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Pharmacokinetics and pharmacodynamics of the new podophyllotoxin derivative NK 611

A study by the AIO groups PHASE-I and APOH

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Abstract NK 611 is a new podophyllotoxin derivative in which a dimethyl amino group replaces a hydroxyl group at the sugar moiety of etoposide. This results in profound physico-chemical differences: NK 611 is much less hydrophobic than etoposide. Preclinical studies have shown that NK 611 is advantageous in terms of bioavailability and of the potency of its anticancer activity. A clinical phase I study was performed in cancer patients within the framework of the AIO. Additionally, its pharmacokinetics and pharmacodynamics were investigated. NK 611 was given to 26 patients at doses ranging from 60 to 140 mg/m^2 [maximum tolerated dose (MTD) 120 mg/m^2] in a 30-min infusion. Plasma and urine samples were collected from 25 patients and analyzed using a validated high-performance liquid chromatography (HPLC) assay procedure. The concentration versus time curve of total NK 611 in plasma samples was best described by a three-compartment model. The overall median pharmacokinetic values were as follows (ranges are given in parantheses): mean residence time (MRT) 16.5 (5.4–42.3)h, terminal half-life 14.0 (8.2–30.5)h, volume of distribution at steady state (V_{ss}) 11.4 (7.9–18.1) l/m^2, and plasma clearance (Cl_p) 15.1 (3.6–36.4) ml min^{-1} m^{-2}. The total systemic drug exposure, represented by the area under the curve (AUC), varied between 53.4 and 532.0 μg ml^{-1} h. The mean AUC ($\pm$ SD) increased with the dose from 78.7 $\pm$ 3.7 μg ml^{-1} h at 60 mg/m^2 up to 202.8 $\pm$ 157.2 μg ml^{-1} h at 120 mg/m^2. The mean urinary excretion (UE) fraction of unchanged drug at 48 h after the end of the infusion varied between 3.0% and 25.8% of the total dose delivered. Analysis of ultrafiltrate samples showed a protein binding of approx. 99%. The percentage reduction in white blood cells (WBC) and neutrophils (ANC) correlated with the dose, AUC, and AUC_{free}. The best relationship between the percentage of reduction in ANC and a pharmacokinetic parameter (AUC) took a nonlinear Hill-type form. The laboratory parameter for kidney or liver function did not correlate with the AUC. The variation of pharmacokinetic parameters within each dose level was profound. The reason for this pharmacological behavior remains unclear and should be investigated in further studies.

Key words Podophyllotoxin derivative · Pharmacokinetics · Pharmacodynamics

Abbreviations *AIO* Arbeitsgemeinschaft Internistische Onkologie (of the German Cancer Society). *PHASE – I* AIO Phase-I Studiengruppe · *APOH* Arbeitsgruppe Pharmakologie in der Onkologie und Hämatologie

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Introduction

NK 611, a new podophyllotoxin derivative, was developed in Japan in the search for podophyllotoxin with attributes better than those of the well-known podophyllotoxin derivatives etoposide and teniposide: better bioavailability, higher potency, and, possibly, a broader spectrum of anticancer activity [4, 5]. Etoposide and teniposide are very hydrophobic agents that

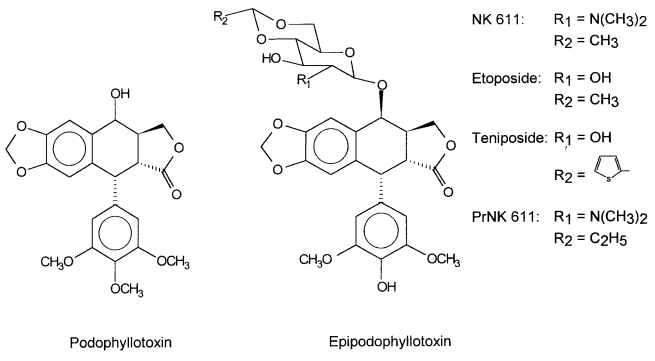

Fig. 1 *Left*: Chemical structure of the purified mandrake-root extract podophyllotoxin. *Right*: Substitution of two binding sites (R_1, R_2) at the sugar moiety of the epipodophyllotoxin leads to the analogues NK 611, etoposide, and teniposide

are soluble only in a complex formulation including polyethylene glycol, polysorbate 80, benzyl alcohol, citric acid, and ethyl alcohol. They are not stable in hydrophilic solutions at concentrations of > 0.5 mg/ml. The oral bioavailability of etoposide is highly variable, ranging from 40% to 80%, depending on the dose delivered [17]. Etoposide is now a major drug in cancer treatment, especially for small-cell lung cancer, testicular cancer, leukemias, and both Hodgkin's and non-Hodgkin's lympomas [8]. The drug is eliminated by hepatic metabolism and renal excretion, with up to 60% being recovered as the parent drug in urine [2, 11]. The structures of etoposide and teniposide differ only in the substitution of a methyl group (etoposide) for the thenylidene (teniposide) at the glucopyranoside sugar. In NK 611 a hydroxy group at the sugar moiety of etoposide is replaced by a dimethyl amino group (see Fig. 1). This leads to 120 to 170-fold higher water solubility [4].

