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Original article

Differential modulation of cisplatin accumulation in leukocytes and tumor cell lines by the paclitaxel vehicle Cremophor EL

A. I. de Vos,[1] K. Nooter,[1] J. Verweij,[1] W. J. Loos,[1] E. Brouwer,[1] P. de Bruijn,[1] E. J. Ruijgrok,[2] M. E. L. van der Burg,[1] G. Stoter[1] & A. Sparreboom[1]

[1]*Laboratory of Experimental Chemotherapy, Pharmacology and Tumor Biology, Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam,* [2]*Department of Clinical Microbiology, Erasmus University Rotterdam, Rotterdam, the Netherlands*

Summary

Background: Several clinical studies have shown that polychemotherapy with the taxanes paclitaxel or docetaxel preceded or followed by cisplatin is associated with important schedule-dependent differences in toxicities, such as leukocytopenia. In general, the pharmacokinetics of both drugs during the combined treatment are unaltered, suggesting that a pharmacodynamic interaction might have occurred.

Materials and methods: In order to gain insight into this pharmacologic interaction, we performed *in vitro* drug accumulation studies using peripheral blood leukocytes and a panel of tumor and non-malignant cell lines with paclitaxel and docetaxel, as wel as with their respective formulation vehicles Cremophor EL and Tween 80.

Results: Our results show a significant reduction in the intracellular cisplatin concentration in leukocytes of up to 42% in the presence of Cremophor EL and Tween 80 as compared to the control. This pharmacodynamic interaction of these surfactants with cisplatin seems to be specific for haematopoietic cells, and does not occur in solid tumor cells.

Conclusion: The present data suggest that the pharmaceutical vehicles Cremophor EL and Tween 80 might contribute to the reduced cisplatin-associated myelotoxicity observed in the clinical combination chemotherapy studies with paclitaxel and docetaxel.

Key words: cisplatin, Cremophor EL, drug accumulation, haematopoietic cells, leukocytes, paclitaxel

Introduction

Paclitaxel (Taxol), an antimicrotubule agent derived from the stem bark of the Western Yew tree (*Taxus brevifolia*), is one of the most promising drugs in the chemotherapeutic armamentory, with proven antitumor activity in wide variety of human neoplastic disorders [1]. Currently, optimization of the usage of paclitaxel is actively persued, particularly by testing the efficacy of this drug in combination chemotherapy. One of the most obvious drug combinations to evaluate is paclitaxel followed by cisplatin (*cis*-Pt), since preclinical data indicated that this combination causes synergistic cytotoxicity in a variety of cell lines [2–4], and human tumor xenograft models [5, 6]. The only partially overlapping side effects of paclitaxel and *cis*-Pt, their different mechanisms of action, and paclitaxel's activity in *cis*-Pt-refractory ovarian cancer also made the combination of both drugs attractive for further clinical exploration [7].

Recently, exciting results have emerged from clinical combination chemotherapy studies with paclitaxel or its semisynthetic analogue docetaxel (Taxotere) and *cis*-Pt, testing two sequences of drug administration [8, 9]. These studies revealed important schedule-dependent differences in frequency and severity of toxicities, with less severe myelotoxicity when the taxane was given before *cis*-Pt. Our own previous investigations demonstrated that in the sequence of docetaxel followed by *cis*-Pt, the reduced leukocytopenia was accompanied by a significant inhibition of *cis*-Pt uptake and DNA-adduct formation in peripheral blood leukocytes as compared to the alternate sequence [10, 11].

At present, convincing data on the fundamental mechanisms behind this clinically important pharmacologic interaction are lacking. This prompted us to further investigate the effects of paclitaxel and docetaxel, as well as of their respective formulation vehicles Cremophor EL and Tween 80, on the intracellular accumulation of *cis*-Pt in haematopoietic and tumor cells *in vitro*. Our results show that not the taxanes themselves, but the polyoxyethylated surfactants Cremophor EL and Tween 80 selectively inhibit *cis*-Pt uptake in peripheral blood leukocytes and bone marrow cells, and suggest that these vehicles are major determinants in the reduced *cis*-Pt-associated myelotoxocity observed in clinical combination chemotherapy studies with paclitaxel and docetaxel.

