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Clinical and pharmacokinetic study of oral NK611, a new podophyllotoxin derivative

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Abstract NK611 is a novel water-soluble podophyllotoxin derivative that has comparable antitumour activity but higher potency and better bioavailability in animals as compared with etoposide. The primary objectives of this study were to determine, after both oral and intravenous administration in the same patient, the bioavailability and the pharmacokinetic profile of NK611. Secondary objectives involved evaluation of the toxicity and the antitumor activity. Patients were randomly assigned to receive oral or intravenous (30-min infusion) doses of 5, 10, and 20 mg/m^2 on day 1, when pharmacokinetic studies were performed. A daily oral dose of 20 mg/m^2 was then given from day 4 through day 7 for respective total doses of 85, 90, and 100 mg/m^2. NK611 and its metabolites were determined in plasma and urine by two different high-performance liquid chromatography (HPLC) methods with UV detection. A total of 21 adult patients entered the study and received the complete first cycle and at least the 1st day of cycle 2; 17 of them received at least 2 complete cycles of treatment. After intravenous administration, the plasma decay curve of NK611 followed a two-exponential model, and after oral administration it declined monoexponentially in most cases. At all dose levels, bioavailability values were around 100%. At concentrations between 10 and 20 mg/m^2 after both routes of administration, the pharmacokinetics were nonlinear; the terminal half-life, plasma clearance, and volume of distribution were significantly different; and the area under the plasma concentration-time curve was not correlated to the dose. The urinary excretion of NK611 corresponded to 10–15% of the dose after administration by both routes, whereas that of *N*-demethyl NK611 and its picroform was highly variable. The features of neutropenia were comparable with those noted for etoposide involving a high degree of interpatient variability and recovery within 1 month after treatment. A daily dose of 20 mg/m^2 for 5 consecutive days every 4 weeks is the recommended regimen for phase II studies in patients who have never been treated or have undergone previous chemotherapy only once.

Key words Podophyllotoxin derivative · Bioavailability · Pharmacokinetics.

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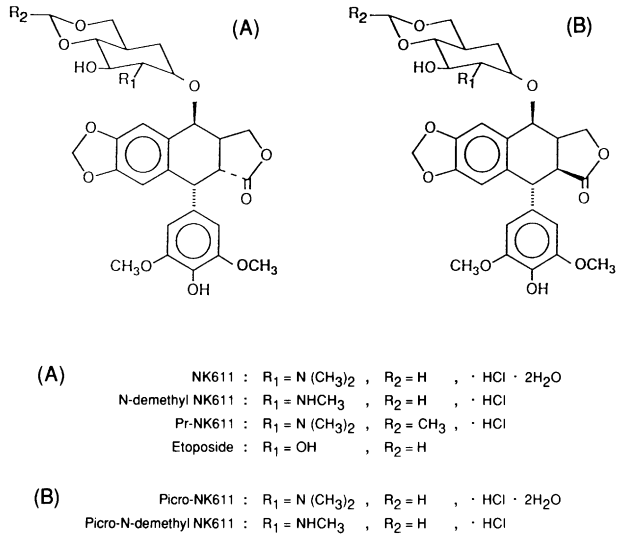
Introduction

NK611 is a novel water-soluble podophyllotoxin derivative with a dimethylamino group substituted in the D-glucose moiety of etoposide (E; Fig. 1). NK611 has a mechanism of action similar to that of E, whereas its cellular uptake is approximately twice as great as that of E [6]. Comparative studies with oral and intravenous E and NK611 have been performed to evaluate the antitumor activity of the two compounds in a variety of in vitro and in vivo models. The cytotoxicity of NK611 has been shown to be higher than that of E in 20 human tumor cell lines, including lung, gastrointestinal, ovarian, testicular, breast, and head and neck cancers and leukemia, and comparable antitumor activity has been reported in murine tumors [4].