This new drug was investigated in a clinical phase I study within the framework of the AIO [16]. A pharmacokinetics and pharmacodynamics study complemented this work since a highly sensitive and validated high-performance liquid chromatography (HPLC) assay for determination of total and unbound NK 611 in plasma and urine samples had been developed and was available [9].

Patients and methods

Patients and protocol

A total of 26 patients entered the clinical phase I study to establish the maximal tolerated dose (MTD). All patients had a performance status of ≤ 2 (WHO) and a life expectancy of at least 3 months. Neither chemotherapy nor irradiation had been performed for at least 4 weeks prior to their entry into the study. All patients had normal creatinine and bilirubin levels. Due to metastasis, liver-enzyme values [aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ-glutamyl transpeptidase (γ-GT), alkaline phosphatase (AP)] were elevated in several patients. The comedication was sometimes complex and included phenytoin in one patient (07 HH). Blood counts were always normal before the start of therapy. All patients gave their informed consent before entry into the clinical trial. The study protocol was approved by an ethics committee. The patients' characteristics are listed in Table 1.

NK 611 was given as a short-term infusion in 250 ml 5% glucose over a period of exactly 30 min by a microprocessor-controlled pump. The starting dose was 60 mg/m^2 body surface area, and the escalation steps included 80, 100, 120, and 140 mg/m^2. Blood samples (10 ml) were collected into heparinized tubes (NH_4-heparinate Monovette, Sarstedt, Germany) and centrifuged (2,000 g, 10 min). The plasma was separated and stored frozen in aliquots (1.3 ml) at -80°C until analysis. A total of 26 complete pharmacokinetics (PK) determinations were performed in 25 patients. In patient 08 M, PK values were established during the first and fourth treatment courses because incorrect sample collection during the first course could not be reliably excluded; since the PK values obtained in the two courses were nearly identical, the fourth course was included in the overall analysis. All other patients were studied after the first course. One patient (06 M) was excluded from the PK and pharmacodynamics (PD) evaluation because the terminal half-life determined by the standard compartment model was calculated from only two measured concentrations. Blood samples were drawn before treatment, at the end of the infusion, and at 10, 20, 40, and 60 min as well as 2, 4, 8, 10, 24, and 48 h after drug administration. Urine was collected in opaque plastic containers filled with 100 ml buffer (10 m*M* ammonium acetate, pH 3.5); sampling periods included 0–6, 6–12, 12–18, 18–24, and 24–48 h. The cumulative volume was recorded and 10 ml urine from each interval was kept frozen until analysis was performed. Ultrafiltration of 1.5 ml plasma was performed with a Centrisart I ultrafiltrate device with a molecular weight cutoff of 10,000 Da (SM 13239 Sartorius, Göttingen, Germany).

Drug assay

NK 611 was measured in plasma, ultrafiltrate, and urine samples using a sensitive and specific HPLC assay [9]. Plasma samples were diluted and extracted prior to analysis by a solid-liquid extraction procedure with a sample preparation unit (SPU; Merck, Darmstadt, Germany) and C_{18} columns (3 cm^3; Varian, Harbor City, USA); 900 µl plasma was added to 100 µl internal standard (PrNK 611). The sample-preparation columns were conditioned with 2 ml methanol and 4 ml distilled water. After introduction of the sample onto the column, it was purged from all unbound material with 4 ml 20 m*M* phosphate buffer (pH 7). The bound material was eluted from the column by the addition of 4 ml methanol: acetonitrile (50: 50, v/v). The organic solvents were evaporated in a metal heating block under a stream of nitrogen. The residue was redissolved in 200 µl mobile phase and sonicated for 10 min. After centrifugation at 16,000 g for 2 min, samples were split into two fractions and 20 µl of each fraction was injected into the HPLC unit.