Materials and methods

Drugs and chemicals

The *cis*-Pt reference material was supplied by Pharmachemie (Haarlem, the Netherlands) as a solution containing 1 mg/ml of drug in D5NS (lot 95F29MC). Paclitaxel powder, the clinical paclitaxel formulation in Cremophor EL and dehydrated ethanol USP (1 : 1, v/v; Taxol) and carboplatin were obtained from Bristol-Myers Squibb (Princeton, NJ, USA). Docetaxel and the clinical docetaxel formulation in Tween 80 (Taxotere) originated from Rhône-Poulenc Rorer (Antony Cedex, France). Camptothecin lactone (Pharmacia Inc., New Mexico), doxorubicin (Farmitalia Carlo Erba, Milan, Italy), methotrexate (Pharmachemie) and vincristine (Eli Lilly, Nieuwegein, the Netherlands) were used as their pharmaceutical formulations for clinical use without further purification. Cremophor EL (polyoxyethyleneglycerol triricinoleate 35; lot 32H0925), Tween 80 (polyoxyethyleneglycerol sorbitan monooleate; lot 95H09251), castor oil and ricinoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Five reversed-phase HPLC fractions of Cremophor EL were kindly donated by Dr David Kessel (Wayne State University School of Medicine, Detroit, USA) [12]. Formic acid, tetrahydrofuran, ammonium hydroxide and Triton X-100 were from Baker (Deventer, the Netherlands). Coomassie Brilliant Blue G-250 was purchased from Bio-Rad Laboratories (Munchen, Germany). All other chemicals and reagents were of analytical or HPLC grade and were obtained from Rathburn (Walkerburn, UK). Water was filtered and deionized with a Milli-Q-UF Plus system (Millipore, Bedford, MA, USA), and was used in all aqueous solutions.

Cell cultures

The human ovarian carcinoma cell lines A2780 and IGROV-1, the breast carcinoma cell line MCF-7, the human promyelocytic leukaemia cell line HL-60 and the murine lymphoid leukaemia cell line P388 were grown and maintained in RPMI1640 medium (Brunschwig, Amsterdam, the Netherlands). The non-malignant chinese hamster ovary (CHO) and the African green monkey kidney (VERO) cell lines were maintained in Dulbecco's modified Eagle's Medium (DMEM; Brunschwig). The cells were cultured in a humidified atmosphere in 5% CO_2 at 37 °C in media supplemented with 10% (w/v) of heat-inactivated bovine calf serum (Hyclone, Logan, UT, USA), 100 U/ml of penicilline, 100 µg/ml of streptomycin and 2 mM freshly added L-glutamine (all from Life Technologies, Gaithersburg, MD, USA). The cytotoxicity of Cremophor EL against the cells was estimated with the sulforhodamine B assay [13].

For peripheral blood leukocytes (PBL) isolation, 55 ml of a freshly prepared buffy coat suspension obtained from healthy volunteers was mixed with 50 ml of 0.1 M phosphate buffer and 300 ml of red cell lysing buffer (composed of 0.83% (w/v) of ammonium chloride and 0.1% (w/v) of potassium carbonate in 1 mM of EDTA). Prior to drug exposure, the mixture was incubated for 20 min at 4 °C in a water bath. After centrifugation, the PBL were washed with PBS (Unipath Ltd., Basingstoke, UK) and maintained in RPMI1640 medium for 45 min.

Isolation of granulocytes and mononuclear cells from heparinized human blood was performed by sequential ficoll-gradient centrifugation and methyl cellulose sedimentation at room temperature as described [14]. Contaminating erythrocytes were removed by hypotonic lysis in 0.22% (w/v) of sodium chloride for five minutes. Both post-ficoll cell fractions were handled further as described above for whole PBL.

