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Pharmacokinetics of MEN-10755, a novel anthracycline disaccharide analogue, in two phase I studies in adults with advanced solid tumours

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Abstract The doxorubicin analogue MEN-10755 has been identified as a compound with promising antitumour activity based on structure-activity studies of a new series of anthracycline disaccharides. The high antitumour activity of MEN-10755 in human tumour xenografts, including doxorubicin-resistant xenografts, and its unique pharmacological and biological properties made this novel disaccharide analogue an interesting candidate for clinical evaluation. Two pharmacokinetic phase I studies with different dosing schedules were performed in adults with solid refractory malignancies. The pharmacokinetics of MEN-10755 were studied after a 15-min i.v. infusion given once every 3 weeks or once every week for 3 weeks followed by 1 week rest. Plasma and urine levels of MEN-10755 were measured by HPLC with fluorescent detection. It was possible to combine the pharmacokinetic results of the two studies because there was no accumulation of MEN-10755 before the next infusion of MEN-10755 in the weekly study with 1 week rest. The administered dose levels on day 1 in this study were all in the lower range from the 3-weekly study. The postinfusion plasma kinetics of MEN-10755 were best described by a triexponential model. The plasma peak levels (C_{max}) of MEN-10755 showed a linear relationship with the administered dose. Peak plasma MEN-10755 levels ranged between 474 and 21,587 μg/l. The mean elimination half-life ($T_{1/2\gamma}$) was 20.7 ± 9.0 h. The $AUC_{0-\infty}$ was proportional to the administered dose. The mean plasma clearance of MEN-10755 was 6.0 ± 2.2 l/h per m^2 with a mean volume of distribution (V_{ss}) of 95.6 ± 43.4 l/m^2. The mean renal excretion of unchanged drug within 24 h was $4.3 \pm 1.8\%$. Compared to epirubicin and doxorubicin, the pharmacokinetics of MEN-10755 were characterized by an approximately twofold shorter terminal half-life, a much lower total plasma clearance and a much smaller volume of distribution.

Keywords Pharmacokinetics · Anthracycline · Phase I · Solid tumours

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Introduction

The anthracycline antibiotics, of which doxorubicin is the most representative, are well-known chemotherapeutic agents with well-established clinical effects in many different human cancers [1]. Although doxorubicin is still considered to be the antineoplastic drug with the broadest spectrum of antitumour activity, there are some solid tumours (e.g. colon cancer, melanoma and renal cancer) in which doxorubicin is ineffective [1]. In addition, side effects, such as myelotoxicity and

cardiotoxicity, together with the appearance of multidrug resistance, limit its clinical effect. Much effort has been expended in the development of new anthracycline derivatives with improved curative properties and/or reduced toxicity and in overcoming drug resistance [1, 2].

The second generation analogue epirubicin only marginally overcomes the above drawbacks. Although epirubicin at equimolar doses exhibits less-acute toxicity and cardiotoxicity compared to doxorubicin, at equimyelotoxic doses there is little difference [1]. In the past, the structural features of the amino sugar have been reported to be critical determinants of the pharmacological activity of doxorubicin-related anthracyclines. Later studies have indicated that the presence of the amino group at C-3 of the sugar is not a strict requirement for pharmacological activity, and that the lack of the amino group increases the drug's ability to stimulate DNA cleavage mediated by topoisomerase II [3, 4, 5, 6, 7].

A series of new anthracycline molecules have been synthesized in which the amino group appears at the second sugar residue bound in axial orientation to the first residue, which is characterized by the substitution of the amino for a hydroxyl group [8]. MEN-10755 has been identified as the most promising compound during this course of synthesis and biological evaluation of new third generation anthracyclines, which was based essentially on the hypothesis that elongation of the carbohydrate moiety and its variation might result in improved compounds. The chemical structure of MEN-10755 is shown in Fig. 1.

MEN-10755 shows remarkable cytotoxic effects in vitro against a variety of human tumour cell lines, including ovarian, lung, breast, cervical and colon cancer, with a potency comparable to that of doxorubicin or idarubicin (4-demethoxy-daunorubicin) [9]. MEN-10755 is more efficacious than doxorubicin against a large series of human tumour xenografts in the nude mouse [10, 11]. In particular, in gynaecological tumour models MEN-10755 shows efficacy against the intrinsically doxorubicin-resistant mammary tumour MX-1 and the doxorubicin-sensitive ovarian carcinoma A2780 [10, 11]. With regard to lung tumours, MEN-10755 shows activity against two small-cell lung cancer tumour xenografts, POVD and POSG, that are resistant to doxorubicin, and against the non-small-cell lung cancer H460 which shows only a marginal response to doxorubicin [10, 11].