Acute toxicity studies in mice, rats, and dogs involving the intravenous and oral administration of NK611 have revealed a pattern of toxicity comparable with



(A) NK611 : $R_1 = N(CH_3)_2$, $R_2 = H$, · HCl · $2H_2O$
N-demethyl NK611 : $R_1 = NHCH_3$, $R_2 = H$, · HCl
Pr-NK611 : $R_1 = N(CH_3)_2$, $R_2 = CH_3$, · HCl
Etoposide : $R_1 = OH$, $R_2 = H$

(B) Picro-NK611 : $R_1 = N(CH_3)_2$, $R_2 = H$, · HCl · $2H_2O$
Picro-N-demethyl NK611 : $R_1 = NHCH_3$, $R_2 = H$, · HCl

Fig. 1A,B Chemical structures of NK611 and related compounds

that observed for E, with the major target organs for both routes being the gastrointestinal (GI) tract and bone marrow. After single oral administration, lethal doses of NK611 were approximately 1/20 of the E dose in mice and between 1/20 and 1/40 of the dose in rats.

The schedule dependency of NK611 has been investigated in six different human xenografts [5], including lung, gastric, and ovarian carcinomas; schedule dependency of the antitumor activity was shown, the optimal schedules of treatment being days 1, 5, and 9 for the intravenous route and days 1–4 for the oral route. Single-dose pharmacokinetics (PK) studies in rats and dogs have shown a rapid and high-level absorption of oral NK611 (51% and 84%, respectively), whereas in both species, biliary excretion appears to be the main route of drug elimination [5].

NK611 was brought into clinical evaluation because of the marked improvement of its water solubility and bioavailability over that of oral E in dogs. Four phase I studies have been completed in Japan with different schedules (single intravenous and oral administrations, oral chronic administration, daily × 5 intravenous administration; in particular, the oral chronic protocol established the maximal tolerated dose (MTD) as 6 mg/m^2 per day for 21 consecutive days, producing dose-limiting myelotoxicity and very mild GI toxic effects [1]. In the daily × 5 intravenous study, the MTD for patients with or without prior chemotherapy was 20 and 24 mg/m^2 per day, respectively [7]. Comparable MTD (12.5 mg/day) and dose-limiting toxicity (DLT) values were established in Germany with the same oral chronic schedule [11]. A phase I study of a single intravenous infusion every 4 weeks was also conducted in Germany [15] ; the MTD was fixed at 120 mg/m^2, which was also the recommended dose for phase II studies. In a three-exponential model the distribution half-life ($t_{1/2\beta}$) ranged from 1.0 to 3.34 h and the terminal half-life ($t_{1/2\gamma}$), from 8.2 to 30.5 h. Within each dose level the area under the plasma concentration-time curve (AUC) varied considerably, and the mean AUC ± SD at the MTD was 203 ± 157 μg μl^{-1} h. In a subsequent cohort of patients, doses were further escalated with granulocyte colony-stimulating factor (G-CSF) administration from 140 to 250 mg/m^2. DLTs were neutropenia and thrombocytopenia. Pharmacokinetic analysis of this part of the study remains incomplete [12]. No experience with single oral administration of NK611 is available yet. Although the limited amount of data does not allow a comparison with E, a better tolerability of NK611 is suggested by the studies conducted thus far, with NK611 possibly producing fewer GI side effects than E [7, 15].

The present trial was designed to determine the bioavailability of oral NK611 as compared with the intravenous formulation in the same patient and to evaluate the pharmacokinetic profile of a single dose of oral NK611 at three different dose levels. Secondary objectives were to define the toxicity profile and the antitumor activity of the drug following its administration by the oral or the intravenous route on a mutiple-day schedule.

Patients and methods

Patient eligibility

Adult patients with histologically/cytologically confirmed solid tumors or lymphomas were eligible for this trial. Eligibility criteria also included a performance status of ≤ 2 on the WHO scale; a life expectancy of ≥ 3 months; an absolute neutrophil count (ANC) of ≥ 2.0×10^3/μl and a platelet (Pt) count of ≥ 100×10^3/μl; a direct bilirubin value of < 35 μmol/l; ALAT and ASAT values of < 2.5 times the upper limit of normal; a creatinine level of < 135 μmol/l; and a 24-h creatinine clearance of > 60 ml/min. Patients with a history of gastric disorders assumed to influence absorption (e.g., gastric resection, chronic nausea and vomiting) were excluded. A D-xylose test result within the normal range was also required, as was the patients' written informed consent.

