the only consequence being that the relevant parameters will be based on the sum of the two concentrations rather than on $C_{unbound}$ alone. Thus, $C_{non\text{-}micellar}$ is the sum of $C_{unbound}$ and C_{bound}, C_{plasma} is the sum of $C_{micellar}$ and $C_{non\text{-}micellar}$, whereas C_{blood} is the hematocrit-weighted average of C_{rbc} and C_{plasma}.

The only feature that distinguishes the model used from standard linear compartmental pharmacokinetic models is the possibility of paclitaxel to be included in micelles and thereby undergo a change in the pharmacokinetic properties. In the present model, these CrEL-paclitaxel micelles were assumed to exist in plasma, and with plasma rapidly equilibrating organs and tissues. The initial model for $C_{micellar}$ assumed it to be proportional to both the unbound paclitaxel concentration and the total CrEL concentration. Several modifications to this assumption were

explored, including nonlinear dependence on paclitaxel and/or CrEL concentrations as well as time dependent changes in the proportionality constant governing the relationship. The information about the relationship between paclitaxel, CrEL and $C_{micellar}$ as entered into the model came from the nonlinear behavior of the plasma concentration-time curve and the varying ratios of C_{blood} to C_{plasma}. Therefore, the data were best utilized in a simultaneous fit of the model to both. The ratio between C_{rbc} and $C_{non\text{-}micellar}$, a necessary part of the model, was estimated as the parameter θ_{rbc}. Whereas nonlinear mixed effects ('population') modeling is the accepted method of analyzing sparse pharmacokinetic data, rich pharmacokinetic data sets are more commonly analyzed by individual modeling. However, when complex models are used, there is a gain in analyzing data from several subjects together even if data are rich (11,23). Indeed, if many parameters are to be estimated from a single individuals pharmacokinetic profile, there are usually some parameters for which the information is slight. Thus, in a sense, with complex pharmacokinetic models, individual data are no longer 'rich' in information. This analysis has been performed as population modeling using the first-order method as implemented in the NONMEM program (version V; S.L. Beal and L.B. Sheiner, San Francisco, CA, 1998). As the number of individuals was small, there has been no model building in the traditional sense, where both the structural, statistical and covariate models are refined in an integrated process. Covariates, apart from the CrEL concentrations, were absent. Interindividual variability parameters can be expected to be poorly estimated, but will be included in the model as long as the termination is successful and can provide estimates of parameter precision. Interindividual variability was included as log-normally distributed and residual variability was initially modeled as combined additive and proportional, where either component may be omitted if superfluous. The assessment of statistical significance of the additional parameters was based on the difference between the objective function values (part of the output from NONMEM) between models being compared. This difference is minus twice the log likelihood for the models and is approximately χ-squared distributed. A difference of greater than 10.83 (one degree of freedom) is significant at the 0.1% level, which was used in discriminating between hierarchical models.

Introducing the CrEL concentrations into the model requires knowledge of CrEL at all time points, including those in-between sampled

CrEL observations. In the present modeling, we have used the individual predictions from a two-compartment model generated as a part of a separate analysis of CrEL pharmacokinetics in a larger population that included 67 complete concentration-time courses (not shown). Although this model is a simplification of CrEL pharmacokinetics, it adequately describes the individual profiles, which is what was needed for the present purpose.

Table 1 Characteristics of patients undergoing concomitant blood and plasma sampling during and after paclitaxel infusion

Characteristic		No. of patients
Total no.		7
No. of courses		19
Age (years)		
Median	54	
Range	32 - 64	
Gender		
Male		3
Female		4
WHO** performance status		
0		2
1		4
2		1
Primary tumor site		
Bladder		3
Breast		1
Lung		1
Unknown		1
Weak tissue (sarcoma)		1
Pretherapy clinical chemistry *		
serum creatinine (μM)	92 (69 - 138)	
total bilirubin (μM)	9 (3 - 15)	
ASAT (units/L)	19 (10 - 28)	
ALAT (units/L)	16 (6 - 31)	
total protein (mg/mL)	76 (74 - 78)	
serum albumin (mg/mL)	42 (34 - 49)	

* Mean values with range in parentheses.

** WHO, World Health Organization; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase.

RESULTS

Patient Characteristics

Seven patients (3M/4F) with various malignant solid tumors were studied (Table 1). The median age was 54 years (range, 32–64 years), and all patients had normal hematopoietic and liver functions at the time of study. One patient had slightly increased serum creatinine values and a lower creatinine clearance (138 μM and 57 mL/min, respectively), but was accepted based on the known limited role of renal function in paclitaxel disposition (8). Two patients did not receive the last course (135 mg/m^2) because of progressive disease after two paclitaxel courses. Eventually, 19 courses were available for pharmacokinetic evaluation.

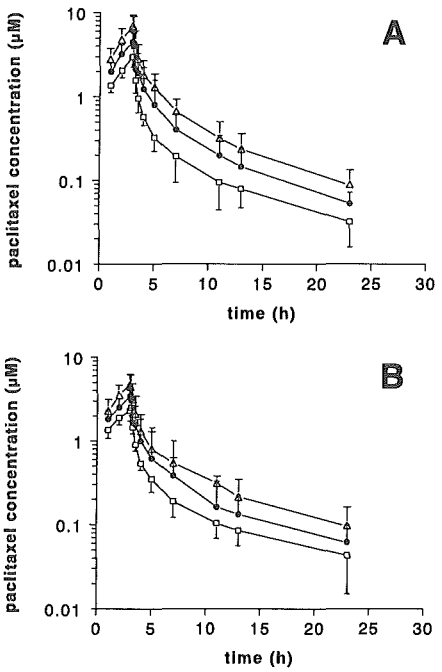

Figure 1 Plasma (panel A) and blood (panel B) concentration-time profiles of paclitaxel in patients treated with paclitaxel at dose levels of 135 mg/m^2 (□), 175 mg/m^2 (•) and 225 mg/m^2 (Δ). Data are presented as mean values (symbols) ± SD (error bars).

Paclitaxel Blood Distribution

The mean plasma and blood concentration-time profiles of paclitaxel at the three different dose levels tested are displayed in Fig. 1. The relationships between the administered dose and paclitaxel C_{max} and AUC in plasma indicated a substantial deviation from linearity (Fig. 2A), whereas this disproportionality was less pronounced with data based on whole blood measurements (Fig. 2B).

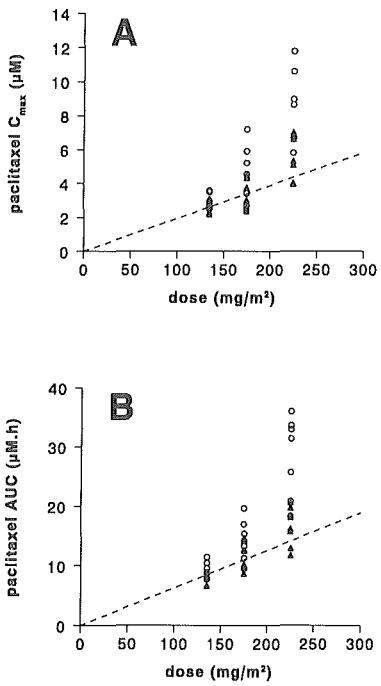

Figure 2 Relationships between the administered dose and paclitaxel C_{max} (panel A) and paclitaxel AUC (panel B) in plasma (o) and whole blood (▲). Dotted lines indicate linearity of the relationships in case of proportional increases of C_{max} and AUC with dose.

Table 2 Summary of non-compartmental paclitaxel pharmacokinetics*

Dose** (mg/m^2)	Matrix	C_{max}*** (μM)	AUC ($\mu M.h$)	CL ($L/h/m^2$)	$T_{1/2}$ (h)
135	plasma	3.04±0.493	9.62±1.43	16.7±2.54	7.70±4.15
	blood	2.63±0.387	8.06±2.92	18.8±2.30	8.15±2.92
175	plasma	4.54±1.73	14.3±3.40	14.0±3.76	7.17±1.92
	blood	3.31±0.883	11.8±2.30	17.9±3.64	6.96±1.63
225	plasma	8.75±2.76	28.7±7.04	9.75±2.78	6.77±2.45
	blood	5.58±1.32	17.9±4.81	15.7±4.34	5.99±1.67

* Data were obtained from 7 patients after treatment with a 3-h i.v. infusion of paclitaxel, each receiving consecutive 3-weekly doses of 225 ($n=7$), 175 ($n=7$) and 135 mg/m^2 ($n=5$). Pharmacokinetic parameters represent mean values ± SD.

** Corresponding dose levels of CrEL, 11.3, 14.6, and 18.8 mL/m^2, respectively.

*** C_{max}, peak concentration; AUC, area under the concentration-time curve up to the last sampling-time point with detectable paclitaxel concentration; CL, apparent clearance; $T_{1/2}$, apparent half-life of the terminal disposition phase.

In line with previous findings (9,15,24), the apparent plasma clearance of paclitaxel was clearly dose-dependent and significantly decreased from 16.7±2.53 $L/h/m^2$ (at 135 mg/m^2) to 9.75±2.78 $L/h/m^2$ (at 225 mg/m^2; $P=0.030$) (Table 2). The whole blood clearance of paclitaxel, however, appeared to be independent of the administered dose ($P=0.063$), and averaged 17.5±3.43 $L/h/m^2$. Examination of blood:plasma concentration ratios (C_b/C_p) revealed that the discrepant kinetic profiles of paclitaxel in plasma and blood is most closely associated with a concentration-dependent change in movement of drug within the central compartment during the distribution phase. At C_{max}, C_b/C_p ratios decreased in a dose-dependent manner from 0.83±0.11 (135 mg/m^2) to 0.80±0.18 (175 mg/m^2) and 0.68±0.07 (225 mg/m^2). The estimated terminal half-life was relatively consistent in all subjects and not different between dose levels, exhibiting mean values of 7.21±0.623 h and 6.91±0.485 h for plasma and blood, respectively (plasma *versus* blood, $P=0.69$). This finding is further substantiated by the fecal recovery patterns of paclitaxel and its three main hydroxylated metabolites during the first 24 h after initiation of the infusion,

that were qualitatively and quantitatively independent of the dose given. Fecal excretion of unchanged drug accounted for only 2.8%, 3.7% and 3.7% of the total dose administered at the 135, 175 and 225 mg/m^2 dose levels, respectively. This is in line with previous findings (31), and with murine data indicating that fecal excretion pathways of paclitaxel are independent of the administered dose and the vehicle used for drug formulation (27).

