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Report

Phase I clinical and pharmacokinetic study of PNU166945, a novel water-soluble polymer-conjugated prodrug of paclitaxel

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Intravenous administration of paclitaxel is hindered by poor water solubility of the drug. Currently, paclitaxel is dissolved in a mixture of ethanol and Cremophor EL; however, this formulation (Taxol®) is associated with significant side effects, which are considered to be related to the pharmaceutical vehicle. A new polymer-conjugated derivative of paclitaxel, PNU166945, was investigated in a dose-finding phase I study to document toxicity and pharmacokinetics. A clinical phase I study was initiated in patients with refractory solid tumors. PNU16645 was administered as a 1-h infusion every 3 weeks at a starting dose of 80 mg/m^2, as paclitaxel equivalents. Pharmacokinetics of polymer-bound and released paclitaxel were determined during the first course. Twelve patients in total were enrolled in the study. The highest dose level was 196 mg/m^2, at which we did not observe any dose-limiting toxicities. Hematologic toxicity of PNU166945 was mild and dose independent. One patient developed a grade 3 neurotoxicity. A partial response was observed in one patient with advanced breast cancer. PNU166945 displayed a linear pharmacokinetic behavior for the bound fraction as well as for released paclitaxel. The study was discontinued prematurely due to severe neurotoxicity observed in additional rat studies. The presented phase I study with PNU166945, a water-soluble polymeric drug conjugate of paclitaxel, shows an alteration in pharmacokinetic behavior when paclitaxel is administered as a polymer-bound drug. Consequently, the safety profile may differ significantly from standard paclitaxel. [© 2001 Lippincott Williams & Wilkins.]

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Introduction

Paclitaxel (Taxol®) is a well-known antitumor agent with proven efficacy in ovarian, breast and non-small cell lung cancer.[1–4] However, the i.v. administration of paclitaxel is associated with unpredictable side effects, which are considered to be related to the pharmaceutical vehicle.[5–9] Due to poor water solubility, paclitaxel is pharmaceutically formulated in a mixture of ethanol and Cremophor EL (polyoxyethyleneglycerol triricinoleate 35), the latter being at least partially responsible for the hypersensitivity reactions observed after i.v. administration. Therefore the development of a safer i.v. formulation of paclitaxel without these additives is an important investigational aim. PNU166945 is a novel polymer-conjugated derivative of paclitaxel. The compound consists of a hydroxypropyl-methacrylamide (HPMA) polymer, which is linked through an amino acid chain at the 2′ position of paclitaxel (Figure 1). As the polymer-bound drug is very water soluble (>2 compared to >0.0001 mg/ml for paclitaxel), it can be dissolved and administered i.v. without Cremophor EL. PNU166945 was selected for clinical evaluation, among several candidates, because of its high antitumor activity and low toxicity in preclinical studies.[10] No signs of hypersensitivity, neurotoxicity or cardiovascular toxicity were observed in the acute and subchronic toxicity studies with