Bone marrow samples were collected from female Wistar rats obtained from the Erasmus Animal Experimental Centre (Rotterdam, the Netherlands), that were housed in conventional plastic cages covered with filter bonnets, and provided with food and water *ad libitum*. The animals were sacrified by cervical dislocation under diethylether anaesthesia, and femurs were removed surgically, scraped free of muscular tissues and other adhering debris, while maintaining the integrity of the knee and hip joints during dissection. The marrow was harvested by snipping the tip of the femur and flushing with ice-cold PBS using a syringe fitted with a 27-gauge needle. Cell suspensions were stored on ice and used within one hour of collection.

Cellular drug accumulation

All cell lines were grown to 60%–70% confluence in 75 cm^2 cell culture flasks (Greiner, Alphen a/d Rijn, the Netherlands), and incubated simultaneously for two hours with Cremophor EL and *cis*-Pt (10 µg/ml). Immediately after drug incubation, the cells were rinsed twice with PBS, harvested by trypsinization, and washed three times with PBS.

For the accumulation studies in PBL and bone marrow cells, 6 ml of the cell suspension were incubated in 12.5 ml polypropylene screw-cap tubes (Greiner) with various concentrations of the drugs or surfactants and *cis*-Pt for two hours. During this incubation, the tubes were vortex-mixed at 30 min-intervals to prevent clogging of the cells. Next, samples were processed as described above for tumor cells.

Drug analyses

Cell pellets were digested with 500 µl of Triton X-100-water (1 : 500, v/v) by vortex-mixing (five minutes) and ultrasonication (15 minutes). A 100 µl-aliquot of this solution was removed for determination of the protein content by the method of Bradford [15]. The remaining cell lysate was then analyzed for total platinum by flameless atomic absorption spectrometry using a Perkin Elmer Model 4110 ZL atomic absorption spectrometer with Zeeman-background correction (Perkin Elmer Corp., Rockville, MD, USA) as described previously [16]. Each sample was analysed in duplicate by external standardization with a weighted linear regression analysis ($1/x^2$), and the absorbance readings were averaged.

For the analysis of intracellular paclitaxel and docetaxel concentrations, PBL pellets were digested in 1000 µl of water, followed by liquid-liquid extraction and reversed-phase HPLC according to methods reported in detail previously [17, 18]. The HPLC equipment was composed of a constaMetric 3200 pump (LCD Analytical, Rivera Beach, CA, USA), a Waters Model 717 plus autosampler (Milford, MA, USA) and a UV-2000 detector (Spectra Physics, San Jose, CA, USA). Separations were achieved on an Inertsil ODS-80A column (GL Science, Tokyo, Japan) and a mobile phase of methanol-tetrahydrofuran-20% (w/v) ammonium hydroxide (60 : 2.5 : 37.5, v/v/v; pH 6.0), delivered at 1.00 ml/min. Detection was performed at 230 nm, with peak recording performed using the Chrom-Card data analysis system (Fisons, Milan, Italy). Calibration curves were fitted by weighted regression analysis ($1/x^2$).

Cremophor EL concentrations in plasma collected from two patients receiving 100 and 225 mg/m^2 of paclitaxel (three-hour i.v. infusion), respectively, followed by 70 mg/m^2 of *cis*-Pt (three-hour i.v. infusion) were measured by a novel microassay that will be described in detail elsewhere [19]. In brief, the method is based on a selective binding of Coomassie Brilliant Blue G-250 to Cremophor EL following plasma protein precipitation with acetonitrile and liquid-liquid extraction with *n*-butylchloride. The increase in the absorption maximum of the dye from 465 to 595 nm after binding to Cremophor EL was monitored using a Titertec automated microplate reader (Flow Laboratories, Irvine, UK). Calibration curves constructed in blank human plasma over the concentration range of 0.05%–1.0% (v/v) of Cremophor EL were fitted by using unweighted second-order polynomial (quadratic) regression analysis.

Results

In order to gain insight into the pharmacologic mechanism of interaction involved in our clinical studies with paclitaxel or docetaxel and *cis*-Pt, *in vitro* drug accumulation studies were performed using both PBL, isolated

Table 1. Cellular accumulation of *cis*-Pt in PBL following a two-hour simultaneous *in vitro* incubation of various drugs and pharmaceutical formulation vehicles and *cis*-Pt (10 μg/ml). Data are presented as a mean percentage relative to a 100% control value ± SD.