CrEL Pharmacokinetics

The plasma clearance of CrEL at the tested dose levels of 18.8, 14.6, and 11.3 mL/m^2 was independent of the administered dose ($P=0.61$) and averaged 320±53.2 mL/h/m^2, which is within the same range as described for this compound previously when based on calculations using AUC_{0-t} (30). Mean CrEL plasma concentration-time profiles at the various dose levels are shown in Fig. 3.

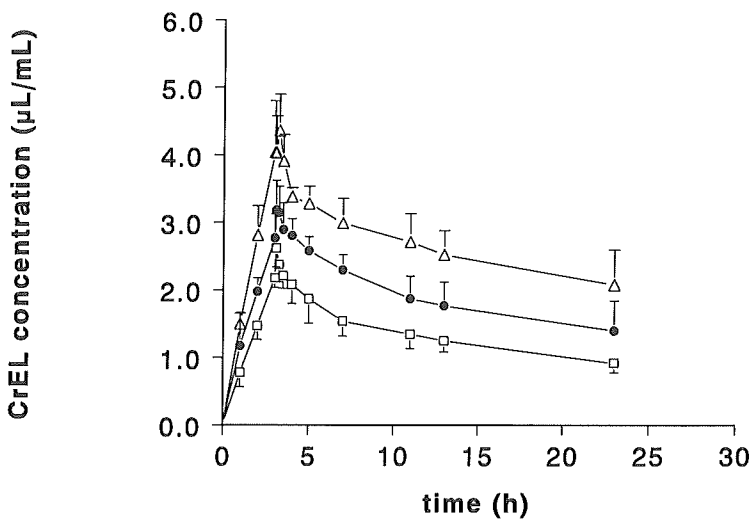

Figure 3 Plasma concentration-time curves of CrEL in patients treated with paclitaxel at dose levels of 135 mg/m^2 (□), 175 mg/m^2 (•) and 225 mg/m^2 (Δ). Data are presented as mean values (symbols) ± SD (error bars).

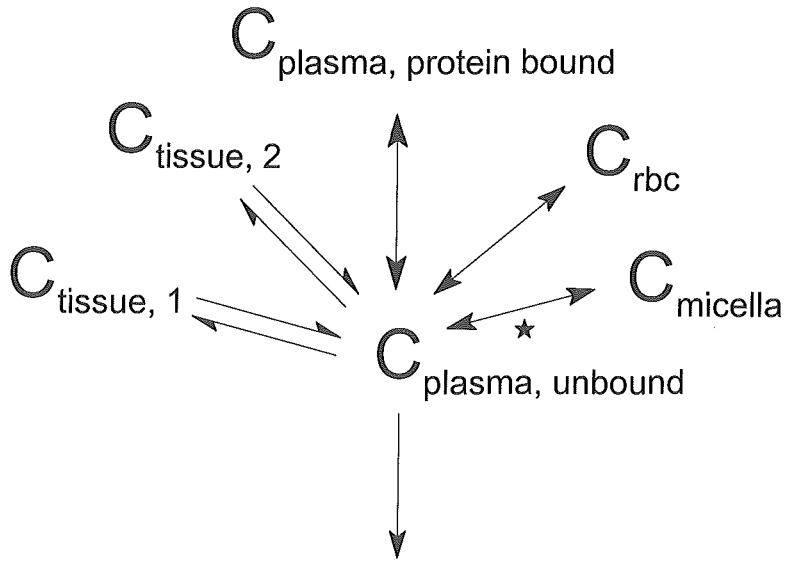

Figure 4 Pharmacokinetically distinguishable forms of paclitaxel in blood compartment assumed to exist in the proposed model for paclitaxel pharmacokinetics. Double-headed arrows denote processes with assumed instantaneous equilibrium. The star indicates a non-linear process.

Pharmacokinetic Model

We sought to define a pharmacokinetic model that would explain the nonlinearity observed in paclitaxel plasma disposition. The distribution of paclitaxel to tissues was best described by a three compartment model (based on considerations presented in Fig. 4), and was characterized by the following parameters (±SD): inter-compartmental clearance for peripheral compartment one, 30 ± 8 L/h; volume of paclitaxel for peripheral compartment one, 51 ± 14 L; inter-compartmental clearance of paclitaxel for peripheral compartment two, 34 ± 8 L/h; volume of paclitaxel for peripheral compartment two, 340 ± 81 L. The central volume of distribution of paclitaxel was 41 ± 14 L, whereas the proportionality constant relating C_{rbc} to $C_{non\text{-}micellar}$ was 1.7 ± 0.2. The overall clearance of paclitaxel based on $C_{non\text{-}micellar}$ was 71 ± 13 L/h. The relationship between $C_{micellar}$ and $C_{non\text{-}micellar}$ could be well described by the following function:

$$C_{micellar} = C_{non\text{-}micellar} \times C_{CrEL} \times \theta_{micellar} \times \{1 - (T \times \theta_{time})\}$$

where C_{CrEL} is the CrEL plasma concentration in μL/mL, T is time after start of infusion, and $\theta_{micellar}$ and θ_{time} are parameters estimated to be 0.97 ± 0.22 and 0.039 ± 0.004, respectively. Before arriving at this final model for the relationship, several others were tried, including those containing functions with nonlinear relationships for both $C_{non\text{-}micellar}$ and C_{CrEL}. The fit of the current model to the observed paclitaxel plasma and blood concentration gave a good description of the data without any trends in the residuals over time or paclitaxel concentration (Fig. 5).

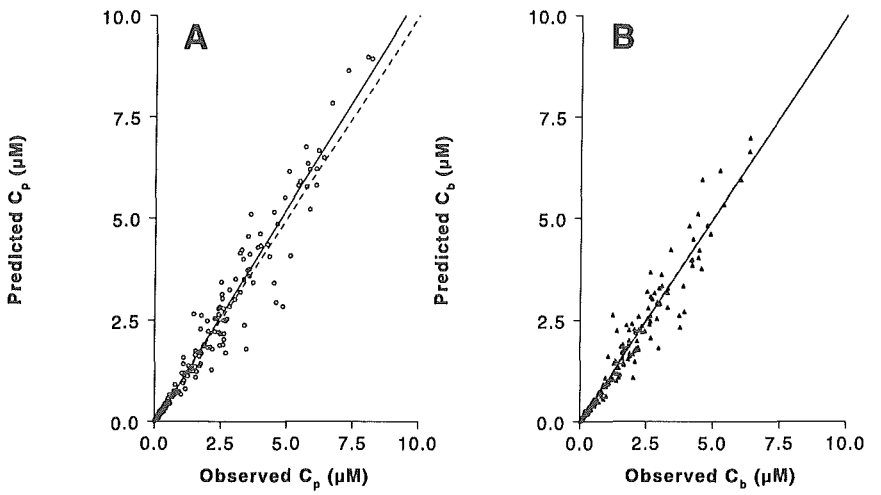

Figure 5 Observed *versus* predicted plasma (panel A) and blood (panel B) concentrations using the proposed pharmacokinetic model. Dotted lines indicate the lines of identity, whereas solid lines indicate linear regression fits ($y = 1.0654x - 0.0802$ for plasma and $y = 1.0008x - 0.0263$ for blood).

The presently developed model gave an unbiased prediction of the C_b/C_p ratio over time (Fig. 6A), and it could also predict the free fraction of paclitaxel at any CrEL concentration compared to the free fraction in absence of CrEL. This estimated ratio shows good agreement with previously published data obtained from a single patient treated with paclitaxel at a dose level of 157.5 mg/m^2 (31) (Fig. 6B). An example of

concentration-time data for the various pharmacokinetically distinguishable forms of paclitaxel in blood from a representative patient is given in Fig. 7.

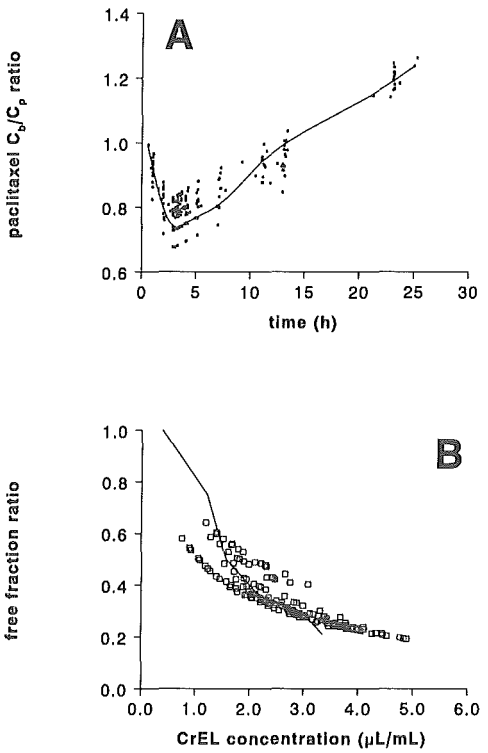

Figure 6 Observed (symbols) and model predicted (line) ratios (C_b/C_p) of the paclitaxel blood:plasma concentrations over time (panel A), and the ratio between the free fraction of paclitaxel at zero CrEL concentration to the free fraction of paclitaxel in the presence of CrEL at various concentrations [*i.e.* $C_{non\text{-}micellar}$ / ($C_{non\text{-}micellar}$ + $C_{micellar}$)]. The continuous line is based on data from Ref. 31 [see Table 3 therein], whereas the squares are model based estimates for all observations up to 20 h after paclitaxel administration (panel B).

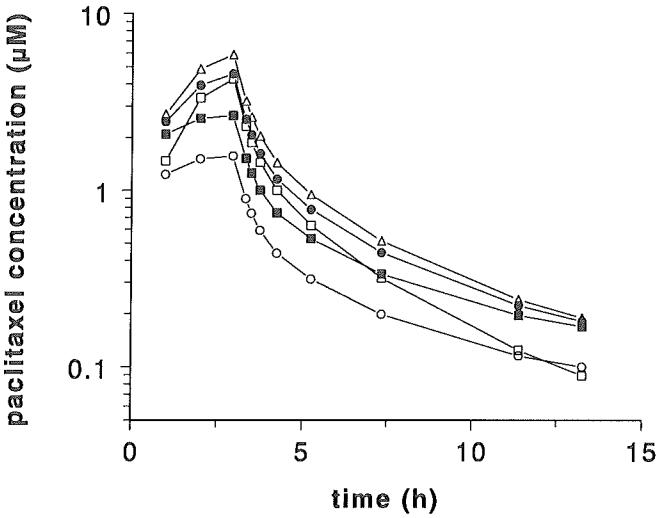

Figure 7 Paclitaxel concentration-time profiles using model-fit curves in a representative patient treated with paclitaxel at a dose level of 175 mg/m^2. C_{plasma} [*i.e.* the sum of $C_{micellar}$ and $C_{non\text{-}micellar}$] (Δ), C_{blood} [*i.e.* the hematocrit-weighted average of C_{rbc} and C_{plasma}] (•), $C_{non\text{-}micellar}$ [*i.e.* the sum of $C_{unbound}$ and C_{bound}] (o), $C_{micellar}$ (□) and C_{rbc} (▩).