The chromatography system consisted of an L-6200 pump, an AS-2000 autosampler (set to a "cut"-mode), an L-4250 UV detector (wavelength set to 215 nm), and a D-2500 integrator (all Merck-Hitachi, Darmstadt, Germany). Separation was performed with a Microspher C_{18} guard column connected to a Microspher C_{18} column (200 × 3 mm) (all Chrompack, Middleburg, The Netherlands). The mobile phase was acetonitrile/20 m*M* phosphate buffer (pH 3.5; 30 : 70, v/v). The flow rate was 1 ml/min. The limit of detection (LOD) in 1 ml plasma was 10 ng/ml, and the limit of quantitation (LOQ) was 35 ng/ml. The calibration curves were linear from 50 to 1,000 ng/ml with a correlation coefficient (r) of 0.999 ± 0.0003. The relative error in terms of accuracy and precision for low (118 ng/ml) and high (955 ng/ml) NK 611 concentrations was 6.8% and 3.8% for within-day measurements and 6.6% and 3.2% for day-to-day measurements, respectively. NK 611 was stable in plasma samples at different temperatures (37°C, ambient temperature, and 4°C) for 24 h as well as in a frozen status for several weeks.

Table 1 Patients' baseline characteristics (*M* München, *HD* Heidelberg, *HH* Hamburg)

Patient number, center	Diagnosis	Age (years)	Weight (kg)	Height (cm)	Sex	ASAT (U/I)	ALAT (U/I)	γ-GT (U/I)	Bilirubin (mg/dl)	AP (U/I)	Creatinine (μmol/l)	Tot. protein (g/dl)	Albumin (g/dl)
01 M	Head & neck cancer	69	71	171	M	9	5	12	0.3	135	0.8	7.9	4.6
02 M	Malignant melanoma	49	80	170	M	10	12	45	0.5	174	0.9	8.4	5.0
01 HD	Small-cell lung cancer	41	65	173	M	7	13	29	0.5	108	0.9	6.1	3.1
02 HD	Non-small-cell lung cancer	37	43	164	F	4	7	51	–	599	0.7	7.2	3.2
03 M	Non-small-cell lung cancer	50	65	164	F	8	12	8	0.4	126	0.6	6.8	4.2
04 M	Brenner's carcinoma	52	66	162	F	10	9	11	0.1	140	1.0	7.2	4.0
05 M	Head & neck cancer	61	81	178	M	6	6	14	0.6	97	0.7	7.3	4.6
01 HH	Adenocarcinoma of the colon	52	69	186	M	13	9	22	0.4	179	1.1	5.3	2.8
02 HH	Hepatocellular carcinoma	42	58	170	F	24	12	24	0.2	174	0.6	7.3	4.0
07 M	Small-cell lung cancer	68	62	168	M	6	8	26	0.4	178	0.6	6.9	4.2
08 M	Unidentified primary malignancy	56	84	178	M	13	14	24	0.7	111	0.9	6.6	–
09 M	Rhabdomyosarcoma	52	74	171	M	8	10	32	0.3	152	0.9	7.8	4.2
10 M	Head & neck cancer	49	60	172	M	6	9	41	0.3	254	0.8	7.6	4.8
03 HH	Fibrotic histiocytoma	69	77	172	M	9	11	28	0.4	136	0.9	7.6	3.9
04 HH	Carcinoma of the rectum	67	73	173	M	15	6	221	0.9	573	0.7	5.9	3.1
05 HH	Carcinoma of the colon	43	72	162	F	43	50	89	0.5	300	0.9	6.9	4.2
11 M	Oropharyngeal malignancy	46	44	175	M	7	4	7	0.2	181	0.9	7.5	4.1
12 M	Non-small-cell lung cancer	55	87	170	M	9	7	18	0.2	90	1.1	6.6	3.6
13 M	Non-small-cell lung cancer	54	88	166	M	10	14	47	0.3	234	0.9	7.4	3.3
15 M	Head & neck cancer	73	78	176	M	12	9	22	0.8	132	1.0	7.8	4.7
06 HH	Malignant melanoma	50	78	175	M	32	6	67	0.4	180	0.9	6.8	3.3
07 HH	Carcinoma of the rectum	41	65	175	M	14	8	153	0.5	420	0.9	7.1	1.7
08 HH	Carcinoma of the colon	62	82	181	M	16	13	57	1.3	302	0.9	7.7	4.3
14 M	Malignant melanoma	54	54	164	M	11	12	7	0.54	154	0.6	7.1	–

PK evaluation

All concentration versus time [c(t)] curves obtained for NK 611 were best described by a three-compartment model. The decision was made to evaluate the c(t) curves on the basis of statistics presented by the software program TOPFIT 2.0, a validated PK and PD analysis system that includes the *F*-test, the Akaike information criterion, the Imbimbo criterion, the Schwarz criterion, and the residuals [7]. The free drug content was calculated by comparison of the concentrations in plasma and ultrafiltrate samples as mean values across all concentrations. The area under the concentration versus time curve for the free drug (AUC_{free}) was calculated in percent from the AUC for the total drug. The total urinary excretion was determined by comparison of the total amount of unchanged drug excreted in the urine during 48 h and the delivered drug dose. The PK parameter change between the different dose levels was tested for statistical significance by the Mann-Whitney *U*-test and the Wilcoxon rank-sum test (one-tailed *P*).