Drug (formulation)	Concentration	*cis*-Pt accumulation (%)	*n*
Paclitaxel (Cremophor EL)	1.0 μg/ml	67 ± 12[a]	3
Paclitaxel (DMSO)	1.0 μg/ml	99 ± 8	2
Docetaxel (Tween 80)	1.0 μg/ml	73 ± 13[a]	3
Docetaxel (DMSO)	1.0 μg/ml	101 ± 13	3
Cremophor EL	1.0%	52 ± 9[a]	2
	0.01%	58 ± 10[a]	11
	0.001%	62 ± 11[a]	5
	0.0001%	66 ± 11[a]	2
	0.00001%	75 ± 2[a]	2
Tween 80	0.01%	58 ± 8[a]	3
Ethanol	0.01%	94 ± 7	3
Camptothecin	1.0 μg/ml	105 ± 9	3
Doxorubicin	1.0 μg/ml	101 ± 3	3
Methotrexate	1.0 μg/ml	104 ± 9	3
Vincristine	1.0 μg/ml	94 ± 16	3

[a] $P < 0.05$ *versus* the control value.

Table 2. Cellular accumulation of paclitaxel, docetaxel and carboplatin in PBL following a two-hour simultaneous *in vitro* incubation with Cremophor EL or Tween 80 (0.01%). Data are presented as a mean percentage relative to a 100% control value ± SD.

Drug (formulation)	Concentration	Drug accumulation with Cremophor EL	*n*	Drug accumulation with Tween 80	*n*
Paclitaxel (DMSO)	1.0 μg/ml	94 ± 11	6	105 ± 14	4
Docetaxel (DMSO)	1.0 μg/ml	94 ± 6	3	96 ± 7	3
Carboplatin (water)	100 μg/ml	112 ± 13	4	95 ± 4	3

from freshly prepared buffy coat suspensions, and various tumor and normal cell lines. The total cellular accumulation of the drugs used in these experiments was quantitated as a percentage relative to the 100% control value, which was assigned to the drug level achieved without co-incubation at the specified treatment interval.

Simultaneous incubation of PBL with Taxol (i.e., paclitaxel formulated in Cremophor EL) or Taxotere (i.e., docetaxel formulated in Tween 80) and *cis*-Pt resulted in a similar magnitude of *cis*-Pt uptake inhibition, with the intracellular concentrations of *cis*-Pt in PBL being significantly reduced by 33% and 27% for Taxol and Taxotere, respectively, as compared to the control values (Table 1).

Because the possibility existed that the reduced *cis*-Pt accumulation in PBL after co-incubation with Taxol or Taxotere was due to the large amounts of Cremophor EL and Tween 80 added to the cells, PBL were also treated with Cremophor EL or Tween 80 alone prior to *cis*-Pt (Table 1). In these experiments, a significant reduction in the intracellular *cis*-Pt concentration in PBL of 42% compared to the control was observed in the presence of 0.01% (v/v) of Cremophor EL or Tween 80 (i.e., the same concentrations present in 1.0 μg/ml of Taxol and Taxotere, respectively). Further studies revealed a clear concentration-dependent effect of Cremophor EL on the *cis*-Pt uptake in PBL, with maximal inhibition (approximately 50%) achieved at an extracellular concentration of 1.0% of Cremophor EL. It is of interest to note, however, that Cremophor EL concentrations as low as 0.00001% still resulted in a mere 25% decrease in *cis*-Pt uptake as compared to the control (Table 1). The increased *cis*-Pt levels observed in PBL incubated in higher concentrations of Cremophor EL ($\geq 10\%$) was not due primarily to interference with uptake, but rather to gross cellular damage (data not shown). The *cis*-Pt accumulation was unaffected in a similar set of experiments in which cells were exposed to paclitaxel or docetaxel formulated without Cremophor EL or Tween 80 (viz. DMSO) (Table 1). These findings indicate that the inhibition of *cis*-Pt accumulation in PBL observed with Taxol and Taxotere is exclusively caused by their formulation vehicles Cremophor EL and Tween 80.