DISCUSSION

By prospectively measuring paclitaxel concentrations in whole blood and plasma in cancer patients given different dose levels of paclitaxel and serving as their own control, we demonstrated that the AUC of paclitaxel in whole blood is a linear function of the dose administered. By calculating paclitaxel C_b/C_p ratios, we found that the fraction of this drug that is bound in plasma changes appreciably with concentration in the concentration range of interest. Recently, we showed *in vitro* that CrEL causes a profound alteration of paclitaxel accumulation in erythrocytes in a concentration-dependent manner, by reducing the free drug fraction available for cellular partitioning, thereby suggesting that paclitaxel trapping occurred in CrEL micelles (31). These findings were later independently confirmed by

Knemeyer, *et al.* (17) and Van Tellingen, *et al.* (33) using similar *in vitro* experiments. Yet, the findings of our present study demonstrate for the first time that CrEL affects the clinical pharmacokinetics of paclitaxel by a disproportional accumulation process in plasma, and lends further support to our prior supposition that the ability of CrEL to modulate the murine disposition of paclitaxel may have important clinical ramifications. Moreover, our data indicate that the nonlinear pharmacokinetics of paclitaxel are not related to saturable tissue binding but are caused by paclitaxel-dose related levels of CrEL in blood. Although our study population included only seven patients, the results of the paclitaxel plasma pharmacokinetics were highly consistent with previously described data by Gianni, *et al.* (9) obtained from a larger group of patients. For example, the paclitaxel plasma AUC varied from 10.9±1.1 to 18.5±3.0 and 24.3±6.8 μM.h in their study *versus* 9.6±1.4, 14.3±3.4 and 28.7±7.0 μM.h in our study, at dose levels of 135, 175 and 225 mg/m^2, respectively, indicating that our small patient population was indeed representative.

The other speculated causes of the nonlinearity of paclitaxel as mentioned in the introduction became unlikely by our findings; the established decrease in C_b/C_p ratio of paclitaxel in our study exclude the role of P-glycoprotein (10), because modulation of endogenous P-glycoprotein expressed in certain blood cells (e.g. white blood cells) by CrEL should lead to an increase of this ratio. The hypothesis of altered P-glycoprotein-mediated hepatobiliary secretion is based on experiments in an isolated perfused rat-liver system in which paclitaxel is administered as a bolus dose both in the absence of CrEL and after the administration of either 80 or 800 μL of CrEL (7). Total and biliary clearance of paclitaxel decreased in a dose-dependent manner not through alterations in the metabolism of paclitaxel under influence of CrEL but logically by the same principle as defined in our study, *i.e.* by micellar formation preventing paclitaxel from reaching the sites of metabolism and excretion. Altered protein binding as primary cause of the nonlinear paclitaxel pharmacokinetics (32) was already excluded as an explanation by our previous *in vitro* study, in which the CrEL-induced alteration of paclitaxel accumulation in erythrocytes was also observed in the absence of any plasma proteins (31).

The model that we presented here accurately described the pharmacokinetics of paclitaxel in patients at all dose levels studied, and could be proven useful for prediction of paclitaxel disposition at as yet

untested dose levels. In the past, the first attempts to model the nonlinear pharmacokinetics of paclitaxel used a nonlinear distribution mechanism and a nonlinear elimination based on plasma measurements (24). Later a model where the saturable process was replaced by a saturable binding to sites outside plasma was shown to describe data equally well, and be more probable based on the known pharmacologic properties of paclitaxel (9). In the present report we have used an alternative approach for describing the nonlinear pharmacokinetics of paclitaxel. As opposed to the previous models, only one nonlinear component was required to describe both the apparent nonlinear distribution and elimination kinetics. The sequence of models developed for paclitaxel illustrates the strive for mechanistic models that is a dominant principle in contemporary modeling. It also illustrates the principle that to discriminate between complex models, there is often a necessity to obtain information on more than one component of the system. In this case, we have, in addition to CrEL levels, measured both blood and plasma concentrations. Although it cannot be excluded that the saturable binding of paclitaxel to tubulin may play a role in its pharmacokinetic behavior (14), the present model can explain not only the nonlinear distribution and elimination of paclitaxel, but also the changing C_b/C_p ratio, something that no previous model had explained. We have not performed a comparison between the fit of the present model to data with the fit that previous models might have given. One reason for this is that the previous models from the nature of their development had no component for predicting the blood concentrations. As blood concentrations are an integral part of the present model, without which the model cannot be evaluated, no direct comparison could be made.

Our model makes several assumptions and approximations that may not be necessary if additional information on these processes are being gathered. Three assumptions regarding the micellar binding may be particularly approximate. Firstly, the amount of paclitaxel in micellar form is, at any time, assumed directly proportional to the total concentrations of the drug and CrEL. We know that at very low CrEL concentrations micelles cannot be formed, although it has been shown that in aqueous solution CrEL micelles can persist for several hours after dilution below the critical micellar concentration, estimated to be 0.009% (w/v) at equilibrium (16). It appeared, however, that this critical micellar concentration in plasma is low compared to the range of concentrations experienced during the

observation period of these treatments. Secondly, even above the critical micellar concentration there may be deviation from the simple direct proportionality that was postulated. Although literature data on CrEL micellar kinetics are lacking, preliminary experiments indicated that the *in vitro* equilibrium between paclitaxel in micelles and free paclitaxel is reached within 1 min,[1] and thus can be considered instantaneous on the time scale of paclitaxel pharmacokinetics. It is possible, however, that with time CrEL is able to form micelles with other components, such that less is available for forming paclitaxel micelles. Thirdly, the change in the micellar concentration with time is modeled as a linear relationship although it may well be a more complex function of both degradation of CrEL and/or binding to other blood constituents. Indeed, we have found recently, that compounds such as CrEL and polysorbate 80 are prone to a degradation pathway induced by serum carboxylesterases that causes a release of fatty acids (ricinoleic acid in case of CrEL and oleic acid in case of polysorbate 80) (34). Although this metabolic route is likely to be very slow for CrEL, given the long terminal half-life of CrEL of around 80 h (30), it is likely to impact on the CrEL-paclitaxel interaction. Hence, the assumptions regarding linear plasma protein and red cell binding as well as linear tissue distribution and elimination may in the end prove false under further scrutiny. In the present data set, however, such nonlinearities were not indicated. It is also noteworthy in this context that plasma protein binding of paclitaxel in human samples, determined by both equilibrium dialysis and ultrafiltration techniques, has been shown previously to be independent of the paclitaxel concentration within the therapeutic range associated with 3-h i.v. infusions (18). We are currently exploring the relevance of this principle for the *in vivo* situation by defining free (unbound) area-dose relationships in cancer patients treated with paclitaxel.

Our current findings will have significant implications for interpretation of the relationship between the pharmacokinetic parameters and pharmacodynamic outcome of paclitaxel treatment. Over the last few years, various hypotheses regarding relationships between plasma pharmacokinetics and hematological toxicity (e.g. neutropenia) have been postulated, including those using a step function (threshold model) (12,15) and more general models that used a nonlinear continuous function for the time-dissociated component (13,22). A common feature of these models

[1] Sparreboom A, Verweij J: unpublished data.

has been the use of the total paclitaxel plasma concentration, which is, in view of our current findings, unlikely to be the exposure measure best linked to observed toxicity profiles. Since the nonlinear pharmacokinetics of paclitaxel in plasma can be explained by CrEL concentration-dependent changes in drug movement within the central compartment, the effects of entrapment of paclitaxel in the plasma compartment will be less with prolonged (e.g. 24-h) infusion schedules associated with lower concentrations of CrEL (35). Thus, total plasma levels of paclitaxel measured in these schedules will represent a higher fraction of free paclitaxel, and this may, in part, explain the increased incidence of severe hematological toxicity seen in the 24-h infusion schedules as compared with infusion of the same dose over 3 h (6,9).

The existence of CrEL in blood as large polar micelles with a highly hydrophobic interior may also have important consequences for other drugs that are formulated in this vehicle. CrEL is currently used in i.v. preparations of many hydrophobic drugs including other anticancer agents (e.g. teniposide, didemnin B, halomon), anesthetics, vitamins and immunosuppressive agents (e.g. cyclosporin A, tacrolimus). It is admissible that the pharmacokinetic behavior of these drugs will also be influenced by the formulation vehicle, although the impact is likely to be smaller than for paclitaxel, since the amounts of this vehicle co-administered are much less. For example, cyclosporin A is known to exhibit disproportional dose-AUC relationships, and it can be postulated this phenomenon is related, in part, to the presence of CrEL and micellar entrapment similar to that described here for paclitaxel, in addition to saturable binding to erythrocytes (19). It can also be anticipated that CrEL may alter the distribution of some compounds co-administered with paclitaxel. For example, clinically significant pharmacokinetic interactions between paclitaxel and doxorubicin have been reported (10), and both preclinical and clinical evidence suggests that these are directly attributable to CrEL (4,21,37). Similarly, differences in the plasma concentration-time profile of CrEL imparted by the paclitaxel administration schedule could also account for the apparent sequence and schedule-dependent effect of paclitaxel on doxorubicin pharmacokinetics (5).

In conclusion, we have shown that micellar encapsulation of paclitaxel in CrEL can, as a single principle, explain both the nonlinear distribution and nonlinear elimination processes of paclitaxel in plasma. This

micellar formation is likely to affect other hydrophobic drugs either those formulated in CrEL themselves, or those given in combination with paclitaxel, leading to changes in the pharmacokinetic and pharmacodynamic behavior of these compounds that can have significant clinical implications. Our pharmacokinetic model could accurately describe the generated paclitaxel blood and plasma data, and will help guide further development and refinement of clinical protocols. Future studies will focus on defining the exposure measure best linked to paclitaxel-induced antitumor effects and toxicities, not only to expand our understanding of paclitaxel pharmacology but to provide insight into considerations of optimal dosage and schedule of drug administration. Ultimately, a more rational and selective chemotherapy with paclitaxel should be possible and thus the treatment of cancer improved.