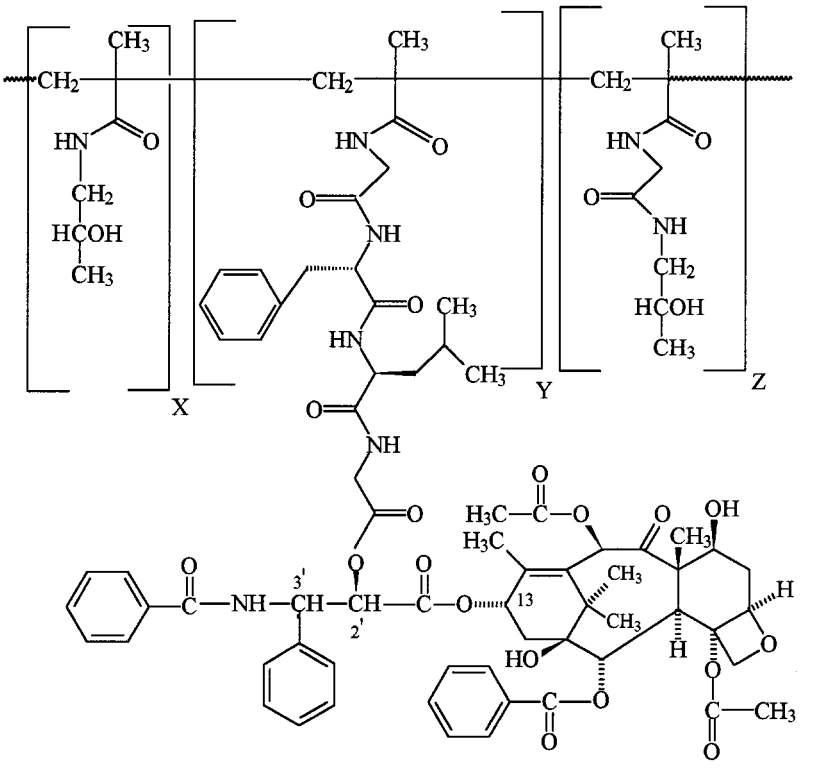

Figure 1. Structural formula of PNU166945.

PNU166945 in rodents or dogs.[11] A second advantage of polymeric drug delivery systems is that they provide the opportunity for specific tumor targeting. Attachment of a cytotoxic drug to a macromolecule reduces its systemic cellular uptake, whereas accumulation may occur at the tumor site due to the increased vasopermeability of many tumors.[12] Enhanced local efficacy can be achieved, provided that the drug is able to release itself from the carrier at the tumor site. We conducted a clinical phase I and pharmacologic study to investigate the toxicity and pharmacokinetics of PNU166945. PNU166945 was administered i.v. as a 1-h infusion every 3 weeks in patients with solid malignant tumors not amenable to other treatment. Objectives of our study were to determine the maximal tolerated dose (MTD) and dose-limiting toxicities (DLTs), and to determine the plasma and urine pharmacokinetic profile of PNU166945 and polymer-released paclitaxel.

Patients and methods

Patient population

Patients with histologically confirmed solid tumors for whom no recognized therapy was available were amenable to the study treatment. Previous radiotherapy and/or chemotherapy were allowed provided that the last cytotoxic drug treatment was at least 4 weeks prior to entry in the study (or 6 weeks for nitrosoureas and mitomycin C). Prior taxane therapy was allowed unless this was discontinued due to the occurrence of hypersensitivity reactions and patients must have recovered from all acute toxic effects from any prior therapy. Patients had to have adequate bone marrow (absolute neutrophil count (ANC) $\geqslant 2000/mm^3$ and platelets $\geqslant 100\,000/mm^3$), liver (serum bilirubin $\leqslant 25\ \mu M$) and renal (creatinine < 1.5 mg/dl) function, and a WHO performance status $\leqslant 2$. Patients were excluded if they had a cardiovascular history of myocardial infarction within the last 6 months, or congestive heart failure, angina pectoris, atrio-ventricular or bundle branch blocks, arrhythmias requiring medication, or if they were on chronic treatment with medications which might alter the cardiac conduction. Patients with known brain or leptomeningeal disease were also excluded. The study protocol was approved by the ethics committee of the Institute and all patients gave written informed consent.

Treatment plan and study design

PNU166945 was supplied by Pharmacia & Upjohn (Milan, Italy) as a porous, off-white, freeze-dried mass in colorless glass vials, containing 30 mg as paclitaxel. The polymer:paclitaxel ratio was approximately 19:1 w/w. The content of the vial was primary reconstituted with 15 ml of 0.9% sodium chloride sterile solution and the obtained solution was further diluted in 0.9% sodium chloride sterile solution for injection to obtain an adequate fluid volume for infusion (about 500 ml). The appropriate dosage was administered as a 1-h continuous i.v. infusion at a steady rate, using a controlled infusion device. The starting dose was 80 mg/m^2 as paclitaxel equivalents, which is approximately equivalent to one-third of the highest tolerated dose in dogs (i.e. 250 mg/m^2), to be administered every 3 weeks. Dose escalation in second and subsequent dose levels was based on the safety profile observed in the previous level. Hematologic toxicities grade 3 or higher in severity (grade 4 for neutropenia) and non-hematologic toxicities grade 3 or higher (grade 2 for newly occurring neurotoxicity) were considered dose-limiting. Initially, three patients were entered at each dose level. If one-third of patients developed a DLT, three additional patients were entered and toxicity evaluated before continuing dose escalation. The MTD was based on the NCI CTC[13] and defined as the dose level producing DLTs in at least two out of three or three out of six patients, occurring during the first cycle of treatment and attributable to PNU166945. Hypersensitivity reactions were not expected to occur at the frequency observed with Cremophor EL–ethanol-formulated paclitaxel,[2] due to the absence of Cremophor EL in the pharmaceutical formulation, and therefore no premedication was given.