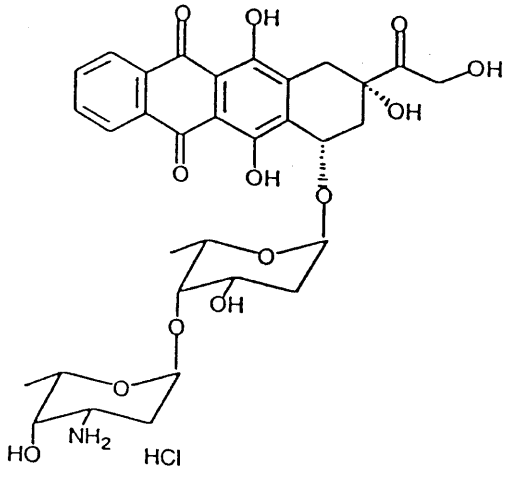

Fig. 1 The chemical structure of MEN-10755. Molecular formula $C_{32}H_{37}NO_{13}$·HCl; relative molecular mass 680.11

Most of the tumours refractory to doxorubicin and responsive to MEN-10755 are characterized by overexpression of the antiapoptotic protein Bcl-2 [11]. In MX-1 breast carcinoma tumours in mice, MEN-10755 induces phosphorylation of Bcl-2 after a single treatment at therapeutic doses [11]. An equimyelotoxic regimen of MEN-10755 in rats produces fewer ECG alterations, smaller impairment of the ventricular response to adrenergic stimulation, and less-severe myocyte lesions than doxorubicin. Unlike the effects of doxorubicin, the histological and functional cardiotoxic effects in rats are not progressive, being similar at 4 and 13 weeks after the last treatment [12].

Antitumour anthracyclines are intercalating agents, a DNA double-stranded molecule being their biological receptor. The nuclear enzyme DNA topoisomerase II, which relaxes DNA supercoils arising during replication and gene transcription, has been identified as the specific target [7]. The DNA cleavage induced by MEN-10755 shows the same sequence specificity as the reference compounds doxorubicin and idarubicin [9]. Thus, topoisomerase-II-mediated DNA cleavage produced by MEN-10755 occurs at a specific nucleic acid sequence. The antitumour efficacy of MEN-10755 is probably related to its intrinsic cytotoxic potential, as predicted by cellular pharmacokinetics [10]. The peculiar feature of MEN-10755 could be the result of different interactions at critical genomic sites and/or a different cellular response to topoisomerase-mediated DNA lesions, possibly related to the disaccharide moiety [11].

The high antitumour activity of MEN-10755 against human tumour xenografts, including doxorubicin-resistant xenografts, and its unique pharmacological and biological properties make this disaccharide analogue an interesting candidate for clinical evaluation. Two phase I dose tolerability and pharmacokinetic studies with different dosing schedules of MEN-10755 have been performed in patients with refractory malignancies. Preliminary results have been presented in abstract form [13, 14].

The objective of the current study was to characterize the pharmacokinetics of MEN-10755 when administered as a 15-min intravenous (i.v.) infusion given once every 3 weeks or once every week for 3 weeks followed by 1 week rest. The aim of using the latter schedule was to achieve a higher total drug dose than the 3-weekly regimen.

Patients and methods

Eligibility

Patients with a histologically or cytologically confirmed diagnosis of a solid tumour not amenable to established forms of treatment were eligible. Other eligibility criteria were: evaluable or measurable disease; age ≥18 years; performance status ≤ 2 on the WHO scale; life expectancy ≥3 months; written informed consent; no chemo-, immuno- or radiotherapy for at least 4 weeks prior to entry to the study (6 weeks for nitrosoureas, mitomycin-C and high-dose carboplatin and extensive radiotherapy); no prior use of anthracyclines or anthracenediones; adequate bone marrow function (absolute neutrophil count ≥1500/mm^3, platelet count ≥100,000/mm^3); adequate hepatic function (serum bilirubin ≤ 25 μmol/l; other liver function tests not more than twice the normal upper limit, unless related to liver metastases in which case not more than five times the upper limit of normal); adequate renal function (serum creatinine ≤ 120 μmol/l or a creatinine clearance ≥60 ml/min); adequate cardiac function, established by a left ventricle ejection fraction of ≥50% as measured by MUGA scan; no active infections; no history of alcoholism, drug addiction or psychotic disorders; suitable for adequate follow-up; and no clinical signs of brain involvement or leptomeningeal disease.

Study design

Both studies were open, non-randomized, two-centre, dose-escalating trials. Study 1 was performed in Denmark and Norway and study 2 in Belgium and The Netherlands. The protocol was approved by the Medical Ethical Committees of the participating hospitals. Each patient gave written informed consent.