Study design

In the first cycle, patients were assigned to oral or intravenous treatment by randomization; in the second cycle, patients who had received oral NK611 in the first cycle received the same dose intravenously, and vice versa. The drug was given on day 1 and then, to allow PK sampling after an adequate washout period, on days 4–7. Day-1 doses were 5, 10, and 20 mg/m^2; on days 4–7 a fixed dose of 20 mg/m^2 per day was given at all dose levels. Six patients had to be enrolled at each level. Assignment of patients to the different dose levels was sequential and nonrandomized.

Day-1 doses were chosen so as to include a low dose, to be used in a subsequent 21-day chronic oral study, and a higher dose, to be evaluated in a subsequent daily × 5 trial. The fixed dose of 20 mg/m^2 per day was selected by taking into account the maximal total doses investigated in previous clinical trials (120 mg/m^2 in the single intravenous trial in Germany and 126 mg/m^2 in the chronic oral study in Japan) [1, 15].

Treatment was repeated every 4 weeks, provided that an ANC of $\geq 2.0 \times 10^3/\mu l$ had been reached. In case of grade 4 neutropenia, the total dose given on days 4–7 was decreased by 20% during subsequent cycles, whereas day-1 doses were left unchanged. Complete blood cell counts with differential were done weekly and at least twice a week in instances of toxicity of at least grade 3. Chemistry [including electrolytes, ALAT/ASAT, bilirubin, total protein, albumin, creatinine, urea, glucose and lactate dehydrogenase (LDH)], urinalysis, and physical examination were performed weekly. Toxicity was evaluated according to the National Cancer Institute (NCI) Common Toxicity Criteria[1]. The relative decrease in ANC was calculated as:

$$\frac{\text{Pretreatment ANC} - \text{ANC nadir}}{\text{Pretreatment ANC}} \times 100 .$$

All patients with measurable disease who had received at least one cycle of therapy were evaluable for efficacy; tumor response was assessed according to WHO criteria [10].

Drug formulation and administration

NK611 was supplied by Asta Medica AG (Frankfurt) as white film-coated tablets containing 5, 20, and 50 mg of product or as vials of 20 mg NK611 on an anhydrous basis. The latter had to be reconstituted with 5 ml of 5% dextrose to a final concentration of 4 mg/ml. The prescribed amount of drug was then diluted in 250 ml of 5% dextrose and given as a 30-min infusion into the lateral entry of an intravenous line. For bioavailability studies, patients fasted overnight and were given only 5-mg tablets. Prophylactic antiemetics were not routinely prescribed. Prophylactic oral antibiotics were given in instances of an ANC of $< 0.5 \times 10^3/\mu l$ and were continued until an ANC of $\geq 1.0 \times 10^3/\mu l$ was reached.

Sample collection

Blood samples (5 ml) were collected in tubes containing ethylenediaminetetracetic acid (EDTA) as an anticoagulant on day 1 of both the first and second cycles (i.e., after oral and intravenous administration, or vice versa). PK studies were performed in all 21 patients who entered the trial, whereas bioavailability was determined in 18 of them, 6 for each of the 3 dose levels tested. Blood samples were taken before (T_0), immediately after, and at 10, 20, 30, and 60 min as well as 2, 4, 8, 10, 24, 36, and 48 h after intravenous administration and were obtained before and at 15, 30, 45, 60, and 90 min as well as 2, 4, 8, 10, 24, 36, and 48 h after oral administration. Blood was immediately placed in an ice bath and centrifuged at 4 °C (2000 g, 10 min), and the separated plasma was stored at − 20 °C in cryotubes (NUNC A-S, Roskilde, Denmark) until analyzed.

Urine samples were collected at intervals of 0–6, 6–12, 12–24, 24–48, and 48–72 h after administration during both cycles. To prevent isomerization reactions of the compounds excreted into the picro form at pH ≥ 5, 100 ml of ammonium acetate buffer (0.5 *M*, pH 3.5) was added to the containers used for urine collection. Aliquots of 10 ml were then stored at − 20 °C in cryotubes (NUNC) until analyzed.