Treatment assessment

Pretreatment evaluations were performed within 1 week prior to the start of treatment, and included a complete medical history and physical evaluation. Tumor assessment was performed within 3 weeks prior to the start of treatment and every other cycle thereafter. Weekly evaluations while on treatment included physical examination, complete blood counts, blood chemistry and electrocardiography. The criteria for grading adverse events were the CTC of the NCI.[13]

Pharmacokinetic studies

A primary purpose was to estimate the individual patient's exposure to PNU166945 and free paclitaxel, and to assess its relationship to selected hematological variables. Pharmacokinetics were monitored in at least three patients per dose level during the first course. Whole blood samples of 5 ml each were collected in heparinized tubes at 14 time points up to 48 h after PNU166945 administration. The samples were placed on ice immediately after withdrawal and subsequently centrifuged at 0–4°C over 10 min. The obtained plasma fraction was divided into four aliquots of 0.5 ml each. Two aliquots were immediately frozen in a CO_2–ethanol bath (−70°C) and two other aliquots were stabilized with 0.5 ml KH_2PO_4 0.5 M before they were frozen in the CO_2–ethanol bath. All samples were stored at −70°C until analysis. Urine was collected in 24-h fractions up to 48 h post-dosing.

To determine free paclitaxel levels in plasma, a reversed-phase high-performance liquid chromatography (HPLC) method with UV detection (limit of quantification 10 ng/ml) was developed, preceded by a solid-phase extraction (SPE).[14] SPE was performed within 1 week after storage of the samples to ensure a minimum of *in vitro* paclitaxel release from the polymer. For the quantification of total paclitaxel concentrations, the same HPLC method was used after a suitable hydrolysis procedure to release paclitaxel from its polymeric carrier. Levels of PNU166945 were determined by subtraction of free paclitaxel from total paclitaxel concentrations, obtained after quantitative release from the polymer.[14]

The pharmacokinetic parameters were calculated using standard non-compartmental methods.[15] The area under the plasma concentration versus time curve (AUC) was estimated by the linear trapezoidal rule with extrapolation to infinity. The terminal half-life ($t_{\frac{1}{2}}$) was calculated as ln2/k, where k is the rate constant estimated by linear regression analysis of the logarithmic plasma concentration-time curves. The plasma clearance (Cl) was defined as dose/AUC and the volume of distribution at steady state (V_{SS}) as $Cl \cdot t_{\frac{1}{2}}$/ln2. The peak concentration (C_{max}) and the time to peak (T_{max}) were the highest observed values. The time duration above a threshold concentration of 0.1 μM ($T > 0.1$ μM) was derived graphically from the pharmacokinetic curves.

Pharmacokinetic–pharmacodynamic interactions

Relationships between pharmacokinetics and pharmacodynamics were investigated using plots of percentage decrease in white blood cells (WBC) or ANC versus the AUC of free paclitaxel and versus the $T > 0.1$ μM of free paclitaxel, after the first course. The percentage decrease is defined as:

$$\text{Percentage decrease} = \frac{\text{Pretreatment value} - \text{Value of the nadir}}{\text{Pretreatment value}} \times 100\%$$

Hematologic values were obtained by measurement of full blood cell counts twice weekly and the nadir after the first course was used to calculate the percentage decrease after one course.

Results

Patient characteristics

Twelve patients were accrued for the study. Patient characteristics are listed in Table 1. A total of 51 courses of PNU166945 was delivered with a median of 4 courses (range 1–9) per patient. The starting dose was 80 mg/m^2 and dose escalation was performed in three steps: 100, 140 and 196 mg/m^2. Three patients received 80 mg/m^2, three patients 100 mg/m^2, four patients 140 mg/m^2 and three patients 196 mg/m^2. One patient started at a dose of 80 mg/m^2, went off study after 7 courses and re-entered the study at a dose of 196 mg/m^2. However, because the treatment-free interval in this patient was more than 9 weeks, no pharmacokinetic interaction is expected and she was considered to be evaluable for pharmacokinetics at the dose of 196 mg/m^2 as well. We also included the course at a dose of 196 mg/m^2 in the toxicity analysis.