Incubation of PBL with pure castor oil and ricinoleic acid, two major components present in the Cremophor EL vehicle prior to polyoxyethylation, showed no evidence of any decrease in *cis*-Pt accumulation (data not shown). Furthermore, experiments with five reversed-phase HPLC fractions of Cremophor EL (each with progressively increased hydrophobicity) indicated that the inhibitory effects of the unfractionated vehicle on *cis*-Pt uptake is not observed with the more hydrophilic components present in fraction I, mainly containing polyethylene glycol and oxyethylated glycerol (data not shown). The maximum concentration of ethanol, another component of the paclitaxel formulation, to which the cells were exposed was 0.01% (v/v). Although it was unlikely that such low concentrations would contribute to the observed effects, it was necessary to rule out this organic solvent as an important contributor to the decreased *cis*-Pt accumulation. To date, in the cases where cells were exposed to ethanol in the absence of drug, the cellular accumulation of *cis*-Pt was identical to the control (Table 1). In addition, a number of other drugs, formulated without a polyoxyethylated surfactant, including camptothecin, doxorubicin, methotrexate and vincristine had no effect on the accumulation of *cis*-Pt at the specified concentrations (Table 1).

PBL were also treated with paclitaxel, docetaxel (both formulated in DMSO) or the *cis*-Pt analogue carboplatin and the surfactants, to determine whether the observed mechanism of interaction was specific for *cis*-Pt. At drug concentrations chosen close to achievable plasma levels *in vivo*, both Cremophor EL and Tween 80 had negligible effects on drug accumulation (Table 2).

To test whether the pattern of *cis*-Pt uptake in the presence of Cremophor EL (at non-cytotoxic concen-

Table 3. Cellular accumulation of *cis*-Pt in various cell lines following a two-hour simultaneous *in vitro* incubation of Cremophor EL (0.01%) and *cis*-Pt (10 μg/ml). Data are presented as a mean relative to a 100% control value ± SD.

Cell line	Origin	*cis*-Pt accumulation	*n*	Cremophor EL IC_{50} (%)[a]	*n*
Malignant					
A2780	Human ovarian carcinoma	98 ± 8	3	0.047 ± 0.009	2
IGROV-1	Human ovarian carcinoma	96 ± 10	3	ND	–
MCF-7	Human breast carcinoma	102 ± 9	2	0.068 ± 0 008	2
HL-60	Human promyelocytic leukaemia	77 ± 12[b]	5	ND	–
P388	Murine lymphoid leukaemia	84 ± 5[b]	4	ND	–
Non-malignant					
CHO	Chinese hamster ovary	93 ± 4	3	0.092 ± 0.010	2
VERO	African green monkey kidney	111 ± 11	3	0.093 ± 0.015	2
Granulocytes	Human blood	82 ± 1[b]	4	ND	–
Mononuclear cells	Human blood	76 ± 3[b]	4	ND	–
Bone marrow	Wistar rat	84 ± 1[b]	2	ND	–

[a] Dose of Cremophor EL to produce a 50% inhibition of normal cell growth during a five-day continuous exposition.
[b] $P < 0.05$ *versus* the control value.