Drug administration

MEN-10755 was supplied by Menarini Ricerche (Rome, Italy) as a lyophilized powder in 10-ml glass vials containing 10 mg active material (MEN-10755 hydrochloride) with 50 mg lactose as excipient. The vials were easily reconstituted with 5–50 ml normal saline or water for injection. The solution was stable in glass and PVC bags at 25°C for at least 48 h [15]. MEN-10755 has to be stored in the dark, but protection from light is not necessary during administration. In both studies, MEN-10755 was administered as a single 15-min infusion via a free-flowing peripheral infusion system. In study 1, MEN-10755 was administered once every 3 weeks. In study 2, MEN-10755 was administered every week for 3 weeks followed by 1 week rest.

Dose-escalation procedures

Based on studies of single i.v. administration of MEN-10755 in rats and mice, the starting dose in study 1 was 4 mg/m^2, which was one-tenth of the mouse LD_{10}. The starting dose in study 2 was 15 mg/m^2, which was half the upper non-toxic dose level reached in study 1. If no toxicity was observed, doses were escalated in 100% steps. When toxicity was observed, doses were escalated by 100–66–40–25% increments (Fibonacci-like) depending upon the type and severity of the toxicity at previous levels, and upon prior treatment and pharmacokinetic data. There was no dose escalation in individual patients.

Pharmacokinetics

Sampling procedure

Pharmacokinetics was investigated in all patients during the first administration of MEN-10755. Peripheral blood (7-ml samples) was obtained from the arm contralateral to the infusion site and was collected in heparinized Vacutainer tubes. Blood samples were collected immediately before drug administration and at 10, 15, 20, 25, 30, 45 min and 1, 2, 4, 6, 9, 12 and 24 h after infusion. In study 1, extra blood samples were collected after 36 and 48 h and in study 2, on days 8 and 15 just prior to the start of the new infusion. Samples were immersed in ice-water at the bedside. Plasma was obtained by immediate centrifugation at 2100 *g* for 5 min at 4°C and 2 ml plasma from each sample time-point was stored frozen in polypropylene tubes at –20°C for subsequent analysis. Urine was collected every 8 h over 24 h. In study 1, urine was further collected until 48 h. During collection, urine was stored in a refrigerator. Two 10-ml aliquots of urine were taken from each collection interval, transferred to polypropylene tubes and stored at –20°C until analysis for a maximum period of 3 months. MEN-10755 was proven to be stable under these conditions in both human plasma and urine.

Analytical procedure

The study was conducted in compliance with the Good Laboratory Practice (GLP) regulations of the US FDA and with the Dutch and Italian GLP regulations.

Plasma and urine levels of MEN-10755 were determined by a validated high-performance liquid chromatographic (HPLC) method [16, 17]. In study 1, the HPLC system consisted of a model 116 solvent delivery pump (Beckman), equipped with an automatic sample injector model 232 (Gilson), a Spheri-5 RP-18 250×4.5 mm (Brownlee), a fluorescence detector (Shimadzu, model RF-551) an analogue interface 406 (Beckman) and an IBM computer. The mobile phase consisted of a mixture of 0.1 *M* sodium dihydrogen phosphate monohydrate and acetonitrile (70:30), with the pH adjusted to 3 using orthophosphoric acid. The HPLC system operated isocratically at room temperature. For plasma sample preparation daunomycin (internal standard) and sodium chloride were added. The samples were extracted with a mixture of chloroform and isopropyl alcohol. For urine samples the internal standard and sodium acetate pH 4 were added. The samples were vortexed and loaded onto a Supelco Supelclean LC-Diol cartridge (500 mg) which had been preconditioned with methanol and HPLC water. The substance was eluted with 0.06 *M* potassium bromide in methanol. The lower limit of quantitation (LLQ) was 0.25 ng/0.5 ml in human plasma and 1 ng/0.1 ml in human urine.

The absolute recovery from plasma was 79.5%. The values of the intra- and interassay variability (precision) expressed as coefficient of variation (percent) were less than 2.3% in the concentration range 5–50 ng/0.5 ml, whereas the values of the intra- and interassay variability for the 0.25 ng/0.5 ml (the LLQ for plasma) were 2.7% and 3.2%, respectively. The accuracy evaluated at the same concentrations and expressed as relative error (percent) ranged from –2.9% to 5.7%.

The absolute recovery from urine was 87.1%. The values of the intra- and interassay variability (precision) expressed as coefficient of variation (percent) were less than 2.3% in the concentration range 5–150 ng/0.1 ml, whereas the values of the intra- and interassay variability for the 1 ng/0.1 ml (the LLQ for urine) were 4.5% and 3.1%, respectively. The accuracy evaluated in the same samples and expressed as relative error (percent) ranged from –2.7% to 2.2%.