Pharmacodynamic evaluation

The AUC and dose correlated with the relative reduction in white blood cell count (WBC), neutrophil count (ANC), platelet count (PLT), and hemoglobin count (Hb) at the nadir. Furthermore, the blood chemistry data on liver and renal function correlated with the AUC and ANC. The E_{max} model used for correlation finding was the asymmetric sigmoid form (allosteric Hill kinetics):

$$f(x) = \min + \frac{\max - \min}{1 + (x/x50)^{-p}}.$$

Statistical tests were the same used for PK evaluation.

Results

PK data were evaluated for 24 of 26 patients. In one patient, two c(t) curves were determined during the first and fourth treatment courses (patient 08 M). Another patient (06 M) was excluded from the overall analysis. The mean c(t) curves generated for total NK 611 at the 80-, 100- and 120-mg/m^2 dose levels are given in Fig. 2 [curves for 60 mg/m^2 ($n = 2$) and 140 mg/m^2 ($n = 1$) are not shown for reasons of clarity]. The PK values recorded for each patient are given in Table 2, and a summary of the data obtained at each dose level is shown in Table 3.

Statistical tests investigating changes in PK and PD values at different dose levels did not prove the changes to be significant. "Free" NK 611 was detectable for up to 4 h after the end of the infusion and, in four patients,

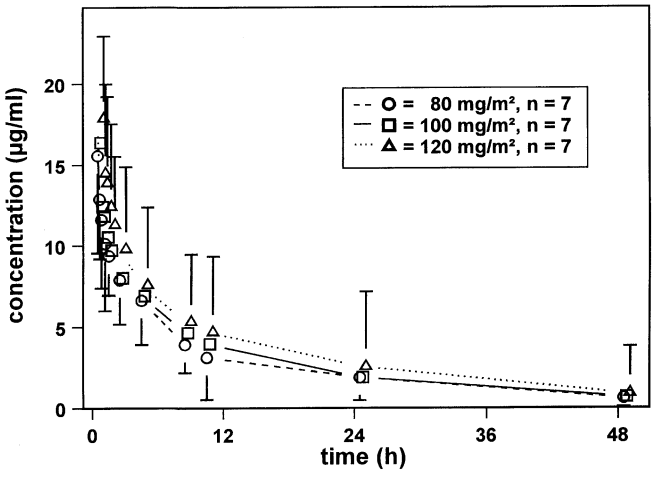

Fig. 2 Mean c(t) curves obtained for the 80–100, and 120-mg/m^2 dose levels

even longer (in one case for 8 h, in two cases for 10 h, and in one case for 24 h). The mean free drug concentration was 1.1% of that of total NK 611. The amount of free drug was constant in each individual and did not depend on the time point of sample collection. Renal excretion of NK 611 was highly variable and was not dose-dependent. Of the total dose given to the patients, 13.1 $\pm$ 5.9% was excreted as the unchanged drug; 40% of this small portion was excreted within the first 6 h. Peaks appearing between the front peak (hydrophilic material with a UV signal at 215 nm) and the NK 611 peak were suggestive of metabolites, but precise and reliable analysis was impossible because analytical standards for metabolite identification and quantitation were not available.

Increasing AUCs correlated with increasing doses ($r^2 = 0.978$; Fig. 3), but the standard deviation was remarkably high. The percentage of reduction in neutrophils at the nadir correlated with the dose and AUC (Fig. 4, 5). Additionally, the AUC_{free} correlated with the reduction in ANC (Fig. 6). No correlation was found between the values recorded for AUC, AUC_{free}, Cl_p or V_{ss}, on the one hand, and the clinical chemistry data, on the other (ASAT, ALAT, γ-GT, AP, bilirubin, creatinine, total protein, and albumin; determined 1 day before treatment).

Deviation of PK parameters was observed in one patient (07 HH) who received seizure prophylaxis with phenytoin; his AUC value was the lowest recorded, his Cl_p value was the largest noted within the study, and his V_{ss} value was near the median. This patient had the lowest rate of urinary excretion of the unchanged drug, whereas that of the free drug was within the normal range. The ANC reduction for this patient was not evaluable (tumor-related ileus made surgery and treatment with granulocyte colony-stimulating factor necessary). No other comedication could be identified as having substantial influence on the PK parameters, although several patients had a low AUC value due to a high level of clearance (1 HD, 3 M, 2 HH, 9 M, 10 M, 5 HH, 7 HH, 8 HH, 14 M).