One patient at a dose of 140 mg/m^2 refused to participate in the pharmacokinetic studies and was therefore not evaluable for pharmacokinetics. All 12 patients were considered evaluable for response and toxicity. Eleven patients were evaluable for assessing pharmacokinetic–pharmacodynamic interactions.

Table 1. Patient characteristics

Total number (M:F)	12 (4:8)
Age [median (range)]	52 (36–74)
WHO PS	
0	3
1	8
2	1
Tumor	
ovarian	4
breast	2
colon	2
lung (small cell lung cancer)	1
others	3
Previous treatment	
chemotherapy	2
surgery/chemotherapy	5
radiotherapy/chemotherapy	1
surgery/radiotherapy/chemotherapy	4

Toxicity

Flu-like symptoms common to paclitaxel therapy developed in most patients and never exceeded grade 2 in severity. Nausea and vomiting were mild (grade 1 or less) and seldom present for more than one episode. Neurotoxicity grade 2 occurred temporarily in two patients at a dose of 140 mg/m^2 after the fourth course; in both patients, however, neuropathy grade 1 had been pre-existent to their entry in the study (which was not documented as acute toxic effect of earlier treatment with chemotherapy). One patient at a dose of 196 mg/m^2 developed a grade 3 neuropathy after the fourth course and went off-study. The most striking feature was the absence of alopecia. The maximum observed hematologic toxicity was grade 4 anemia which occurred in one patient after the sixth course at a dose of 100 mg/m^2. In this patient transfusions with packed red blood cells were necessary to increase the hemoglobin level (Hb). Five other patients developed grade 2 anemia (two patients at a dose of 80 mg/m^2, one patient at a dose of 100 mg/m^2, one patient at a dose of 140 mg/m^2 and one patient at a dose of 196 mg/m^2), which in three patients started after their second course. It should be noted, however, that mild anemia was present in nine patients prior to study entry. No relationship existed between the administered dose and the occurrence of anemia. Further hematologic toxicity was also mild and dose independent. Additional toxicity figures are presented in Table 2. No DLTs were observed at the studied dose levels (up to 196 mg/m^2 as paclitaxel equivalents) and therefore the MTD was not reached. However, the study had to be discontinued due to severe neurotoxicity observed in subsequent rat studies combined with the observed neurotoxicity in our clinical study.

Responses

One breast cancer patient developed a partial remission of her skin metastases after two courses of PNU166945 at a dose of 100 mg/m^2. Two other patients at a dose of 140 mg/m^2 had stable disease, both after the second course. All other patients went off study because of progressive disease.

Pharmacokinetic studies

Blood samples were obtained from 11 patients for a total of 12 courses. In Figures 2 and 3 the mean plasma concentration–time curves for total and free paclitaxel concentrations at each dose level are shown. The bound fraction is found by subtraction of the curve for free paclitaxel from the curve of total paclitaxel.

Table 2. Toxicity

Symptoms	Patients (courses)
Alopecia	0
Flushes	3 (4)
Flu-like symptoms	
myalgia	8 (22)
fatigue	7 (23)
headache	3 (6)
Gastrointestinal symptoms	
nausea	5 (10)
vomiting	2 (5)
mucositis	5 (5)
diarrhea	7 (8)
Peripheral neuropathy	
grade 1	5 (16)
grade 2	3 (3)
grade 3	1 (1)
Hematologic toxicity	
anemia	
grade 1	8 (18)
grade 2	6 (15)
grade 3	1 (1)
grade 4	1 (1)
leukopenia	
grade 1	6 (11)
grade 2	3 (3)
granulocytopenia	
grade 1	2 (4)
grade 2	1 (1)
grade 3	1 (1)
Other toxicity	
local skin reactions	4 (7)
epistaxis	2 (2)

Pharmacokinetic parameters of total and free paclitaxel for all dose levels are presented in Tables 3 and 4. Plasma levels of free paclitaxel were approximately 100-fold lower than total paclitaxel levels. The mean T_{max} of the free paclitaxel concentrations ranged from 1.17 to 1.37 h after start of the infusion and often coincided with the end of the infusion. The mean duration of free paclitaxel level above the pharmaceutically relevant threshold concentration of 0.1 μM ranged from 9.5 to 21.2 h and when plotted against the dose, a linear increase could be detected (Figure 4). Dose linearity also existed for the AUC of free paclitaxel (Figure 5) as well as for total paclitaxel (results not shown).