REFERENCES

1. Bauer LA, Blouin RA: Phenytoin Michaelis-Menten pharmacokinetics in Caucasian paediatric patients. Clin Pharmacokin 8:545, 1983
2. Brouwer E, Verweij J, Hauns B, Loos WJ, Nooter K, Mross K, Stoter G, Sparreboom A: Linearized colorimetric assay for Cremophor EL: application to pharma-cokinetics after one-hour paclitaxel infusions. Anal Biochem 261:198, 1998
3. Chen T-L, Passos-Coelho JL, Noe DA, Kennedy J, Black KC, Colvin OM, Grochow LB: Nonlinear pharmacokinetics of cyclophosphamide in patients with metastatic breast cancer receiving high-dose chemotherapy followed by autologous bone marrow transplantation. Cancer Res 55:810, 1995
4. Colombo T, Parisi I, Zucchetti M, Sessa C, Goldhirsch A, D'Incalci M: Pharmacokinetic interactions of paclitaxel, docetaxel and their vehicles with doxorubicin. Ann Oncol 10:391, 1999
5. Duska LR, Penson R, Supko JG, Finkelstein DM, Makastorsis T, Gallagher J, Borden K, Goodman A, Fuller AF, Nikrui N, Seiden MV: A phase I study of continuous infusion doxorubicin and paclitaxel chemotherapy with granulocyte colony-stimulating factor for relapsed epithelial ovarian cancer. Clin Cancer Res 5:1299, 1999
6. Eisenhauer EA, Ten Bokkel Huinink WW, Swenerton KD, Gianni L, Myles J, Van der Burg MEL, Kerr I, Vermorken JB, Buser K, Colombo N, Bacon M, Santabárbara M, Onetto N, Winograd B, Canetta R: European-Canadian randomized trial of paclitaxel in relapsed ovarian cancer: high-dose *versus* low-dose and long *versus* short infusion. J Clin Oncol 12:2654, 1994
7. Ellis AG, Webster LK: Inhibition of paclitaxel elimination in the isolated perfused rat liver by Cremophor EL. Cancer Chemother Pharmacol 43:13, 1999
8. Gelderblom H, Verweij J, Brouwer E, Pillay M, De Bruijn P, Nooter K, Stoter G, Sparreboom A: Disposition of [*G*-^{3}H]paclitaxel and Cremophor EL in a patient with severely impaired renal function. Drug Metab Dispos 27:1300, 1999

9. Gianni L, Kearns CM, Giani A, Capri G, Vigano L, Locatelli A, Bonadonna G, Egorin MJ: Nonlinear pharmacokinetics and metabolism of paclitaxel and its pharmacokinetic/pharmacodynamic relationships in humans. J Clin Oncol 13:180, 1995
10. Gianni L, Vigano L, Locatelli A, Capri G, Giani A, Tarenzi E, Bonadonna G: Human pharmacokinetic characterization and *in vitro* study of the interaction between doxorubicin and paclitaxel in patients with breast cancer. J Clin Oncol 15:1906, 1997
11. Hempel G, Karlsson MO, De Alwis DP, Toublanc N, McNay J, Schaefer HG: Population pharmacokinetic-pharmacodynamic modeling of moxonidine using 24-hour ambulatory blood pressure measurements. Clin Pharmacol Ther 64:622, 1998
12. Huizing MT, Keung AC, Rosing H, Van der Kuij V, Ten Bokkel Huinink WW, Mandjes IM, Dubbelman AC, Pinedo HM, Beijnen JH: Pharmacokinetics of paclitaxel and metabolites in a randomized comparative study in platinum-pretreated ovarian cancer patients. J Clin Oncol 11:2127, 1993
13. Karlsson MO, Molnar V, Bergh J, Freijs A, Larsson R: A general model for time-dissociated pharmacokinetic-pharmacodynamic relationship exemplified by paclitaxel myelosuppression. Clin Pharmacol Ther 63:11, 1998
14. Karlsson MO, Molnar V, Freijs A, Nygren P, Bergh J, Larsson R: Pharmacokinetic models for saturable distribution of paclitaxel. Drug Metab Dispos 27:1220, 1990
15. Kearns CM, Gianni L, Egorin MJ: Paclitaxel pharmacokinetics and pharmacodynamics. Semin Oncol 22:16, 1995
16. Kessel D: Properties of Cremophor EL micelles probed by fluorescence. Photochem Photobiol 56:447, 1992
17. Knemeyer I, Wientjes MG, Au JL-S: Cremophor reduces paclitaxel penetration into bladder wall during intravesical treatment. Cancer Chemother Pharmacol 44:241, 1999
18. Kumar GN, Walle UK, Bhalla KN, Walle T: Binding of taxol to human plasma, albumin and alpha 1-acid glycoprotein. Res Commun Chem Pathol Pharmacol 80:337, 1993
19. Legg B, Rowland M: Saturable binding of cyclosporin A to erythrocytes: estimation of binding parameters in renal transplant patients and implications for bioavailability assessment. Pharm Res 5:80, 1988
20. Ludden TM: Nonlinear pharmacokinetics. Clin Pharmacokinet 20: 429, 1991
21. Millward MJ, Webster LK, Rischin D, Stokes KH, Toner GC, Bishop JF, Olver IN, Linahan BM, Linsenmeyer ME, Woodcock DM: Phase I trial of Cremophor EL with bolus doxorubicin. Clin Cancer Res 4:2321, 1998
22. Minami H, Sasaki Y, Saijo N, Ohtsu T, Fujii H, Igarashi T, Itoh K: Indirect-response model for the time course of leukopenia with anticancer drugs. Clin Pharmacol Ther 64:511, 1998
23. Schoemaker RC, Cohen AF: Estimating impossible curves using NONMEM. Br J Clin Pharmacol 42:283, 1996
24. Sonnichsen DS, Hurwitz CA, Pratt CB, Shuster JJ, Relling MV: Saturable pharmacokinetics and paclitaxel pharmacodynamics in children with solid tumors. J Clin Oncol 12:532, 1994
25. Sparreboom A, Huizing MT, Boesen JJB, Nooijen WJ, Van Tellingen O, Beijnen JH: Isolation, purification and biological activity of mono- and dihydroxylated paclitaxel metabolites from human faeces. Cancer Chemother Pharmacol 236:299, 1995

26. Sparreboom A, Van Tellingen O, Nooijen WJ, Beijnen JH: Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle Cremophor EL. Cancer Res 56:2112, 1996
27. Sparreboom A, Van Tellingen O, Nooijen WJ, Beijnen JH: Preclinical pharmacokinetics of paclitaxel and docetaxel. Anti-Cancer Drugs 9:1, 1998
28. Sparreboom A, De Bruijn P, Nooter K, Loos WJ, Stoter G, Verweij J: Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. J Chromatogr 705:159, 1998
29. Sparreboom A, Loos WJ, Verweij J, De Vos AI, Van der Burg MEL, Stoter G, Nooter K: Quantitation of Cremophor EL in human plasma samples using a colorimetric dye-binding microassay. Anal Biochem 255:171, 1998
30. Sparreboom A, Verweij J, Van der Burg MEL, Loos WJ, Brouwer E, Vigano L, Locatelli A, De Vos AI, Nooter K, Stoter G, Gianni L: Disposition of Cremophor EL in humans limits the potential for modulation of the multidrug resistance phenotype *in vivo*. Clin Cancer Res 4:1937, 1998
31. Sparreboom A, Van Zuijlen L, Brouwer E, Loos WJ, De Bruijn P, Gelderblom H, Pillay M, Nooter K, Stoter G, Verweij J: Cremophor EL-mediated alteration of paclitaxel distribution in human blood: clinical pharmacokinetic implications. Cancer Res 59:1454, 1999
32. Sykes E, Woodburn K, Decker D, Kessel D: Effects of Cremophor EL on distribution of Taxol to serum lipoproteins. Br J Cancer 70:401, 1994
33. Van Tellingen O, Huizing MT, Nannan Panday VR, Schellens JHM, Nooijen WJ, Beijnen JH: Cremophor EL causes (pseudo-) non-linear pharmacokinetics of paclitaxel in patients. Br J Cancer 81:330, 1990
34. Van Tellingen O, Beijnen JH, Verweij J, Scherrenburg EJ, Nooijen WJ, Sparreboom A: Rapid esterase-sensitive breakdown of polysorbate 80 and its impact on the plasma pharmacokinetics of docetaxel and metabolites in mice. Clin Cancer Res 5:2918, 1999
35. Van Zuijlen L, Gianni L, Verweij J, Mross K, Brouwer E, Loos WJ, Sparreboom A (2000) Interrelationships of paclitaxel disposition, infusion duration and Cremophor EL kinetics in cancer patients. Anti-Cancer Drugs 11:331, 2000
36. Wagner JG: Nonlinear pharmacokinetics. In: Fundamentals of clinical pharmacokinetics. Second edition. Drug Intelligence Publications, Inc., Hamilton, IL, p247, 1979
37. Webster LK, Cosson EJ, Stokes KH, Millward MJ: Effect of the paclitaxel vehicle, Cremophor EL, on the pharmacokinetics of doxorubicin and doxorubicinol in mice. Br J Cancer 73:522, 1996

Summary and conclusions

Over the last 10 years the taxanes paclitaxel and docetaxel, both belonging to the so-called naturally occurring drugs because of their origin from the yew trees, have obtained a prominent place in cancer treatment. Many publications have discussed their pharmacodynamic characteristics and pharmacokinetic behavior as well as attempts to increase activity by combining them with various other agents. Combined therapy also has to be evaluated on pharmacodynamic and pharmacokinetic modulations. This thesis highlights the last subject with specific attention to two particular problems: 1) the deliberate pharmacologic modulation of docetaxel by the new P-glycoprotein inhibitor R101933, and 2) the unintended pharmacokinetic modulation of paclitaxel by its formulation vehicle Cremophor EL.

P-glycoprotein and MRP (multidrug resistance-associated protein), both transmembrane transport proteins, have been identified to be involved in the phenomenon of multidrug resistance. The clinical relevance of these drug transporters and the attempts to inhibit them are reviewed in **chapter 1** with specific focus on P-glycoprotein and its intentionally designed 'second generation' inhibitors. Potential explanations of the limited clinical success rate of these inhibitors are given with pharmacokinetic interaction as the first and maybe most important one. This interaction results in unintended and unacceptable increases in severity and frequency of side-effects of the co-administered anticancer drug due to competition at the level of cytochrome-P450 3A isozymes, the major metabolic route of many drugs. Recommendations for the development of new P-glycoprotein inhibitors and for the design of future studies with these agents are provided.