Table 2 PK data of individual patients (*M* München, *HD* Heidelberg, *HH* Hamburg)

Patient number, center	Dose (mg/m^2)	Absolute dose (mg)	$AUC_{0.5-\infty}$ (µg ml^{-1})	Cl_p (ml min^{-1} m^{-2})	V_{ss} (I/m^2)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)	MRT (h)	Free drug (%)	Urinary excretion (%)
01 M	60	108	82.4	12.2	8.7	0.05	1.9	9.6	12.1	–	14.5
02 M	60	114	75.0	13.3	10.3	0.05	1.9	10.1	12.9	1.9	16.8
01 HD	80	140	65.8	19.7	12.8	0.06	2.0	8.7	11.0	1.8	–
02 HD	80	114	341.0	4.0	7.9	0.12	1.6	24.2	33.2	–	–
03 M	80	136	67.4	19.8	11.2	0.05	2.3	8.8	9.5	1.9	25.0
04 M	80	136	136.0	9.8	8.3	0.22	2.2	11.5	14.1	3.3	7.6
05 M	80	160	143.0	9.3	9.1	0.18	1.1	11.6	16.2	–	8.5
01 HH	80	150	135.0	9.8	10.9	0.48	4.7	17.3	16.2	0.6	7.7
02 HH	80	135	70.1	18.9	11.2	0.13	3.1	13.2	9.9	0.7	–
07 M	100	170	131.0	12.7	8.4	0.15	1.8	8.2	10.9	1.0	13.1
08 M	100	200	118.0	14.2	11.2	0.35	1.7	9.7	13.2	–	13.5
09 M	100	185	92.1	17.6	12.3	0.05	2.4	9.9	11.7	1.5	15.0
10 M	100	185	54.4	30.6	17.1	0.06	2.5	13.7	9.3	1.6	6.4
03 HH	100	185	317.0	5.4	9.7	0.05	4.2	23.3	30.1	0.8	25.8
04 HH	100	185	368.0	4.4	9.7	0.15	2.7	27.3	36.6	0.6	14.8
05 HH	100	180	86.2	19.3	14.9	0.07	3.2	11.4	12.8	0.7	14.9
11 M	120	180	159.0	12.6	8.5	0.05	2.2	10.5	11.4	0.8	20.5
12 M	120	240	317.0	6.3	8.5	0.23	1.4	16.3	22.4	0.7	11.7
13 M	120	220	532.0	3.6	9.2	0.08	1.9	30.5	42.3	0.6	11.5
15 M	120	230	116.0	17.4	14.6	0.05	1.0	10.4	13.9	1.6	18.8
06 HH	120	230	178.0	11.3	12.7	0.05	2.9	15.3	18.7	0.8	8.7
07 HH	120	210	53.4	36.4	11.8	0.26	2.5	15.0	5.4	0.7	3.0
08 HH	120	240	64.2	31.2	18.1	0.05	3.3	9.8	9.7	1.8	5.6
14 M	140	220	103.0	22.2	15.6	0.07	2.1	9.1	11.7	–	12.1

Table 3 Mean PK values ± SD obtained at each dose level; ranges are given in parentheses

Dose (mg/m^2)	$AUC_{0.5-\infty}$ ($\mu g\,ml^{-1}\,h$)	Cl_p ($ml\,min^{-1}\,m^{-2}$)	V_{ss} (I/m^2)	$t_{1/2\gamma}$ (h)	MRT (h)	Free drug (%)	Urinary excretion (%)
60 mg, $n = 2$	78.7 ± 3.7 (75.0–82.4)	12.8 ± 0.6 (12.2–13.3)	9.5 ± 0.8 (8.7–10.3)	9.9 ± 0.3 (9.6–10.1)	12.5 ± 0.4 (12.1–12.9)	1.9	15.7 ± 1.2 (14.5–16.8)
80 mg, $n = 7$	136.9 ± 89.5 (65.8–341.0)	13.0 ± 5.9 (4.0–19.8)	10.2 ± 1.7 (7.9–12.8)	13.6 ± 5.1 (8.7–24.2)	15.7 ± 7.6 (9.5–33.2)	1.7 ± 1.0 (0.6–3.3)	12.2 ± 7.4 (7.6–25.0)
100 mg, $n = 7$	166.7 ± 114.3 (54.4–368.0)	14.9 ± 8.3 (4.4–30.6)	12.0 ± 2.9 (8.4–17.1)	14.8 ± 6.9 (8.2–27.3)	17.8 ± 10.1 (9.3–36.6)	1.0 ± 0.4 (0.6–1.6)	14.8 ± 5.3 (6.4–25.8)
120 mg, $n = 7$	202.8 ± 157.2 (53.4–532.0)	17.0 ± 11.5 (3.6–36.4)	11.9 ± 3.3 (8.5–18.1)	15.4 ± 6.7 (9.8–30.5)	17.7 ± 11.3 (5.4–42.3)	1.0 ± 0.5 (0.7–1.8)	11.4 ± 6.0 (3.0–20.5)
140 mg, $n = 1$	103.0	22.2	15.6	9.1	11.7	–	12.1
Overall mean	158.5 ± 121.4 (53.4–532.0)	15.1 ± 8.6 (3.6–36.4)	11.4 ± 2.9 (7.9–18.1)	14.0 ± 6.1 (8.2–30.5)	16.5 ± 9.3 (5.4–42.3)	1.2 ± 0.7 (0.6–3.3)	13.1 ± 5.9 (3.0–25.8)