Drug assay and pharmacokinetic analysis

NK611 and its metabolite (*N*-demethyl NK611) were determined in plasma and urine using two different high-performance liquid chromatography (HPLC) methods with UV detection at 205 nm that were recently developed in our laboratory. The method for plasma involves the addition of pr-NK611 as the internal standard (IS) and solid-phase extraction on a C18 cartridge [16]. The two compounds, together with their isomerization products (picro-NK611 and picro-*N*-demethyl NK611), were determined in urine after the addition of the same IS and chloroform extraction [2]. The lower limit of quantitation (LLQ) of NK611 and *N*-demethyl NK611 in plasma was 20 ng/ml, and the LLQ in urine was 100 ng/ml for each of the four compounds assayed.

The NK611 plasma concentrations versus time-points after oral (p.o) and intravenous (i.v.) administration for each patient were fitted to the standard equations for a monoexponential or a two-exponential model [13] using a nonlinear fitting computer program [14] to determine the half-life of the first distribution phase ($t_{1/2\alpha}$) the half-life of the elimination phase (terminal half-life, $t_{1/2\beta}$), the volume of distribution (Vdβ) , and the plasma clearance (Clp). The AUC was calculated by the trapezoidal rule from T0 to the last measurable point (48 h for the majority of cases studied) and extrapolated to infinity (AUC_{inf}) using the elimination rate constant (β) , which is the slope of the elimination phase of the plasma concentration-time curve. The bioavailability (F) was calculated by the formula:

$$F = \frac{AUC_{inf}\ \text{(p.o.)}}{AUC_{inf}\ \text{(i.v.)}} \times \frac{\text{dose i.v.}}{\text{dose p.o.}} \times 100 .$$

PK parameters were compared among the groups using Kruskal-Wallis' test.

Results

From July 1993 to October 1994, 21 patients entered the study and 17 of them completed at least 2 cycles. Three patients discontinued the treatment after the first cycle because of rapid tumor progression (PD), and one patient received only the first dose of the second cycle and was then lost to follow-up. The characteristics of the patients treated are summarized in Table 1. The

Table 1 Characteristics of patients (*NSCLC* Non-small-cell lung cancer, *SCLC* small-cell lung cancer)

	Number of patients
Entered	21
M/F	14/7
Age (years) :	
Median	60.5
Range	39 –75
Performance status (WHO):	
0	16
1	5
Prior therapy:	
Chemotherapy alone	5
Radiotherapy	2
Chemo- + radiotherapy	5
None	9
Tumor type:	
NSCLC	11
SCLC	2
Colon	3
Melanoma	1
Other	4

[1] Common Toxicity Criteria from the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland

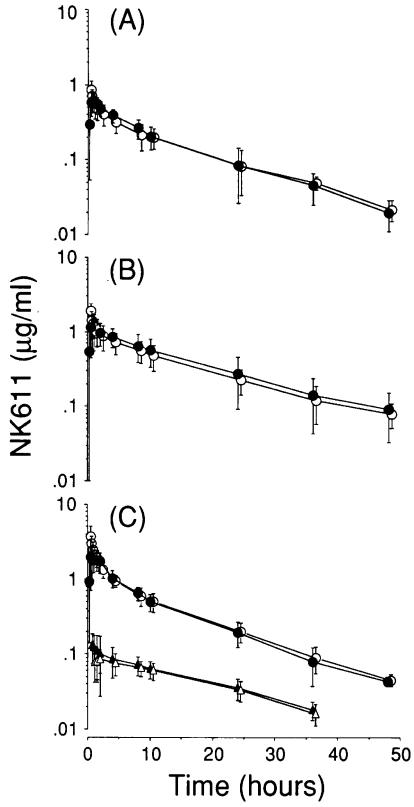


Fig. 2A–C NK611 plasma levels (mean ± SD) determined after oral (*black circles*) and i.v. (*white circles*) administration of **A** 5 mg/m^2 ($n = 8$), **B** 10 mg/m^2 ($n = 7$), and **C** 20 mg/m^2 ($n = 6$) NK611. Note the decay curves of *N*-demethyl NK611 (**C**; *black triangles* oral administration, *white triangles* i.v. administration)

most common tumor type was non-small-cell lung cancer, present in 11 patients, 8 of whom had not received previous chemotherapy.