Chapter 2 describes a phase I and pharmacokinetic study with the new P-glycoprotein inhibitor R101933, the major metabolic pathway of which is cytochrome-P450 3A4 independent, in combination with docetaxel. Fifteen patients received oral R101933 alone at a dose escalated from 200 to 300 mg twice daily (cycle 0), an escalating intravenous dose of docetaxel (60, 75, 100 mg/m^2) as a 1-hour infusion (cycle 1), and the combination of both (cycle 2 and further). Dose limiting toxicity consisting of mucositis and neutropenic fever was reached at the docetaxel dose of 100 mg/m^2 combined with R101933 300 mg twice daily, and the maximum tolerated dose was set at docetaxel 100 mg/m^2 with R101933 at a dose of

200 mg twice daily. Plasma concentrations of R101933 achieved in patients were in the same range as those required in preclinical rodent models to overcome paclitaxel resistance. The plasma pharmacokinetics of docetaxel were not influenced by the R101933 co-treatment at any dose level tested, as indicated by plasma clearance values of 26.5 ± 7.78 L/h/m^2 and 23.4 ± 4.52 L/h/m^2 (P=0.15) in cycle 1 and 2, respectively. These findings indicated that the contribution of a P-glycoprotein inhibitor to the activity of anticancer chemotherapy can now be assessed in patients independent of its effect on anticancer-drug pharmacokinetics.

In five patients, apart from plasma samples also complete stool collections for docetaxel analysis were obtained for up to 31 h after the start of drug administration during cycle 1 and 2. This way the role of P-glycoprotein in the metabolic disposition of docetaxel in humans could be studied as described in **chapter 3.** The cumulative fecal excretion of docetaxel was markedly reduced from 8.47±2.14% (mean±SD) after dosing the single agent to less than 0.5% in the presence of R101933 (P=0.0016). Levels of the major cytochrome P450 3A4-mediated metabolites of docetaxel in feces were significantly increased following combination treatment with R101933 (P=0.010), indicating very prominent and efficient detoxification of re-absorbed docetaxel into hydroxylated compounds before reaching the systemic circulation. The similarity of the terminal disposition phases in plasma of docetaxel between treatment courses as shown in chapter 2 indicated that the reduced fecal excretion of the parent drug in the patients receiving R101933 is unlikely related to diminished (P-glycoprotein-mediated) biliary secretion. It is more likely a consequence of intestinal P-glycoprotein playing a principal role in the fecal elimination of docetaxel by modulating re-absorption of the drug following hepatobiliary secretion. In addition, the results indicate that inhibition of P-glycoprotein activity in normal tissues by effective modulators, and the physiological and pharmacological consequences of this treatment, cannot be predicted based on plasma drug monitoring alone.

The study of docetaxel in combination with the orally administered R101933 described in chapter 2 indicated that a further increase of the level of P-glycoprotein inhibition could not be reached by oral administration due to low oral bioavailability of R101933. Therefore, **chapter 4** presents a

study to assess the feasibility of combining docetaxel with intravenously administered R101933. Treatment consisted of R101933 i.v. alone at a dose escalated from 250 to 500 mg on day 1 (cycle 0), docetaxel 100 mg/m^2 given as a 1-hour infusion on day 8 (cycle 1) and the combination given every three weeks thereafter (cycle 2 and further). Twelve patients were entered of whom nine received the combination treatment. Single treatment with R101933 i.v. was associated with minimal toxicity consisting of temporary drowsiness and somnolence. Dose limiting toxicity consisting of neutropenic fever was seen in cycle 1 as well as in cycle 2 or further at both dose levels of R101933, and was due to docetaxel. The plasma pharmacokinetics of docetaxel did not change by co-administration of R101933 at both dose levels tested, as indicated by plasma clearance values of 22.5 ± 6.2 L/h/m^2 and 24.2 ± 7.4 L/h/m^2 (P=0.38) in cycle 1 and 2, respectively. Because of the observations reported in chapter 3, analysis of the fecal excretion of docetaxel was performed and showed a significant decrease after the combination treatment from 2.54 ± 2.05% to less than 1% of the administered dose of docetaxel, most likely due to inhibition of the intestinal P-glycoprotein also by i.v. administered R101933. Plasma concentrations of R101933 were similar in cycle 0 and cycle 2 and the achieved concentrations were capable of inhibiting P-glycoprotein in an *ex vivo* assay. It can be concluded that the combination of docetaxel 100 mg/m^2 i.v. and R101933 500 mg i.v. is feasible, lacks pharmacokinetic interaction in plasma, and with evidence of P-glycoprotein inhibition both in an *ex vivo* assay and *in vivo* by the indication of inhibition of intestinal P-glycoprotein.

As reviewed in chapter 1, many agents are known to be inhibitors of P-glycoprotein and Cremophor EL, the formulation vehicle of paclitaxel, is known to be one of these. This feature of Cremophor EL has been hypothesized as one of the explanations of the nonlinear pharmacokinetics of paclitaxel. In the second part of this thesis we report that a completely different cause underlies this phenomenon.

Chapter 5 reviews the biological and pharmacological properties of the non-ionic surfactants Cremophor EL and Tween 80. Both are used as formulation vehicles of many (anticancer) agents including paclitaxel and docetaxel. Their influence on the disposition of the solubilized drugs, with focus on Cremophor EL and paclitaxel, and of concomitantly administered

drugs is discussed in detail. The ability of the surfactants to form micelles in aqueous solution as well as biological fluids (e.g. plasma) appears to be of great importance with respect to the pharmacokinetic behavior of the formulated drugs. Due to drug entrapment in the micelles, plasma concentrations and clearance of free drug change significantly leading to alteration in pharmacodynamic characteristics. Some perspectives related to further studies and development of alternative methods of administration are given.

To give the initial impetus to the correct explanation of the nonlinearity of paclitaxel the *in vitro* cellular distribution of paclitaxel in human blood and the influence of Cremophor EL was evaluated as is presented in **chapter 6**. In the absence of Cremophor EL, the blood:plasma concentration ratio of paclitaxel was 1.07 ± 0.004 (mean ± SD). The addition of Cremophor EL at concentrations corresponding to peak plasma levels achieved after the administration of paclitaxel (175 mg/m^2 i.v. over a 3-h period; i.e. 0.50%) resulted in a significant decrease in the concentration ratio to 0.690 ± 0.005 ($P<0.05$). Kinetic experiments revealed that this effect was due to reduced erythrocyte uptake of paclitaxel by polyoxyethyleneglycerol triricinoleate, the major compound present in Cremophor EL. Using equilibrium dialysis, it was shown that the affinity of paclitaxel for tested matrices was (in decreasing order) Cremophor EL > plasma > human serum albumin, with Cremophor EL present at or above the critical micellar concentration (approx. 0.01%). These *in vitro* findings demonstrated a profound alteration of paclitaxel accumulation in erythrocytes caused by a trapping of the compound in Cremophor EL micelles, thereby reducing the free drug fraction available for cellular partitioning. It was proposed that the nonlinearity of paclitaxel plasma disposition in patients reported previously should be reevaluated prospectively by measuring the free drug fractions and whole blood:plasma concentration ratios.

Before this prospective study could be performed more had to be known about the pharmacokinetics of Cremophor EL. Therefore, as reported in chapter 7, the clinical pharmacokinetics of Cremophor EL were studied and the interrelationships of paclitaxel disposition, infusion duration and Cremophor EL kinetics were examined. The Cremophor EL plasma

clearance, studied in 17 patients for a total of 28 courses, was time-dependent and increased significantly with prolongation of the infusion duration from 1 to 3 to 24 h ($P<0.03$). An indirect response model, applied based on use of a Hill function for Cremophor EL concentration-dependent alteration of *in vivo* blood distribution of paclitaxel, was used to fit experimental data of the 3-h infusion ($r^2=0.733$; $P=0.00001$). Simulations for 1- and 24-h infusions using predicted parameters and Cremophor EL kinetic data revealed that both short and prolonged administration schedules induce a low relative net change in paclitaxel blood distribution. Our pharmacokinetic/pharmacodynamic model demonstrated that Cremophor EL causes disproportional accumulation of paclitaxel in plasma in a 3-h schedule, but is unlikely to affect drug pharmacokinetics in this manner with alternative infusion durations.

In **chapter 8** the prospective re-evaluation of the linearity of paclitaxel disposition in patients using whole blood and plasma analysis is reported, and a new pharmacokinetic model to describe the data is defined. Seven patients with solid tumors were treated with paclitaxel infused over 3 h, each at consecutive 3-weekly dose levels of 225, 175 and 135 mg/m^2 (Cremophor EL dose level, 18.8, 14.6, and 11.3 mL/m^2, respectively). Blood and plasma samples were collected up to 24 h after start of infusion, and analyzed by high-performance liquid chromatography. Paclitaxel peak levels and areas under the curve in whole blood increased linearly with dose, whereas plasma levels showed substantial deviation from linearity. This was shown to be caused by a Cremophor EL-concentration dependent decrease in paclitaxel uptake in blood cells, as reflected by the blood:plasma concentration ratios which altered significantly from 0.83 ± 0.11 (at 135 mg/m^2) to 0.68 ± 0.07 (at 225 mg/m^2). It is concluded that the nonlinear disposition of paclitaxel is related to paclitaxel-dose related levels of the formulation vehicle Cremophor EL, leading to disproportional drug accumulation in the plasma fraction. The developed pharmacokinetic model could accurately describe the data, and will help guide future development and refinement of clinical protocols, especially in defining the exposure measure best linked to paclitaxel effects and toxicities.

Final conclusions and future perspectives

Evaluation of plasma samples to determine the pharmacokinetics of a drug and to exclude a potential pharmacokinetic interaction in case of combined treatment should be a standard procedure at least in all phase I studies. On the other hand the above summarized studies demonstrated that plasma drug monitoring alone can not predict all physiological and pharmacological consequences of the administered treatment.

This is examplified in the studies combining docetaxel with an inhibitor of P-glycoprotein, where analysis of the fecal excretion of docetaxel revealed the importance of intestinal P-glycoprotein in the disposition of this drug. This finding confirmed the physiological role of P-glycoprotein as one of the biochemical barriers to restrict oral bioavailability of xenobiotics such as the 'naturally occurring' anticancer drugs. However, our findings also demonstrate that inhibiting P-glycoprotein alone is not enough to enhance oral bioavailability due to an efficient detoxification of the absorbed anticancer drug by cytochrome P450 isozymes present in the intestinal cells. Attempts to increase the oral bioavailability of several up to now intravenously delivered anticancer drugs such as the taxane drugs and the topoisomerase I inhibitors by co-administration of a P-glycoprotein inhibitor, have to make use of an inhibitor known to compete with the metabolism of the anticancer drug at the level of cytochrome P450 isozymes. A clinical trial to apply this concept to enhance the oral bioavailability of irinotecan, a topoisomerase I inhibitor, is in progress at our institute.