Pharmacokinetic–pharmacodynamic interactions

Because hematological toxicities were insignificant, no significant correlation could be found between either the AUC of free paclitaxel and percentage decrease in WBC or ANC, or between the $T > 0.1$ μM and percentage decrease in WBC or ANC. However, since the study was discontinued prematurely, the data obtained thus far are probably too limited to draw any firm conclusions regarding pharmacokinetic–pharmacodynamic interactions.

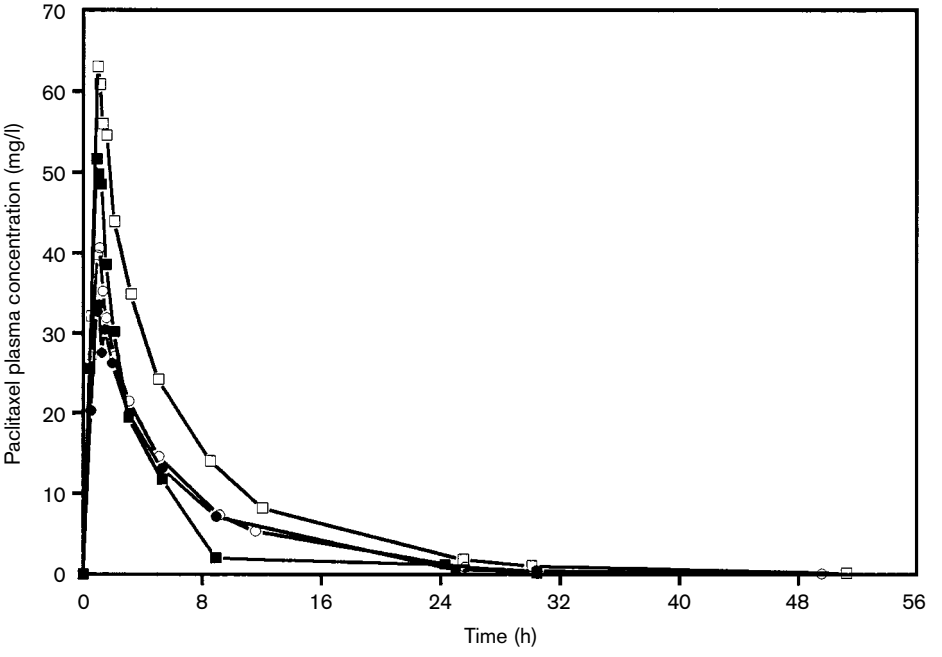

Figure 2. Mean plasma concentration–time curves of total (free + bound) paclitaxel per dose level.

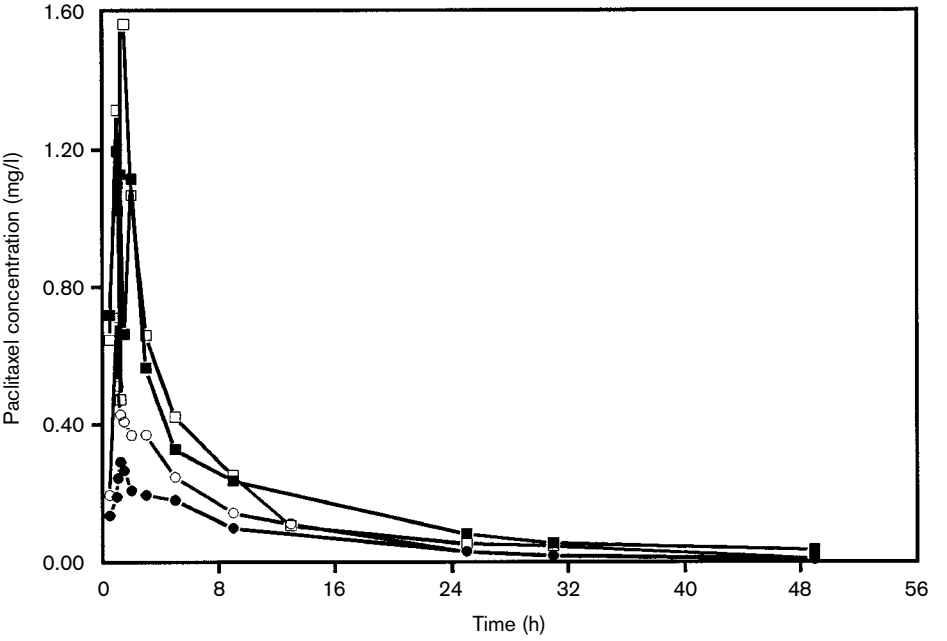

Figure 3. Mean plasma concentration–time curves of free (released) paclitaxel per dose level.