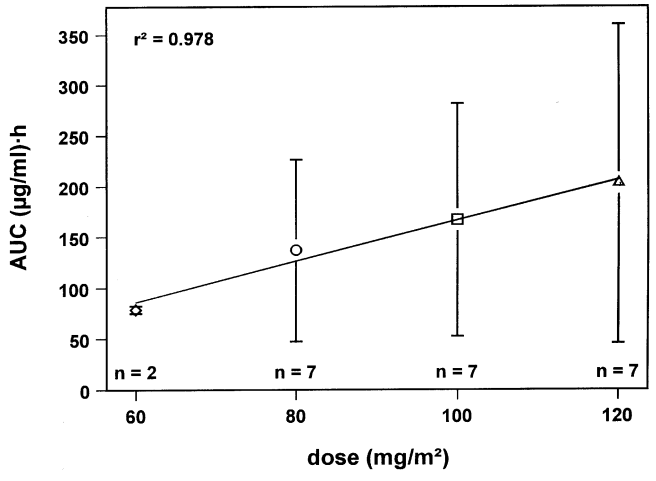

Fig. 3 Plot of dose versus AUC; error bars mark standard deviations

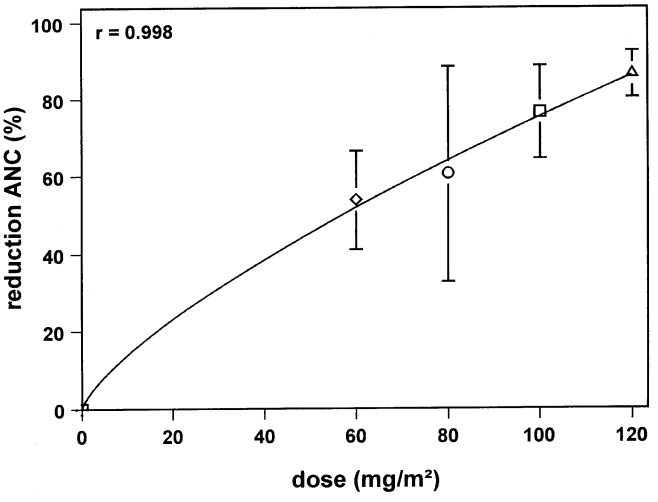

Fig. 4 Plot of dose versus percentage of reduction in neutrophil granulocytes; error bars mark standard deviations

Discussion

NK 611 is a semisynthetic epipodophyllotoxin derived from the mandrake root (mayapple). As compared with etoposide (VP-16) it is much less hydrophobic. This feature favors NK 611 in rapid penetration and crossing of cell membranes. Preclinical studies have indicated a higher degree of potency, better bioavailability, a (possibly) broader spectrum of anticancer activity, and little cross-resistance to VP-16, doxorubicin, and cisplatin in cell-culture systems [19, 20]. In vitro studies have revealed that NK 611's anticancer efficacy depends on the dose regimen, similar to that of VP-16 [3, 19]. The c(t) curves generated after NK 611 administration were best described by a three-compartment model. The majority of authors investigating VP-16 have used a two-compartment model, although recent results suggest a three-compartment disposition for VP-16 [8, 13, 14]. Older PK studies, recently summarized, have described a mean terminal half-life of between 5.6 and 11.1 h for VP-16 [12, 15]. These large interstudy deviations can be explained by differences in assay sensitivity, blood-sampling period, and PK model chosen. With a mean of 14.0 h, NK 611 has a longer half-life during the elimination phase, even if the wide range from 8.2 to 30.5 h is taken into account.