In the past, plasma pharmacokinetics of paclitaxel revealed a nonlinear disposition of this drug leading to extended speculations about the cause and the consequences for daily clinical practice. However, we showed that the cause is related to the limitations of analysing plasma samples alone. The plasma comprises a relatively small fraction of the total volume available for drug distribution and we showed that paclitaxel is equally distributed over plasma and blood cells before delivery to tissues and tumor. However, above a certain plasma concentration of Cremophor EL, the formulation vehicle necessary to enable parenteral administration of paclitaxel, the uptake of the drug in blood cells decreased due to entrapment in Cremophor EL micelles. Consequently, evaluation of plasma samples will show a disproportional increase in the concentration of paclitaxel, but evaluation of whole blood, i.e. plasma plus blood cells, will

reveal a linear disposition. Based on these findings we developed a model that accurately described the pharmacokinetics of paclitaxel at all dose levels studied and that provides a basis for refinement of future clinical protocols such that a certain concentration-time profile of free (unbound) paclitaxel can be targeted. Moreover, using this model, we are presently involved in a re-evaluation of the relationships between free-drug concentration-time profiles and paclitaxel-induced toxicity profiles, to improve the description of the concentration-response relationship and to predict more accurately the ultimate effect of dose and schedule variation.

Samenvatting en conclusies

De taxanen paclitaxel en docetaxel zijn middelen tegen kanker (cytostatica) die behoren tot de 'natuurlijk voorkomende' geneesmiddelen omdat ze afkomstig zijn van taxusbomen. Paclitaxel werd oorspronkelijk onttrokken aan de bast van de *Taxus brevifolia*; docetaxel werd geïsoleerd uit de naalden van de *Taxus baccata*. Sinds ongeveer tien jaar nemen beide taxanen een belangrijke plaats in bij de behandeling van patiënten met kanker. Veel onderzoek is gedaan naar de werking van deze geneesmiddelen (farmacodynamie) en naar de verwerking van deze stoffen door het lichaam (farmacokinetiek). Daarnaast is uitgebreid onderzocht of de werking van deze geneesmiddelen verbeterd kan worden door toediening in combinatie met andere geneesmiddelen, al dan niet andere cytostatica. Hierbij is het van belang de wederzijdse beïnvloeding te onderzoeken, niet alleen met betrekking tot de werking, maar ook ten aanzien van de verwerking van de gecombineerde middelen door het lichaam. Dit laatste aspect is het onderwerp van dit proefschrift toegespitst op twee specifieke problemen: 1) de gevolgen voor de farmacokinetiek van docetaxel van de bewust nagestreefde farmacologische beïnvloeding van dit taxaan door de nieuwe remmer van P-glycoproteïne R101933 en 2) de onbedoelde invloed op de farmacokinetiek van paclitaxel door Cremophor EL, het middel waarin paclitaxel is opgelost om toediening via een infuus mogelijk te maken.

P-glycoproteïne speelt een belangrijke rol bij multidrugresistentie. Multidrugresistentie is het fenomeen dat verantwoordelijk wordt geacht voor de aanwezigheid of het ontwikkelen van ongevoeligheid van kankercellen voor met name 'natuurlijk voorkomende' cytostatica. In dat geval zijn er in de celmembraan eiwitten aanwezig die passief binnengedrongen cytostatica direct naar buiten pompen en hiermee de celdodende werking van de cytostatica voorkomen. Van de klinische relevantie van twee van deze 'uitscheidingspompen', te weten P-glycoproteïne en MRP (multidrug resistance-associated protein), en van de pogingen om deze pompen te remmen wordt een overzicht gegeven in **hoofdstuk 1**. De nadruk wordt hierbij gelegd op P-glycoproteïne en op de specifiek ontwikkelde tweedegeneratie remmers van deze pomp. Mogelijke verklaringen voor het beperkte klinische succes van deze remmers worden gegeven. De belangrijkste verklaring lijkt te zijn dat behandeling met de combinatie van een cytostaticum en een P-glycoproteïneremmer vrijwel altijd leidt tot een toename van de bijwerkingen van het cytostaticum. Dit is

het gevolg van beïnvloeding van de farmacokinetiek van het cytostaticum door competitie met de gelijktijdig toegediende P-glycoproteïneremmer op het niveau van de enzymen die verantwoordelijk zijn voor de stofwisseling van vele middelen (cytochroom P450-isozymen). Dit leidt tot een onbedoelde toename van de concentratie van het cytostaticum in het lichaam. Er worden richtlijnen geformuleerd waaraan de ontwikkeling van nieuwe P-glycoproteïneremmers en waaraan het ontwerp van toekomstige onderzoeken met deze middelen moeten voldoen.

In **hoofdstuk 2** worden de resultaten beschreven van een fase I en farmacokinetisch onderzoek naar de behandeling met docetaxel in combinatie met de nieuwe remmer van P-glycoproteïne R101933. De stofwisseling van R101933 is grotendeels onafhankelijk van cytochroom P450 3A4. Vijftien patiënten deden mee aan dit onderzoek. Voor hen was docetaxel op dat moment het middel van keus of er waren geen andere behandelingsmogelijkheden (meer) aanwezig. In kuur 0 werd alleen R101933 gegeven als drank in een dosering die begon met 200 mg tweemaal daags gedurende vijf dagen. Toen dit goed verdragen bleek te worden werd de dosering verhoogd naar 300 mg tweemaal daags gedurende vijf dagen. In kuur 1 werd docetaxel intraveneus (iv.) toegediend door middel van een 1-uur durend infuus in toenemende doseringen (60, 75, 100 mg/m^2). Hierna werd elke drie weken de combinatiebehandeling gegeven waarbij docetaxel op de derde dag na de start van de inname van R101933 werd toegediend (kuur 2 en verder). De dosisbeperkende bijwerkingen bestonden uit slijmvliesontsteking en koorts optredend in de periode van een tekort aan witte bloedlichaampjes. Deze werden bereikt bij een dosis docetaxel van 100 mg/m^2 in combinatie met een dosis R101933 van 300 mg tweemaal daags. De aanbevolen doseringen voor vervolg-onderzoek met de combinatiebehandeling werden dan ook vastgesteld op docetaxel 100 mg/m^2 en R101933 200 mg tweemaal daags. De concentraties R101933 die bereikt werden in het plasma van de patienten lagen in dezelfde orde van grootte als die welke noodzakelijk waren in preklinische onderzoeken bij knaagdieren om de resistentie van cellen voor paclitaxel tegen te gaan. De plasmaklaring van docetaxel veranderde niet door de gelijktijdige toediening van R101933 bij de geteste doseringen en bedroeg 26,5 ± 7,78 l/h/m^2 en 23,4 ± 4,52 l/h/m^2 (P=0,15) in respectievelijk kuur 1 en 2. Dit geeft aan dat de farmacokinetiek van

docetaxel zoals gemeten in plasma niet werd beïnvloed door R101933, wat betekent dat nu voor het eerst zuiver kan worden beoordeeld wat de bijdrage kan zijn van een remmer van P-glycoproteïne aan de activiteit van een cytostaticum.

Bij vijf patiënten werden niet alleen plasmamonsters onderzocht, maar werd ook de ontlasting gespaard vanaf de start van het infuus met docetaxel tot 31 uur hierna tijdens kuur 1 en 2. Hiermee kon een meer volledig beeld verkregen worden van de rol die P-glycoproteïne heeft in de distributie in en eliminatie uit het lichaam van docetaxel, zoals blijkt uit de resultaten beschreven in **hoofdstuk 3**. De cumulatieve fecale uitscheiding van onveranderd docetaxel bedroeg na kuur 1 (docetaxel alleen) 8,47 ± 2,14% (gemiddelde ± SD) van de dosis en nam duidelijk af tot minder dan 0,5% na toediening van docetaxel in combinatie met R101933 ($P=0,0016$). De concentraties in de ontlasting van de belangrijkste stofwisselingsproducten van docetaxel, die ontstaan door omzetting via cytochroom P450 3A4, waren significant toegenomen na de combinatiebehandeling ($P=0,010$). Dit laatste betekent dat er sprake lijkt te zijn van een efficiënte ontgifting van het via de darm heropgenomen onveranderde docetaxel voordat het de algemene circulatie bereikt. In samenhang met eerdere bevindingen in plasma, beschreven in hoofdstuk 2, betekent dit dat de verminderde fecale uitscheiding van onveranderd docetaxel onder invloed van R101933 niet het gevolg is van vermindering van de (P-glycoproteïne-afhankelijke) uitscheiding via de galwegen, maar vooral veroorzaakt wordt door de aanwezigheid van P-glycoproteïne in de darmen. Op grond van deze resultaten kon dan ook geconcludeerd worden dat de aanwezigheid van P-glycoproteïne in de darmen een belangrijke rol speelt bij de eliminatie van docetaxel via de darmen omdat het de heropname van docetaxel remt nadat het is uitgescheiden via de galwegen. Meer in het algemeen betekenen deze bevindingen dat de fysiologische en farmacologische gevolgen van remming van P-glycoproteïne in normaal weefsel niet kan worden voorspeld louter op basis van metingen in plasma.