Table 3. Pharmacokinetics of total paclitaxel[a]

Dose (mg/m^2)[b]	*n*	AUC (h · μM)	C_{max} (μM)	T_{max} (h)	$t_{1/2}$ (h)	Cl (ml/h)	V_{SS} (l)
80	3	318 ± 99.2	40.1 ± 5.10	1.08 ± 0.19	6.47 ± 0.32	543 ± 220	5.14 ± 2.34
100	3	268 ± 55.9	44.1 ± 10.09	1.15 ± 0.24	5.73 ± 0.60	871 ± 213	7.22 ± 1.87
140	3	413 ± 107.5	61.2 ± 9.27	0.97 ± 0.07	6.54 ± 0.72	758 ± 186	7.24 ± 2.45
196	3	450 ± 19.2	74.9 ± 4.30	1.03 ± 0.04	6.62 ± 0.76	840 ± 95.5	8.10 ± 1.84

[a]Pharmacokinetic parameters of total paclitaxel (means ± SD).
[b]Dose of PNU166945 as paclitaxel equivalents.

Table 4. Pharmacokinetics of free paclitaxel[a]

Dose (mg/m^2)[b]	*n*	AUC (h · μM)	C_{max} (μM)	T_{max} (h)	$T > 0.1$ μM (h)	$t_{1/2}$ (h)
80	3	3.68 ± 0.81	0.35 ± 0.09	1.27 ± 0.26	9.46 ± 3.74	11.7 ± 4.0
100	3	4.88 ± 1.10	0.66 ± 0.39	1.17 ± 0.37	13.2 ± 2.56	8.99 ± 1.27
140	3	10.14 ± 4.06	1.50 ± 0.77	1.33 ± 0.74	18.4 ± 10.0	13.3 ± 6.98
196	3	10.08 ± 2.53	1.85 ± 0.57	1.37 ± 0.32	21.2 ± 9.36	9.51 ± 1.53

[a]Pharmacokinetic parameters of total paclitaxel (means ± SD).
[b]Dose of PNU166945 as paclitaxel equivalents.

Discussion

Polymeric drug delivery systems are often designed to improve the pharmacokinetic profile of an antitumor agent.[12] Synthetic HPMA copolymers are examples of such drug conjugates, which have been developed for over 20 years now in order to control release and targeting of cytostatics.[16–18] Preclinical results from mice studies have been reported for several HPMA-bound drugs among which doxorubicin,[19–21] but to date the results of clinical studies with HPMA copolymers are limited.[22–25] No polymer-related toxicity was observed for HPMA-bound doxorubicin.[22] Acute and subchronic toxicity studies with PNU166945 in mice, rats and dogs indicated that the DLT was myelotoxicity, with no signs of hypersensitivity, neurotoxicity or cardiovascular toxicity.[11] We performed a clinical phase I study with PNU166945, a