The PK parameters of NK 611 derived from a PK/PD study within a clinical phase I trial show several interesting aspects [16]. As one means of describing the systemic drug exposure, the AUC of NK 611 increases with the dose nearly linearly. However, the high standard deviations shown in Fig. 3 illustrate a high degree of variability in the AUC within each dose level. After normalization of all patients to the MTD of 120 mg/m^2, the coefficient of variance was 0.7. In the search for the reasons for this pharmacological behavior, one should focus on drug interactions and the metabolic pathway. As we know from teniposide

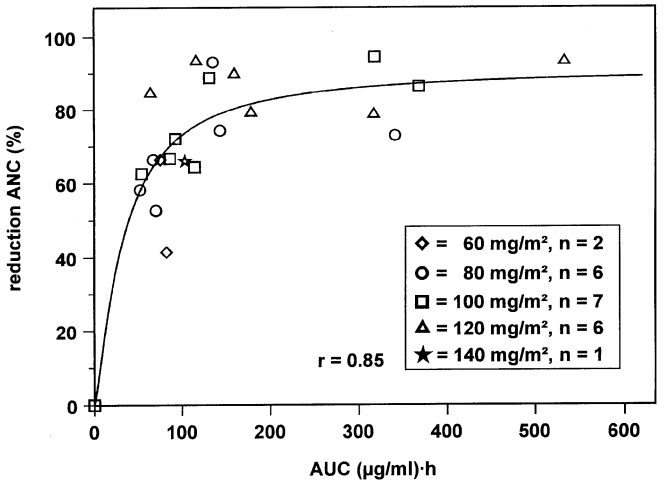

Fig. 5 Plot of AUC versus percentage of reduction in neutrophil granulocytes

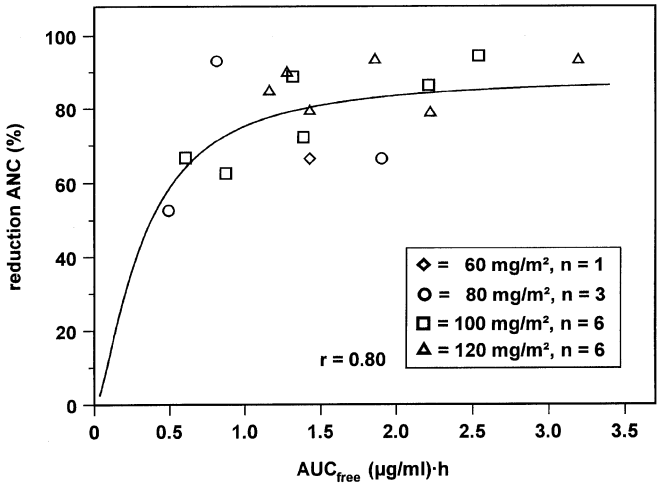

Fig. 6 Plot of AUC_{free} versus percentage of reduction in neutrophil granulocytes

(VM-26), comedication with phenytoin results in a reduction in the AUC to half of the initial value due to increased clearance [1, 13]. This can be explained by the induction of mixed-function oxidase isoenzymes in the hepatic endoplasmic reticulum and, therefore, the enhanced metabolism of VM-26. In looking for such a drug-drug interaction, we identified one patient (07 HH) receiving phenytoin for seizure prophylaxis. This patient was treated at a high dose level (120 mg/m^2), but his AUC value was the lowest recorded and his Cl_p value was the largest noted within the study. In other patients with an AUC of $< 100\ \mu g\,ml^{-1}\,h$ the comedication was sometimes complex, and some did not receive any additional drug therapy at all. For that reason a final conclusion cannot be drawn about the influence of phenytoin and other drugs on the PK behavior of NK 611.

Although we did not measure metabolites in plasma samples, it can be assumed that NK 611's metabolic pathway in the liver is similar to that known for VP-16. Due to the lack of pure standards for metabolites, our assay was optimized and validated for NK 611 and the internal standard. It cannot be ruled out that hydrophilic metabolites might not have been detected because of their disappearance in the front peak on the chromatograms. In future studies the rate of glucuronidation, deamination, and formation of free radicals should be investigated with respect to the unknown cytotoxic potential of these metabolites. Yet, reasons for the high interindividual variation in AUC remain unclear, as no correlation with liver or kidney parameters was found. Possibly a process under genetic control, similar to the bimodal distribution of the population into rapid acetylators and slow acetylators, plays a role.