Uit het onderzoek naar de combinatiebehandeling van docetaxel met R101933 oraal, zoals beschreven in hoofdstuk 2, bleek dat sprake was van een lage biologische beschikbaarheid van R101933. Dit betekende dat geen verdere remming van P-glycoproteïne bereikt kon worden door middel van

verhoging van de orale dosering. Daarom werd een nieuwe studie opgezet naar de haalbaarheid van behandeling met docetaxel gecombineerd met intraveneus toegediend R101933. De resultaten hiervan worden beschreven in **hoofdstuk 4**. De behandeling bestond uit toediening van alleen R101933 iv. in een dosering die in de loop van het onderzoek toenam van 250 tot 500 mg op dag 1 (kuur 0), docetaxel 100 mg/m^2 als 1-uursinfuus op dag 8 (kuur 1) en de combinatiebehandeling elke drie weken hierna (kuur 2 en verder). Twaalf patienten deden mee aan dit onderzoek van wie negen de combinatiebehandeling kregen. Na toediening van alleen R101933 werden geringe bijwerkingen gezien voornamelijk bestaande uit voorbijgaande slaperigheid. De dosisbeperkende bijwerking bestond uit koorts, optredend in de periode van een tekort aan witte bloedlichaampjes, en werd zowel in kuur 1 als in kuur 2 en de vervolgkuren gezien en zowel na R101933 250 mg als na 500 mg. De farmacokinetische parameters van docetaxel in plasma veranderden niet onder invloed van R101933 zoals onder andere bleek uit de klaring die gelijk bleef met waarden van 22,5 ± 6,2 l/h/m^2 en 24,2 ± 7,4 l/h/m^2 (P=0,38) in respectievelijk kuur 1 en 2. Naar aanleiding van de bevindingen beschreven in hoofdstuk 3, werd ook nu de fecale uitscheiding van docetaxel en zijn stofwisselingsproducten gemeten. Overeenkomstig de eerdere resultaten nam de uitscheiding van onveranderd docetaxel significant af na de combinatiebehandeling van 2,54 ± 2,05% van de toegediende dosis docetaxel na kuur 1 tot minder dan 1% na kuur 2. Dit betekent dat waarschijnlijk ook na intraveneuze toediening van R101933 remming van P-glycoproteïne in de darmen wordt bewerkstelligd. De plasmaconcentraties van R101933 verschilden niet tussen kuur 0 en kuur 2 en waren voldoende om P-glycoproteïne te remmen zoals bleek uit een *ex vivo* assay. Geconcludeerd kon worden dat de behandeling met de combinatie docetaxel 100 mg/m^2 en R101933 500 mg iv. haalbaar was. Hierbij werd er geen wederzijdse beïnvloeding gevonden van de plasmafarmacokinetiek. Een *ex vivo* assay en de aanwijzing dat P-glycoproteïne in de darm geremd werd bevestigden dat remming van P-glycoproteïne bereikt werd.

In de literatuur zijn vele remmers van P-glycoproteïne beschreven zoals samengevat is in hoofdstuk 1. Ook Cremophor EL, het oplosmiddel van paclitaxel, staat bekend als een remmer van P-glycoproteïne. Deze eigenschap is aangedragen als één van de verklaringen voor het feit dat er

voor paclitaxel in plasma geen rechtlijnig verband bestaat tussen de toegediende dosis paclitaxel en de farmacokinetische parameters (niet-lineaire kinetiek). In de verdere hoofdstukken laten wij echter zien dat er een heel ander mechanisme aan dit fenomeen ten grondslag ligt.

In **hoofdstuk 5** wordt een overzicht gegeven van de biologische en farmacologische eigenschappen van de oppervlaktespanning-verlagende stoffen Cremophor EL en Tween 80. Beide worden gebruikt als oplosstoffen voor vele (antikanker-) geneesmiddelen inclusief paclitaxel (Cremophor EL) en docetaxel (Tween 80), maar beide stoffen zijn niet fysiologisch neutraal. Gedetailleerd wordt hun invloed op de farmacokinetiek en farmacodynamie van het opgeloste geneesmiddel besproken met nadruk op paclitaxel/Cremophor EL en op gelijktijdig hiermee toegediende andere geneesmiddelen. De eigenschap van Cremophor EL om micellen te vormen in zowel waterige oplossingen als biologische vloeistoffen (bv. plasma) lijkt van groot belang te zijn voor het farmacokinetische gedrag van het opgeloste geneesmiddel of een gelijktijdig toegediend ander geneesmiddel. Ten gevolge van opsluiting van het geneesmiddel in de micellen veranderen de plasmaconcentraties en de klaring van de vrije fractie van het geneesmiddel. Dit leidt aantoonbaar tot verandering in de farmaco-dynamische karakteristieken. Aan het eind van het overzicht worden enkele overwegingen gegeven met betrekking tot verdere ontwikkeling van en onderzoek naar alternatieve methoden voor de toediening van genees-middelen die nu opgelost moeten worden in Cremophor EL of Tween 80.

Als eerste aanzet voor het vinden van de juiste verklaring voor de niet-lineaire kinetiek van paclitaxel werd, zoals beschreven in **hoofdstuk 6**, in een 'reageerbuis' (*in vitro*) de verdeling van paclitaxel over de diverse componenten van bloed en de invloed van Cremophor EL hierop onderzocht. Het bleek dat de verhouding van de concentraties van paclitaxel in bloed en plasma 1,07 ± 0,004 (gemiddelde ± SD) was in afwezigheid van Cremophor EL. Na toevoeging van Cremophor EL, tot concentraties die overeenkwamen met de maximale plasmaconcentraties die bereikt worden na toediening van paclitaxel (175 mg/m^2 iv. in 3-h; d.i. 0,50%) aan patiënten, daalde de verhouding significant tot 0,690 ± 0,005 ($P<0,05$). Kinetiekexperimenten lieten zien dat dit effect veroorzaakt werd door vermindering van de opname van paclitaxel in de rode bloedlichaampjes (erythrocyten) ten gevolge van polyoxyethyleneglyceroltriricinoleaat, de

belangrijkste component van Cremophor EL. Door middel van evenwichtsdialyse werd duidelijk dat de affiniteit van paclitaxel voor de onderzochte bestanddelen als volgt was weer te geven (in afnemende orde): Cremophor EL > plasma > menselijk serum albumine, waarbij de concentratie Cremophor EL gelijk was aan of groter was dan de kritische micelconcentratie (ongeveer 0,01%). Deze *in vitro*-bevindingen bevestigden dat de verandering van de opname van paclitaxel in de erythrocyten werd veroorzaakt door opsluiting van het geneesmiddel in Cremophor EL-micellen. Dit heeft tot gevolg dat de vrije fractie van paclitaxel afneemt zodat minder geneesmiddel beschikbaar is voor cellulaire verdeling. Om te laten zien dat dit ook bij patiënten een rol speelt en dat dit de verklaring is voor het bestaan van niet-lineaire kinetiek van paclitaxel wordt de aanbeveling gedaan prospectief onderzoek op te zetten waarin de bloed:plasma verhouding van de concentratie paclitaxel opnieuw prospectief bepaald moet worden en waarin zo mogelijk de vrije fractie paclitaxel in bloed wordt gemeten.

Voordat deze prospectieve studie kon plaatsvinden was het van belang meer kennis te verkrijgen van de farmacokinetiek van Cremophor EL. In **hoofdstuk 7** wordt dan ook het onderzoek beschreven naar de farmacokinetiek van Cremophor EL en naar de samenhang tussen deze kinetiek, de duur van het infuus en de verdeling van paclitaxel in het bloed. De klaring van Cremophor EL, bestudeerd in 17 patiënten die totaal 28 kuren paclitaxel kregen, bleek afhankelijk te zijn van de infusieduur en significant toe te nemen met een toename van de infusieduur van 1 naar 3 naar 24 uur ($P<0,03$). Door middel van een mathematisch model gebaseerd op de Cremophor EL concentratieafhankelijke verandering van de verdeling van paclitaxel in bloed konden de experimentele gegevens van de 3-uursinfusie beschreven worden ($r^2=0,733$; $P=0,00001$). Simulaties voor 1- en 24-uursinfusies, gebruikmakend van voorspelde parameters en gegevens van de kinetiek van Cremophor EL, gaven aan dat deze beide infusieduren leidden tot een relatief lage nettoverandering in de verdeling van paclitaxel over de bloedbestanddelen. Het model laat dus zien dat, indien er sprake is van toediening van paclitaxel/Cremophor EL in drie uur, Cremophor EL een onevenredige ophoping van paclitaxel in plasma veroorzaakt. Bij alternatieve infusieduren wordt de farmacokinetiek van het geneesmiddel waarschijnlijk niet op deze manier beïnvloed.

In **hoofdstuk 8** wordt de voorgestelde prospectieve studie beschreven naar de verklaring voor de niet-lineaire kinetiek van paclitaxel bij patienten. Hierbij werd gebruikgemaakt van bloed en plasma analyses. Tevens wordt een nieuw farmacokinetisch model gedefinieerd om de gegevens te kunnen beschrijven. Zeven patienten met een solide tumor werden behandeld met paclitaxel toegediend in drie uur, in de opeenvolgende dosering van 225, 175 en 135 mg/m^2 (Cremophor EL-concentratie respectievelijk 18,8, 14,6 en 11,3 ml/m^2) in een driewekelijks schema. Bloed- en plasmamonsters werden verzameld gedurende de 24 uur na de start van het infuus en werden geanalyseerd met behulp van hogedrukvloeistofchromatografie (HPLC). In bloed nam de maximale concentratie en de oppervlakte onder de curve van paclitaxel rechtlijnig toe met de toegediende dosis, terwijl de waarden in plasma van de kinetiekparameters een duidelijke afwijking van de rechte lijn vertoonden. Door berekening van de bloed/plasma-concentratieverhoudingen van paclitaxel, die significant daalden van 0,83 ± 0,11 (bij 135 mg/m^2) naar 0,68 ± 0,07 (bij 225 mg/m^2), werd duidelijk dat de niet-lineaire kinetiek in plasma werd veroorzaakt door een vermindering van de opname van paclitaxel in bloedcellen, die afhankelijk is van de aanwezige concentratie Cremophor EL. Dit leidt tot een onevenredige ophoping van het geneesmiddel in het plasma. Met het ontwikkelde farmacokinetische model konden nauwkeurig de gegevens worden weergegeven.