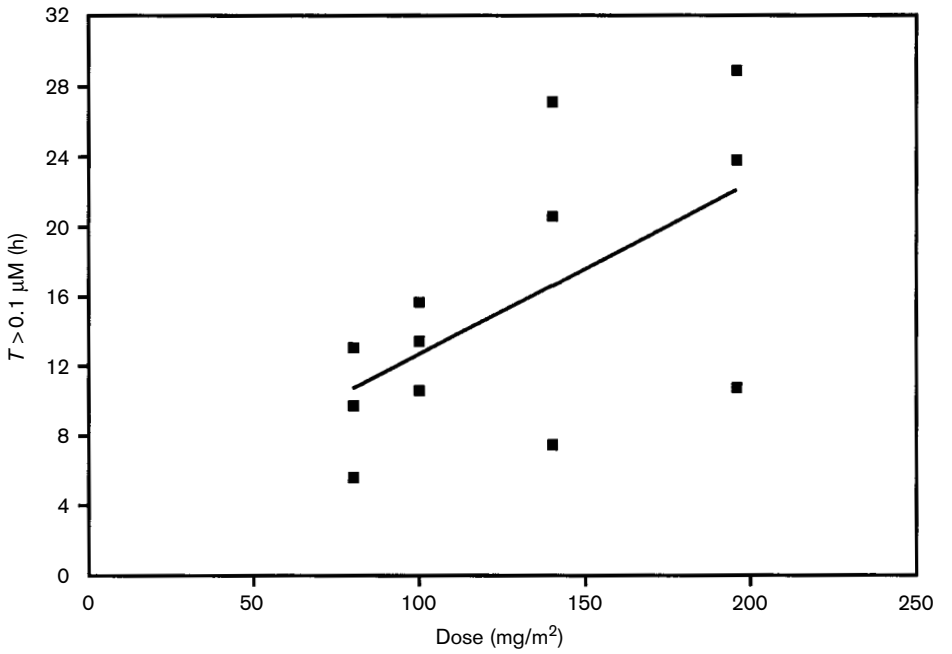

Figure 4. Time period of plasma concentrations of free paclitaxel above 0.1 μM versus the administered dose of PNU166945/m^2 (paclitaxel equivalents).

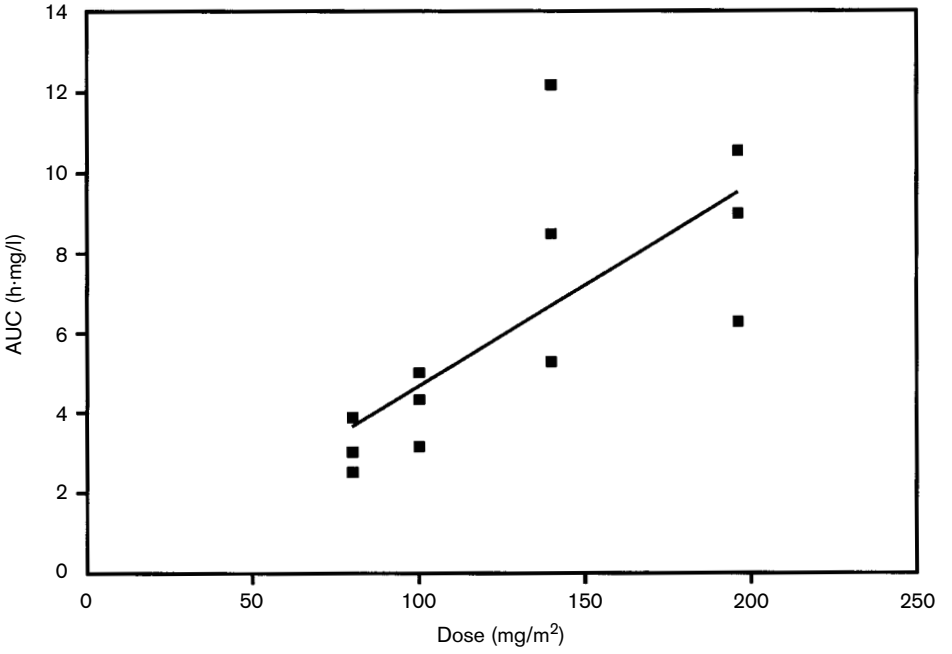

Figure 5. AUC of free paclitaxel versus the administered dose of PNU166945/m^2 (paclitaxel equivalents).

HPMA conjugate of paclitaxel, to investigate its safety and pharmacokinetics at escalating doses.

The highest investigated dose of PNU166945 was 196 mg/m^2 and no DLTs were observed at this dose level. However, at the same time during the course of the clinical study, the results of a 13-week toxicity study in rats indicated severe, motor and sensorial peripheral neurotoxicity, occurring at PNU166945 doses of 60 and 90 mg/kg, corresponding to 440 and 660 mg/m^2, respectively. In the study, 40 rats/group