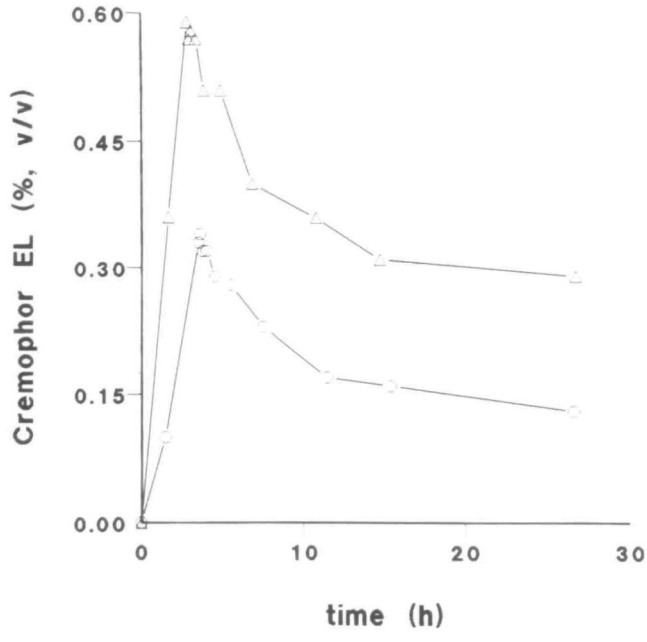

Figure 1. Plasma concentration *versus* time profile of Cremophor EL in two female patients with advanced ovarian cancer, treated with a three-hour intravenous infusion of paclitaxel at dose levels of 100 mg/m^2 and 225 mg/m^2. Paclitaxel was formulated at 6 mg/ml in a mixture of Cremophor EL-dehydrated ethanol USP (1 : 1, v/v), and diluted 20-fold in normal saline to a final concentration of 0.3 mg/ml within three hours before drug administration. Patients one (circles) and two (triangles) received absolute Cremophor EL doses of 13.8 and 27.9 ml, respectively.

trations; Table 3) was also altered in other mammalian cells, *cis*-Pt influx was examined in the tumor cell lines A2780, IGROV-1, MCF-7, HL-60 and P388, the non-malignant cell lines CHO and VERO, and rat bone marrow cells identically to incubations performed in PBL. As depicted in Table 3, a significant decrease in the intracellular *cis*-Pt concentration was only observed in the leukaemic cell lines and the rat bone marrow cells. Furthermore, the effect of Cremophor EL on the *cis*-Pt uptake in PBL was not lineage specific, as indicated by the results from incubation experiments using isolated granulocytes and mononuclear cells (Table 3). In all, these data indicate a selective protection of haematopoietic cells by Cremophor EL against *cis*-Pt uptake at concentrations that can be maintained in humans for several hours after i.v. paclitaxel administration (Figure 1).

Discussion

Cis-Pt is one of the most active anticancer agents. The drug is part of curative treatment in some diseases, however, most patients will eventually relapse after conventional three-weekly *cis*-Pt treatment as a result of cellular resistance to the drug. Our current hypothesis is that dose-intensification by shortening dose-intervals might be an approach to overcome clinical drug resistance. We have recently tested this concept in non-small cell lung cancer and ovarian cancer by giving *cis*-Pt weekly instead of three-weekly and demonstrated increased response rates of up to 52% and 90%, respectively [20, 21]. In these studies, however, *cis*-Pt-induced leukocytopenia appeared to be the dose-limiting toxicity. In contrast, weekly combination chemotherapy with *cis*-Pt and paclitaxel resulted in significantly less haematological toxicities than anticipated (unpublished data). The present study demonstrates that this effect may be related to a selective inhibition of *cis*-Pt accumulation in PBL and bone marrow cells by the paclitaxel vehicle Cremophor EL, which is not lineage specific. These data are in agreement with our pevious findings of reduced *ex vivo cis*-Pt induced DNA-adduct levels in PBL obtained from patients three hours after treatment with paclitaxel compared to PBL from non-pretreated patients [16].

The different effects of Cremophor EL on the *in vitro cis*-Pt uptake in normal haematopoietic cells and (solid) tumor cells, respectively, suggest that administration of Cremophor EL could selectively provide protection against *cis*-Pt uptake in haematopoietic cells, without affecting antitumor activity. Support for this hypothesis can be derived from several clinical and pharmacokinetic phase I studies in patients with solid tumors, applying the sequence paclitaxel or docetaxel followed by *cis*-Pt in one group of patients and that of *cis*-Pt followed by paclitaxel or docetaxel in another group [8, 9]. These combination chemotherapy studies revealed clinically important schedule-dependent differences in frequency and severity of toxicities, whereas antitumor activity did not seem to be affected.