Conclusies en vooruitzichten

Onderzoek naar de farmacokinetiek van een geneesmiddel, op grond van analyse van afgenomen plasmamonsters, en het uitsluiten van een eventuele farmacokinetische interactie tussen geneesmiddelen onderling dient een geïntegreerd onderdeel van fase I-studies te zijn. Uit de onderzoeken beschreven in dit proefschrift blijkt echter ook dat het analyseren van louter plasmamonsters niet het gehele beeld van fysiologische en farmacologische gevolgen van de behandeling kan weergeven. Indien docetaxel gecombineerd wordt met een remmer van P-glycoproteïne blijkt onderzoek van de fecale uitscheiding van docetaxel een aanwijzing te geven voor het belang van de aanwezigheid van P-glycoproteïne in de darmen voor de distributie in en eliminatie uit het lichaam van dit geneesmiddel. Daarmee bevestigt deze bevinding de fysiologische rol van P-glycoproteïne als één van de barrières van het

lichaam die opname tegengaan van oraal binnengekregen giftige stoffen zoals de 'natuurlijk voorkomende' cytostatica. Daarnaast laten de beschreven onderzoeken echter zien dat het omzeilen van deze barrière door het toedienen van remmers van P-glycoproteïne niet voldoende is om de orale beschikbaarheid van cytostatica te vergroten. Indien er wel opname plaatsvindt volgt namelijk een efficiënte ontgifting van de opgenomen stof door cytochroom P450-isozymen die aanwezig zijn in de darmcellen. Voor studies naar het verbeteren van de orale biologische beschikbaarheid met behulp van een remmer van P-glycoproteïne van tot op heden intraveneus toegediende cytostatica, bijvoorbeeld taxanen en topoïsomerase I-remmers, moet dan ook gekozen worden voor een remmer die ook ingrijpt op de stofwisseling van het cytostaticum. Dit gebeurt indien er sprake is van competitie tussen remmer en cytostaticum op het niveau van de cytochroom P450-isozymen. Binnenkort zal in ons ziekenhuis een onderzoek uitgevoerd worden waarin dit principe zal worden toegepast om verbetering van de orale biologische beschikbaarheid van irinotecan, een topoisomerase I-remmer, te bereiken.

Uit vroegere onderzoeken naar de farmacokinetiek van paclitaxel was duidelijk geworden dat bij dit geneesmiddel sprake was van niet-lineaire kinetiek. Dit leidde tot uitgebreide discussies in de literatuur over de mogelijke oorzaak hiervan en over de gevolgen voor de klinische toepassing van paclitaxel. De studies beschreven in dit proefschrift laten echter zien dat de verklaring in verband staat met de beperkingen van het louter evalueren van plasmamonsters. Plasma omvat een relatief klein gedeelte van het totale volume dat een stof voor zijn verdeling over het lichaam ter beschikking heeft. Wij toonden aan dat paclitaxel tot aflevering aan weefsels en tumor gelijkelijk is verdeeld over plasma en bloedcellen. Wanneer echter de concentratie van Cremophor EL, de oplosstof van paclitaxel die nodig is om intraveneuze toediening mogelijk te maken, boven een bepaalde grens komt, vermindert de opname van paclitaxel in de bloedcellen ten gevolge van het ontstaan van Cremophor EL-micellen waarin paclitaxel wordt opgesloten. Als gevolg hiervan laten plasmamonsters, waarin de micellen zich bevinden, een onevenredige toename zien van de concentratie paclitaxel. Evaluatie van bloedmonsters - dit is plasma plus bloedcellen - laat echter een met de dosis evenredige toename van de concentratie paclitaxel zien. Gebaseerd op deze bevindingen werd een model ontwikkeld waarmee de farmacokinetiek van paclitaxel op de juiste

manier kon worden beschreven en waarmee protocollen voor toekomstige studies verbeterd kunnen worden zodat de vrije (niet-gebonden) fractie van paclitaxel het doel van de farmacokinetische studies gaat worden. Daarnaast vindt momenteel een herevaluatie plaats van de relatie tussen de vrije concentratie paclitaxel en de bijwerkingen van dit middel, zodat uiteindelijk het effect van veranderingen in dosis en toedieningsschema's beter voorspeld kan worden.

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CURRICULUM VITAE

Lia van Zuijlen werd op 16 januari 1961 geboren te Katwijk (ZH). In 1979 behaalde zij het VWO-diploma aan het Pieter Groen College te Katwijk. Na uitloting van de studie Geneeskunde begon zij in dit jaar met de studie Psychologie aan de Rijksuniversiteit Leiden. Hoewel zij de colleges van deze studie met plezier volgde, besloot zij toch na inloting in 1980 Geneeskunde te gaan studeren aan voornoemde universiteit. Tijdens de studie was zij werkzaam als student-assistent bij de vakgroep Kindergeneeskunde en als wetenschappelijk assistent bij de vakgroep Interne Geneeskunde. In augustus 1988 behaalde zij haar artsexamen.

Gedurende 2 jaar werkte zij als arts-assistent bij de afdelingen Interne Geneeskunde en Cardiologie van Ziekenhuis Rijnstate, locatie EG, te Arnhem (H. Lamers, L. van Kempen). In oktober 1990 startte zij haar opleiding tot internist in hetzelfde ziekenhuis op de locatie DH (Dr. J.M. Werre, Dr. L. Verschoor), welke in januari 1995 werd voortgezet in het Sint Radboud Ziekenhuis te Nijmegen (Prof. dr J. van der Meer). In oktober 1996 begon zij haar vervolgopleiding in het aandachtsgebied Medische Oncologie in het Academisch Ziekenhuis Rotterdam, locatie Daniel (Prof. dr G. Stoter). In deze periode kwam dit proefschrift tot stand. Momenteel is zij werkzaam als internist-oncoloog in dit ziekenhuis.

PUBLICATIONS

1. Van Wouwe JP, **Van Zuijlen C**: Changes of zinc values in children during malignant disease. Biological Trace Element Res 30:197-204, 1991
2. **Van Zuijlen C**, Oostendorp FMGM, Van Beusekom BR, Cools HJM, Bolk JH, Ligthart GJ: Increasing drug use in nursing homes. Neth J Med 132:1692-1695, 1988
3. **Van Zuijlen C**, Essink AWG, Van Leusen R, Bosch FH: Severe lactate acidosis due to treatment with metformine. Intensive Care Rev 10:86-89, 1995
4. **Van Zuijlen L**, Schellens JHM, Goey SH, Pronk LC, de Boer-Dennert MM, Loos WJ, Ma J, Stoter G, Verweij J: Phase I and pharmacologic study of the arotinoid Ro 40-8757 in combination with cisplatin and etoposide in patients with non-small cell lung cancer. Anti-Cancer Drugs 10:361-368, 1999
5. Sparreboom A, **Van Zuijlen L**, Brouwer E, Loos WJ, De Bruijn P, Gelderblom H, Pillay M, Nooter K, Stoter G, Verweij J: Cremophor EL-mediated alteration of paclitaxel distribution in human blood: Clinical pharmacokinetic implications. Cancer Res 59:1454-1457, 1999
6. Loos WJ, De Bruijn P, **Van Zuijlen L**, Verweij J, Nooter K, Stoter G, Sparreboom A: Determination of 5-fluorouracil in microvolumes of human plasma by solvent extraction and high-performance liquid chromatography. J Chromatogr B 735:293-297, 1999
7. **Van Zuijlen L**, Sparreboom A, Van Der Gaast A, Van Der Burg MEL, Van Beurden V, Bol CJ, Woestenborghs R, Palmer PA, Verweij J: The orally administered P-glycoprotein inhibitor R101933 does not alter the plasma pharmacokinetics of docetaxel. Clin Cancer Res 6:1365-1371, 2000
8. Eskens FALM, Greim GA, **Van Zuijlen L**, Wolff I, Denis LJ, Planting ASTh, Muskiet FA, Wanders J, Barbet NC, Choi L, Capdeville R, Verweij J, Hanauske A-R, Bruntsch U for the EORTC Early Clinical Studies Group: Phase I and pharmacologic study of weekly administration of the polyamine synthesis inhibitor SAM 486A (CGP 48 664) in patients with solid tumors. Clin Cancer Res 6:1736-1743, 2000
9. **Van Zuijlen L**, Nooter K, Sparreboom A, Verweij J: Development of multidrug-resistance convertors: Sense or nonsense? Invest New Drugs 18:205-220, 2000
10. **Van Zuijlen L**, Verweij J, Nooter K, Brouwer E, Stoter G, Sparreboom A: Role of intestinal P-glycoprotein in the plasma and fecal disposition of docetaxel in humans. Clin Cancer Res 6:2598-2603, 2000
11. **Van Zuijlen L**, Gianni L, Verweij J, Mross K, Brouwer E, Loos WJ, Sparreboom A: Interrelationships of paclitaxel disposition, infusion duration and Cremophor EL kinetics in cancer patients. Anti-Cancer Drugs 11:331-337, 2000
12. **Van Zuijlen L**, Verweij J, Sparreboom A: Role of formulation vehicles in taxane pharmacology. Invest New Drugs (in press)
13. **Van Zuijlen L**, Karlsson MO, Verweij J, Brouwer E, De Bruijn P, Nooter K, Stoter G, Sparreboom A: Pharmacokinetic modeling of paclitaxel encapsulation in Cremophor EL micelles. Cancer Chemother Pharmacol (in press)
14. **Van Zuijlen L**, Sparreboom A, van der Gaast A, Eskens FALM, Brouwer E, Bol CJ, de Vries R, Palmer PA, Verweij J: Disposition of docetaxel in the presence of P-glycoprotein inhibition by intravenous administration of R101933. Submitted

Abstracts

1. **Van Zuijlen L**, Sparreboom A, Bol CJ, Van Der Gaast A, Van Der Burg MEL, Palmer PA, Verweij J: R101933, an oral MDR-reversing agent that does not alter the pharmacokinetics of docetaxel in patients with solid tumors. Proc Am Ass Cancer Res #4376, 1999
2. **Van Zuijlen L**, Verweij J, Nooter K, Brouwer E, Stoter G, Sparreboom A: Role of intestinal MDR1 P-glycoprotein in the plasma disposition and fecal elimination pathways of docetaxel in humans. Clin Cancer Res 5(S):518, 1999
3. Sparreboom A, **Van Zuijlen L**, Verweij J, Mross K, Brouwer E, Gianni L: Interrelationships of paclitaxel disposition, infusion duration and Cremophor EL kinetics in cancer patients. Proc AACR-NCI-EORTC Int Conf 1:112, 1999
4. **Van Zuijlen L**, Eskens FALM, Bridgewater J, Sparreboom A, Sklenar I, Planting AST, Choi L, Mueller C, Capdeville R, Ledermann JA, Verweij J: The polyamine synthesis inhibitor SAM486A in combination with 5-FU/LV in metastatic colorectal cancer (MCC): Results of a phase I and pharmacokinetic study. Proc Am Soc Clin Oncol #751, 2000
5. Sparreboom A, Verweij J, **Van Zuijlen L**, and Karlsson MO: A pharmacokinetic model for micellar encapsulation of paclitaxel in Cremophor EL. Proc Am Assoc Cancer Res 41:522, 2000
6. **Van Zuijlen L**, Sparreboom A, Brouwer E, Van Der Gaast A, Eskens FALM, Bol CJ, De Vries R, Palmer PA, Verweij J: Disposition of docetaxel in the presence of confirmed P-glycoprotein inhibition through intravenous R101933 administration. Proc AACR-NCI-EORTC Int Conf #187, 2000