(M:F=1:1) were given 30, 60 and 90 mg/kg/cycle (paclitaxel equivalents) by i.v. bolus every 3 weeks. At a dose of 90 mg/kg, neurotoxicity manifested early (after 1 cycle), while at a dose of 60 mg/kg, signs of slightly delayed neurotoxicity (after 2 cycles) were observed. At both dose levels, neurotoxicity manifested as hind leg weakness with unsteady gait, worsening to the point of paralysis. During the second cycle, forelimb weakness was also noted. Histologic evaluation revealed degeneration of the dorsal and ventral root ganglia. Although the investigated doses were substantially higher than the doses used in our clinical study, the clinical study was discontinued, because of the severity of the anatomical lesions, which might imply irreversibility. In our study mild to moderate neuropathy was observed in five of 12 patients, but in one of them neuropathy was cumulative and progressed to a grade 3 neurotoxicity after the fourth course. Neurotoxicity in this patient manifested as a polyneuropathy with burning sensations and numbness in both hands and feet, which was disabling and eventually resulted in gait disorders. After treatment discontinuation and pain medication, the symptoms improved partly. The findings in animals combined with our clinical observations were the reason for study discontinuation. It is true that the animal data may not accurately predict the clinical outcome, but the severity of the observed lesions was to such a degree that we felt unsafe to continue the clinical study. Critics may argue that the study should have continued, albeit with smaller dose increments, however, the risk of such neurotoxicity outweighed the potential benefits of study continuation.

Hematologic toxicity of PNU166945 at the investigated dose levels was mild, especially with regard to neutropenia. In addition, hematologic toxicity was independent of the administered dose range in our study. Other toxicities consisted of a flu-like syndrome common to paclitaxel. In contrast to paclitaxel administration, administration of PNU166945 did not cause alopecia. No hypersensitivity reactions as seen with i.v. administration of paclitaxel dissolved in Cremophor EL/ethanol (Taxol®) were observed. Activity was observed in three patients, resulting in one partial remission and two stable diseases.

Pharmacokinetics showed dose proportionality of both released paclitaxel and paclitaxel bound in PNU166945. A linear increase was observed in the AUC as well as in the $T>0.1$ μM of free paclitaxel, when plotted against the dose. This may have important implications for the safety profile of dosing paclitaxel. The non-linear pharmacokinetics of paclitaxel dissolved in Cremophor EL/ethanol are at least partly attributed to Cremophor EL and are associated with an unpredictable toxicity profile.[26,27] The time duration of paclitaxel plasma levels above a threshold concentration 0.1 μM is an important pharmacokinetic parameter associated with pharmacologic activity.[4,27,28] In earlier studies with paclitaxel (Taxol®) at a dose of 175 mg/m^2 administered as a 3-h infusion, reported time-periods above the 0.1 μM ranged from 10.54 to 26.31 h (n=9).[28] In this study with PNU166945 we found comparable figures for the $T>0.1$ μM, ranging from 10.75 to 28.90 h (mean 21.16, n=3) at a dose of 196 mg/m^2 and from 7.47 to 27.14 h (mean 18.40, n=3) at a dose of 140 mg/m^2. A relatively low clearance of PNU166945 was found and the volume of distribution was approximately equal to the volume of the intravascular compartment, indicating that elimination of polymer-bound paclitaxel occurs at a low rate and that the drug conjugate resides mainly in the central compartment. Statistical analysis was performed in order to explore relationships between the AUC and the $T>0.1$ μM of free paclitaxel and hematologic toxicity. We did not obtain sufficient data to establish pharmacokinetic–pharmacodynamic relationships, due to the small number of patients included and the few dose escalations performed. Pharmacokinetic–pharmacodynamic relations of paclitaxel (Taxol®), however, are influenced by the pharmaceutical additives, in particular Cremophor EL. PNU166945 offers an administration mode of paclitaxel without this vehicle. The obtained blood levels of free paclitaxel after administration of PNU166945 may therefore have an altered pharmacologic profile, as compared to paclitaxel dissolved in Cremophor EL. The observed neurotoxicity may be the result of this altered pharmacologic profile. However, it remains unclear in which way conjugation to a polymeric carrier would increase the neurotoxicity of paclitaxel.

In summary, PNU166945 administered as a 1-h i.v. infusion in the presented study was well tolerated and did not produce any DLTs up to a dose of 196 mg/m^2. Exposure to (assumed) pharmacologic active levels of paclitaxel was achieved during considerable time periods. In addition, the results suggest an improved pharmacokinetic behavior with potential controlled release of paclitaxel. The concept of polymer-conjugated drugs with favorable pharmacokinetic properties seems worthwhile for future studies.

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