The extracellular levels of Cremophor EL used in our *in vitro* experiments were shown to be maintained in

patients for several hours after i.v. administration of paclitaxel, even at dose levels as low as 100 mg/m^2. These results are consistent with previously published measurements of Cremophor EL concentrations in plasma by pre-column derivatization and reversed-phase HPLC [22, 23]. Previous studies also demonstrated that the distribution volume of Cremophor EL in mice (0.14 l/kg) was less than the volume of the plasma and the extracellular compartment (approximately 0.2 l/kg), indicating that the tissue distribution of Cremophor EL is insignificant [24]. These data imply that even very low doses of Cremophor EL would still result in an extended exposure of bone marrow cells to levels of Cremophor EL providing protection against cellular *cis*-Pt uptake. Recent studies in mice showed that such low i.v. doses of Cremophor EL did not result in localized toxicity or marrow destruction [25], which is consistent with flow cytometric studies demonstrating that even very high concentrations of Cremophor EL (> 10%) did not lyse bone marrow cell membranes [26]. Moreover, the i.v. administration of Cremophor EL was associated with an increase in the incidence of both primitive and committed progenitors, and protected mice from irradiation-induced death [25]. These findings provide a rationale for attempts to circumvent *cis*-Pt-associated myelotoxicity by concomitant administration of Cremophor EL.

Cremophor EL is a complex heterogeneous liquid formed by the reaction of ethylene oxide with castor oil at a molar ratio of 35 : 1 [27]. Separation of Cremophor EL into a series of components based on reversed-phase HPLC provided additional information on the nature of the pharmacologically active constituents. These studies show that maximal inhibition of *cis*-Pt uptake in PBL was achieved with the more hydrophobic components of the mixture, mainly containing polyoxyethyleneglycerol triricinoleate along with fatty acids esters of polyethyleneglycol. As Tween 80 is a polyoxyethylated derivative of sorbitan monooleate, the active agents apparently contain hydrophilic polyoxyethylated side chains attached to hydrophobic moieties with no structural similarities.

The biochemical mechanism of the dose-dependent interaction of Cremophor EL with the cellular uptake of *cis*-Pt is not clear. It has been reported that Cremophor EL can have a major (reversible) effect on the microviscosity of cell membranes, thereby interfering with passive drug diffusion [26]. However, recent experiments demonstrated that *cis*-Pt accumulation in PBL was highly temperature dependent. Lowering the temperature from 37 to 4 °C led to 70% and 90% reduction of intracellular *cis*-Pt in the absence or presence of Cremophor EL, respectively (unpublished data). This would imply that besides passive diffusion, alternative transport systems for *cis*-Pt susceptable to modulation by Cremophor EL are present in PBL. Such mechanisms include facilitated diffusion through gated tunnels regulated by phosphorylation cascades initiated by activation of protein kinase C [28, 29]. These and several other possibilities are currently under investigation.

The lack of interaction between Cremophor EL and the *cis*-Pt analogue carboplatin in our *in vitro* model in PBL is in line with previous clinical observations demonstrating a sequence-independent effect on the myelotoxicity of the combination paclitaxel and carboplatin [30, 31]. In point of fact, in contrast to studies combining paclitaxel or docetaxel and *cis*-Pt, no diminuition in expected leukocytopenia has been reported in any of the trials with paclitaxel/carboplatin combination chemotherapy [32]. Several recent studies, however, found that this combination was better tolerated than anticipated, particularly with respect to thrombocytopenia [33]. The mechanism underlying this observation remains to be elucidated.

In conclusion, this study provides evidence supportive of the conjecture that the pharmaceutical vehicles Cremophor EL and Tween 80 are major determinants in the reduced *cis*-Pt-associated myelotoxocity observed in clinical combination chemotherapy studies with paclitaxel and docetaxel. Furthermore, the pharmacokinetic selectivity for the central blood/bone marrow compartment, combined with a direct protection of normal haematopoietic cells by limitation of *cis*-Pt uptake make Cremophor EL a promising candidate for the selective modulation of *cis*-Pt-induced myelotoxicity in a clinical setting of short-interval *cis*-Pt administration. Presently, this concept is under investigation in a rodent tumor-bearing model.

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Correspondence to:
A. Sparreboom, MD
Department of Medical Oncology
Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and
University Hospital Rotterdam
PO Box 5201
3008 AE Rotterdam
The Netherlands
E-mail: sparreboom@onch.azr.nl