Pharmacokinetic study

Figure 2 shows the plasma decay curves of NK611 concentrations (mean ± SD) as determined in patients after both oral and i.v. administration of 5 mg/m^2 (Fig. 2A, $n = 8$), 10 mg/m^2 (Fig. 2B, $n = 7$) and 20 mg/m^2 (Fig. 2C, $n = 6$) of NK611. Figure 2C also shows the mean (± SD) plasma levels of the main metabolite of NK611, *N*-demethyl NK611. In all patients a two-exponential model gave a good representation of NK611 PK after the 30-min infusion, whereas after oral administration the disappearance of drug from the plasma followed a monoexponential decay in most patients.

The mean (± SD) values of the main PK parameters are reported in Table 2. After oral administration at all three dose levels the peak concentration (C_{max}) was achieved within 1 h. The bioavailability values were around 100% in the majority of cases. A linear relationship was found between the dose and C_{max} after both

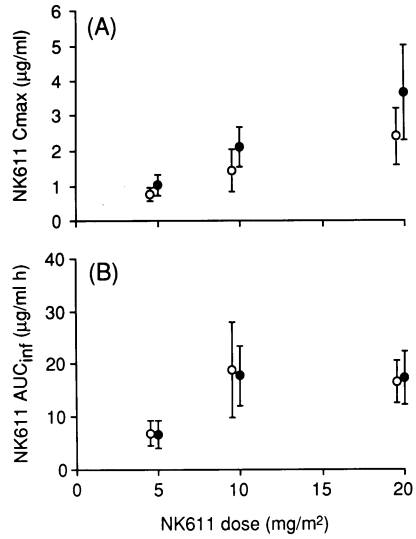


Fig. 3A,B Relationship between the NK611 dose and the **A** plasma C_{max} and **B** plasma AUC_{inf} values determined after oral (*white circles*) and i.v. (*black circles*) administration

oral ($R = 0.993$) and i.v. ($R = 0.995$) administration (Fig. 3A), whereas no significant correlation was found between the dose and the 24-h AUC (p.o. $R = 0.63$, i.v. $R = 0.70$) or AUC_{inf} (p.o. $R = 0.46$, i.v. $R = 0.47$; Fig. 3B). Statistical comparison of the PK parameters revealed significant differences ($P < 0.05$) in the terminal half-life, Clp, and Vdβ values determined at 10 versus 20 mg/m^2 (Table 2). Plasma levels of *N*-demethyl NK611 could be detected in all patients treated at 20 mg/m^2, in three patients who had received 10 mg/m^2, and in two who had received 5 mg/m^2. At 20 mg/m^2 the mean (± SD) AUC and terminal half-lives of the metabolite were, respectively, 3.2 ± 0.6 μg/ml^{-1} h and 14.8 ± 5.9 h after oral dosing and 3.0 ± 0.8 μg ml^{-1} h and 14.4 ± 5.6 h after i.v. administration.

As shown in Fig. 4, the mean amount of NK611 and its picro form in the 72-h urine collection corresponded to 10–15% of the dose after both routes of administration. The interpatient variability was acceptable with a ≤ 30% coefficient of variation (CV). In contrast, a large SD was found for the excretion of *N*-demethyl NK611 and its picro form at the dose of 5 mg/m^2; in particular, one patient excreted as metabolite more than 40% of the oral dose and more than 20% of the i.v. dose. In addition, the percentage of the dose of the metabolite excreted by patients treated at 10 mg/m^2 was significantly lower than the percentage excreted by those treated at 20 mg/m^2 (oral 5.6 ± 2.8% versus 9.8 ± 2.7%, $P = 0.03$; i.v. 4.1 ± 1.7% versus 8.2 ± 2.5%, $P = 0.009$).