Two other aspects in the pharmacology of NK 611 should be considered. First, the high plasma protein binding is striking. Our analysis of ultrafiltrates and comparison of total and free NK 611 revealed that more than 98% of NK 611 is protein-bound drug. This is likely to influence not only the PK in vivo but also the cytotoxic effects. Aberrant protein binding is of potential importance as it may elevate toxicity because the free drug is the active drug that can pass through the cell membrane and act at the target sites. In the case of VP-16 it has been shown that the binding ratio to albumin is linearly correlated with the albumin concentration and that, e.g., bilirubin can alter VP-16 binding to albumin [6, 18]. The high protein binding of NK 611 makes similar effects possible; patients with hypalbuminemia and/or elevated bilirubin levels are in danger of suffering from higher-grade toxicity. A review of the pretreatment data showed that several patients had albumin levels of < 3.5 g/dl and/or total protein levels of < 6.5. Bilirubin levels were within the normal range. The hypothesis that an inverse correlation exists between the AUC (and other PK parameters) and toxicity, on the one hand and protein/albumin levels, on the other, did not prove to be right.

The second aspect worthy of regard is the low-urinary-excretion fraction. Only between 3% and 25.8% of the total drug dose delivered to the patient could be recovered as the unchanged drug in urine samples obtained within 48 h. This finding differs remarkably that reported for VP-16, where up to 60% of the dose is excreted unchanged in the urine [12]. The chromatograms of urine samples showed some additional peaks occurring between the front peak and the NK-611 peak, indicative of more hydrophilic metabolites, e.g., glucuronidated metabolites, as is known for VP-16 [10]. Again, in the absence of pure standards, quantitation of metabolites could not be performed, but the peak height (PH) or the area under the peak (AUP) of these metabolites was in no case greater than the PH or AUP of the NK 611 peak. It can be assumed that no

more than 50% of the total drug will be recovered in the urine either as the unchanged drug or as a metabolite. No correlation between this finding and the PK or PD data was seen.

These results led to the conclusion that research on the metabolic pathways of NK 611 and disease conditions involving liver and kidney impairment should be evaluated to get more insight into the pharmacological profile of NK 611.

The prediction of NK 611-induced myelotoxicity is an important issue. In attempts to find a model to predict myelotoxicity, the dose, AUC, and AUC_{free} proved to correlate with blood cell counts (WBC, ANC, PLT, RBC). A severe toxic effect was seen on neutrophil granulocytes. The fitting was performed using the nonlinear Hill-type form (Figs. 4–6). The dose versus ANC-reduction model showed the highest correlation coefficient, but standard deviations within each dose level were remarkably high. Possibly the model fitting would have been even better if more than two patients had been treated at 60 mg/m^2. According to non-parametric statistical tests, the differences between the means were not significant ($P > 0.05$). However, all patients treated at the 120-mg/m^2 level experienced an ANC reduction of $> 75\%$, though the AUCs determined within this level were spread over the whole AUC range. Therefore, we focused on the models that take AUC and AUC_{free} into account. From Figs. 5 and 6 it becomes clear that there is hardly any difference between the two models and that the correlation coefficients are nearly identical. Hence, we can presume that the key to understanding the PD of this drug does not lie in free NK 611 concentrations and PK. We have to accept that none of these three models allows the precise prediction of ANC reduction. Additionally, the toxic effect might depend on dose scheduling and drug delivery, which can be optimized if the host's PK and metabolism is taken into account.

A search for laboratory parameters for the assessment of liver and kidney function to create a clinically useful pharmacodynamic relationship was not successful. γ-GT, ASAT, ALAT, AP, and creatinine values did not correlate with the AUC, Cl_p, V_{ss}, terminal half-life, MRT, free drug, or urinary excretion. Several patients with liver metastasis had elevated ASAT, ALAT, and AP levels at the time of therapy, but no striking deviation in PK parameters was found. As bilirubin and creatinine were always within the normal range, no conclusion with respect to these laboratory parameters can be drawn.

Comprehensively, this study did not elucidate the interactions among the triangle of dose, PK and PD. As compared with VP-16, the PK profile of NK 611 is advantageous due to the longer half-life but disadvantageous with respect to the enormous variability of PK parameters. Since the reasons for this behavior remain unclear, it is necessary that the PK, metabolism, and PD relationships be studied further clinical studies, e.g., in phase I studies using different schedules of drug administration and in phase II studies. Additionally, it would be of great interest to measure the activity of topoisomerase II and the expression of its complexes with DNA and NK 611. This enzyme seems to be the pharmacological end point of the NK 611 effect. A PD model considering topoisomerase II might lead to more reliability in predicting the toxicity (and therapeutic effect) of podophyllotoxin derivatives.

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