Clinical study

In all, 52 of the 54 courses given were evaluable for hematological toxicity (Table 3). The total dose is

Table 2 Mean pharmacokinetic parameters ± SD determined following i.v. and oral administration of NK611

Dose (mg/m^2)	C_{max} (μg/ml)	T^a_{max} (h)	$t_{1/2\alpha}$ (h)	Terminal half-life (h)	AUC_{24th} ($\mu g\,ml^{-1}\,h$)	AUC_{inf} ($\mu g\,ml^{-1}\,h$)	Clp ($ml\,min^{-1}/m^2$)	Vdβ (l/m^2)	F (%)
5 i.v. ($n = 7$)	1.03 ± 0.30	–	0.6 ± 0.4	10.1 ± 2.3	5.4 ± 2.1	6.6 ± 2.7	15.3 ± 7.8	12.0 ± 2.8	
5 p.o. ($n = 7$)	0.76 ± 0.2	0.75	–	8.6 ± 1.9	5.8 ± 1.9	6.9 ± 2.4			102 ± 25[b]
10 i.v. ($n = 7$)	2.12 ± 0.57	–	0.4 ± 0.3	12.4 ± 3.5*[1]	13.2 ± 4.0	17.8 ± 5.6	10.6 ± 5.2*[2]	10.8 ± 3.4*[3]	
10 p.o. ($n = 6$)	1.43 ± 0.6	0.75	–	11.6 ± 3.6	13.8 ± 5.4	18.9 ± 9.1			106 ± 23[b]
20 i.v. ($n = 6$)	3.66 ± 1.37	–	1.0 ± 0.7	8.8 ± 1.2*[1]	15.4 ± 3.9	17.4 ± 5.0	20.8 ± 6.0*[2]	15.2 ± 3.1*[3]	
20 p.o. ($n = 6$)	2.39 ± 0.8	0.63	–	9.1 ± 2.0	14.5 ± 3.1	16.6 ± 4.1			98 ± 22

*[1] $P = 0.037$; *[2] $P = 0.025$; *[3] $P = 0.030$ according to Kruskal-Wallis' test
[a] Median value
[b] Evaluated in 6 patients per dose

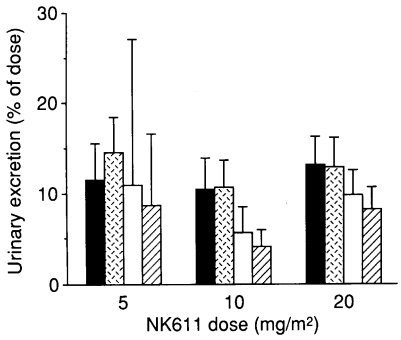


Fig. 4 Urinary excretion (mean ± SD) of NK611, *N*-demethyl NK611, and the picro forms following doses of 5 mg/m^2 (n = 6), 10 mg/m^2 ($n = 6$), and 20 mg/m^2 ($n = 6$): NK611 and its picro form after oral (*black bars*) and i.v. (*hatched bars*) administration; *N*-demethyl NK611 and its picro form after oral (*white bars*) and i.v. (*striped bars*) administration

Table 3 Number of patients and cycles per dose level

Total dose (mg/m^2)	Number of patients	Number of cycles given		
		Initial	Subsequent	Total
75	2	–	8	8
80	2	1	5	6
85	6	6	5	11
90	7	7	5	12
100	5	5	9	14
105	2	2	1	3

calculated by adding the four daily doses of 20 mg/m^2 to the day-1 single dose of 5 (85 mg/m^2), 10 (90 mg/m^2), and 20 mg/m^2 (100 mg/m^2). Two patients inadvertently received 25 mg/m^2 from day 4 to day 7 (105 mg/m^2 total dose). A lower total dose of 80 mg/m^2 (20 mg/m^2 on day 1, 15 mg/m^2 from day 4 to day 7) was given to one patient who had undergone previous chemo- and radiotherapy.

Because of severe myelotoxicity, lower total doses of 75 mg/m^2 (15 mg/m^2 daily) in two cases and 80 mg/m^2 in one case were given during subsequent cycles. A median of 2 (range 1–9) cycles were given. Three of six patients entered at 85 mg/m^2 had been pretreated (one with both chemo- and radiotherapy and two with radiotherapy alone). All but one of the seven patients entered at 90 mg/m^2 had received no previous chemotherapy, whereas all five patients entered at 100 mg/m^2 had previously undergone chemotherapy.

As expected, neutropenia was the main hematological toxicity, with the median time to nadir ranging between day 15 and day 20. The median time to recovery to pretreatment values exceeded 28 days in most cases, but protocol-required values were achieved in all patients at scheduled retreatment visits, with no treatment delay being needed (Table 4). Grade 4 neutropenia developed in only 15% of the cycles, and the median duration of an ANC of $\leq 0.5 \times 10^3/\mu l$ was 6.5 (range 2–11) days.

Neutropenia showed a high degree of interpatient variability at 85 and 90 mg/m^2, with ANC nadirs ranging from 0.4 to $4.0 \times 10^3/\mu l$ and from < 0.1 to $5.5 \times 10^3/\mu l$, respectively. It appeared to be dose-related; grade 3–4 neutropenia was observed in 25% of the cycles at 75 mg/m^2, in 50% at 90 mg/m^2, and in 58% at 100 mg/m^2 (Table 5). Table 5 also shows that the median relative decreases in ANC values were comparable within the dose range from 80 to 100 mg/m^2. Only one episode of neutropenic fever was reported in one patient treated at 105 mg/m^2.

Thrombocytopenia was always associated with neutropenia of grade 3–4. Thrombocytopenia of grade 2 was observed in four patients treated at doses of ≥ 90 mg/m^2, and grade 3 thrombocytopenia was observed in one patient treated at 100 mg/m^2. Anemia was neither dose-related nor cumulative; 17 cycles (32%) were associated with anemia of at least grade 2, and packed red cell transfusions were given on 6 occasions.

Nonhematological toxicity was moderate; complete alopeiac was observed in all patients receiving more than one cycle at all dose levels. Grade 2 nausea was reported in three patients treated at 90 mg/m^2 and was associated with grade 2 vomiting in one patient.

Table 4 Leuko- and neutropenia: median nadir count, time to ANC nadir, and recovery per dose level

Total dose (mg/m^2)	Number of evaluable patients/cycles	Median nadir (range) $\times 10^3/\mu l$		Median time in days to ANC (range)	
		WBC	ANC	Nadir	Recovery
75	2/8	2.3 (2.0–3.4)	1.2 (0.8–1.8)	19 (14–22)	29 (20– > 28)
80	2/6	2.2 (1.2–2.5)	0.5 (0.3–0.8)	18 (12–23)	> 28 (27– > 28)
85	6/11	3.2 (1.4–6.7)	1.8 (0.4–4.0)	20 (12–20)	27 (21– > 28)
90	7/12	2.3 (0.4–7.6)	1.1 (< 0.1–5.5)	16 (14–22)	29 (24– > 28)
100	5/14	2.3 (1.0–3.9)	0.9 (0.2–1.7)	15 (13–22)	29 (22– > 28)
105	2/3	1.8 (1.7–3.3)	0.3 (0.1–0.6)	17 (14–20)	26 (24– > 28)

Table 5 Leuko- and neutropenia: number of cycles with grade ≥ 3 neutropenia and median relative decrease in ANC per dose level

Total dose (mg/m^2)	Number of evaluable patients/cycle	Number of cycles with neutropenia of grade		ANC relative % decrease	
		3	4	median	(range)
75	2/8	2	0	56	(40–88)
80	2/6	3	3	76	(20–90)
85	6/11	3	1	68	(22–93)
90	7/12	4	2	74	(39–99)
100	5/14	5	2	70	(5–94)
105	2/3	1	2	91	(90–95)

Grade 3 stomatitis was observed in one patient receiving 105 mg/m^2.

Two patients with non-small-cell lung cancer, one being chemotherapy-naive and one progressing while on platinum-based chemotherapy, showed disease stabilization of 9 and 4 months' duration, respectively. One patient with lung metastases from melanoma who had been pretreated with combination chemotherapy and one heavily pretreated patient with laterocervical nodes from Fallopian-tube adenocarcinoma showed a disease stabilization of 4 months' duration. One patient with bone and adrenal metastases from lung leiomyosarcoma who had been pretreated with combination chemotherapy, including ifosfamide, Adriamycin, and dacarbazine, showed a disease stabilization of 6 months' duration.

Discussion

NK611 is a novel dimethylamino derivative of etoposide (E) with an antitumor activity in vitro and in vivo that is comparable with that of E [4]. NK611 is more water-soluble than E because of the presence of the dimethylamino group on the sugar moiety. Preclinical studies in animals have shown that oral NK611 is better absorbed than E. The present study suggests that NK611 is better absorbed than E in humans as well. Indeed, the bioavailability was found to be around 100% at all of the three doses tested, i.e., 5, 10, and 20 mg/m^2, with acceptable interpatient variability. We did not investigate the bioavailability of E in those patients, but it has been shown that after the oral administration of 50–100 mg the bioavailability ranges from 38% to 70% [3, 8, 9]. Therefore, the bioavailability of NK611 appears better than that of E, and this suggests that NK611 could more advantageous than E for oral treatment. However, it should be stressed that although the bioavailability was approximately 100% in all cases, a high degree of interpatient variability in the AUC was found after both i.v. and oral administration at doses of 5 and 10 mg/m^2. The reasons for this variability remain to be elucidated. Actually, in all cases but one the AUC of the *N*-demethylated NK611 was less than 20% of the AUC of NK611, and it is unlikely that the main reason for the variability in the NK611 AUC would be the different rate of *N*-demethylation.

Comparison of the Cl and Vdβ values determined at the doses of 10 and 20 mg/m^2 suggests nonlinear PK for NK611. A possible explanation would be saturable plasma protein binding of NK611 associated with an increased drug-tissue distribution and metabolism. In urine a significantly higher percentage of metabolite was found at 20 mg/m^2 than at 10 mg/m^2. In addition, in vitro data obtained by incubation of three different concentrations of NK611 (1, 2.5, and 5 μg/ml) with plasma from healthy donors are consistent with this hypothesis and reveal that the amount of drug bound to plasma proteins was 97%, 96%, and 94%, respectively (unpublished data).

The total renal elimination of NK611, *N*-demethyl NK611, and their picro forms accounted for less than 30% of the NK611 dose. However, it seems likely that a relevant fraction of NK611 would be eliminated as metabolites, such as glucuronides, which were not assessed in this study. That NK611 and its major metabolite undergo conjugation with glucuronic acid has been suggested by Ekimoto et al. [4], who found, after the administration to animals of [^{14}C]-NK611, a relevant amount of radioactivity in urine and bile associated with glucuronated metabolites. It is suggested that NK611 behaves like E since the two drugs possess a similar structure with an OH group on the pendant ring, which reacts with the COOH group of the glucuronic acid. It may therefore be important to assess the urinary excretion of NK611 glucuronides and to evaluate whether the variability in the AUC might be related to a variable degree of conjugation with glucuronic acid among patients.

This study confirms previous data, showing that the toxic effects of NK611 in humans are comparable with those of oral E, involving mild nonhematological side effects, a lack of severe thrombocytopenia, and dose-limiting neutropenia. This study was not primarily designed to establish the MTD for a particular schedule of treatment; however, notwithstanding the differences in patient selection at 90 and 100 mg/m^2 and the overall high degree of interpatient variability in the development of neutropenia, it seems that 100 mg/m^2, corresponding to a daily dose of 20 mg/m^2 for 5 consecutive days, can be recommended for phase II studies in patients that have never been treated or have been pretreated with a maximum of one previous chemotherapy regimen.

Even though the bioavailability of NK611 seems to be satisfactory, the variability in the plasma concentrations of the parent compound and of its more potent *N*-demethyl metabolite (Asta Medica, unpublished results) might be associated with a different risk of toxicity among patients. Therapeutic drug monitoring could be implemented into phase II studies to improve our pharmacodynamic knowledge of NK611 and its metabolities.

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