In study 2, MEN-10755 and daunomycin (internal standard) were extracted from plasma and urine (buffered at pH 8.4 with borate buffer saturated with sodium chloride) by liquid-liquid extraction using a diethylether/*n*-butanol mixture. The organic solution containing MEN-10755 and daunomycin were re-extracted with 0.3 *M* phosphoric acid. The acidic aqueous phase was injected into the HPLC-system, which consisted of a model L-6200S Intelligent pump (Merck Hitachi) equipped with an automatic sample injector (model 717P Waters) with refrigeration (4°C), a fluorescence detector (Shimadzu, RF 10AXL) and a computer with a Chrom Perfect chromatography data integration system.

The within-run precision, evaluated at four different plasma concentrations (0.55, 1.09, 5.47, 54.7 ng/ml) and expressed as relative standard deviation (RSD) ranged from 2.4% to 2.8%. The RSD for the between-run precision evaluated at the same concentrations was lower than 9.7%. The accuracy evaluated in the same plasma samples and expressed as relative error (percent) ranged from –3.6% to 0.5%. The recovery of MEN-10755 during three analytical runs at three plasma concentrations (0.55, 5.47 and 54.7 ng/ml) was 60%. The within-run precision, evaluated at four different urine MEN-10755 concentrations (10.3, 26.0, 260.0, 1041.0 ng/ml) and expressed as RSD ranged from 2.4% to 4.3%. The RSD for the between-run precision evaluated at the same urine concentrations was lower than 5.6%. The accuracy evaluated in the same samples and expressed as relative error (percent) ranged from –5.9% to 6.3%.

The recovery of MEN-10755 from urine samples at three different concentrations (10.3, 260.0 and 1041.0 ng/ml) was 70%. The LLQ of MEN-10755 in plasma was 0.49 ng/ml and in urine 10.3 ng/ml. This HPLC method was robust, precise and capable of accurately quantifying MEN-10755 in plasma in the concentration range 0.49–98.9 ng/ml and in urine in the concentration range 10.3–1546 ng/ml.

Pharmacokinetic and statistical analysis

The Kinfit computer program (MW/Pharm, MediWare, Groningen, The Netherlands) was used to calculate the individual pharmacokinetic parameters [18]. The postinfusion kinetics in both studies were best described by the a triexponential model. The equation describing the triexponential decline in the serum concentration-time curve was derived in each individual by non-linear regression analysis using a weighted least-squares simplex algorithm.

The peak plasma concentration ($C_{max)}$ was the highest measured value, with the corresponding sampling time t_{max}. The area under the curve ($AUC_{0-\infty)}$ was calculated using the linear trapezoidal method with extrapolation to infinity. Total clearance (CL) was calculated by dividing the administered dose by the $AUC_{0-\infty}$. The bioavailability was assumed to be 1. The terminal elimination rate constant (k_{el}) was determined by least-squares regression analysis of the terminal part of the plasma concentration versus time curve. The terminal half-life ($T_{1/2\gamma}$) was calculated as $0.693/k_{el}$. The volume of distribution (V_{ss}) was calculated from the equation $V_{ss} = CL \times MRT$. The MRT (mean residence time) is the MRT in the body of a single drug molecule and was calculated from the following equation: $MRT = AUMC/AUC + t_{inf}/2$. In this equation, t_{inf} is the duration of the infusion. Linear regression analysis was used for testing the pharmacokinetic linearity in the dose range used.

Results

Pharmacokinetics were determined in 32 patients in study 1 and in 11 in study 2. The characteristics of patients in study 1 and study 2 are listed in Tables 1 and 2,

Table 1 Characteristics of the 32 patients in study 1 (*BSA* body surface area; *sCr* serum creatinine values day 1 of treatment, reference range 62–106 μmol/l; *SGOT* serum glutamic-oxaloacetic transaminase day 1 of treatment, reference <25 U/l; *SGPT* serum glutamic-pyruvic transaminase day 1 of treatment, reference <30 U/l)

Patient no.	Sex	Age (years)	Weight (kg)	Height (cm)	BSA (m^2)	sCr (μmol/l)	SGOT (U/l)	SGPT (U/l)	Liver metastases
1	M	59	72.0	190	1.99	80	55	1	–
2	F	59	84.0	172	1.97	69	37	26	–
3	M	58	82.4	184	2.05	89	29	44	–
4	M	69	85.0	182	2.10	94	22	24	–
5	M	50	80.0	184	2.00	73	21	28	–
6	M	55	92.5	189	2.20	71	23	42	–
10	M	65	83.0	186	2.08	90	25	23	–
11	F	59	47.0	163	1.50	66	33	16	–
12	M	31	77.5	184	2.00	74	23	19	–
16	M	44	101.5	191	2.31	66	22	24	–
17	M	49	72.0	178	1.89	56	48	41	+
18	F	51	48.0	167	1.52	62	23	27	+
24	M	34	60.0	185	1.72	79	16	16	–
25	M	53	58.9	177	1.73	81	29	29	–
26	F	57	58.4	163	1.63	61	33	25	–
27	F	43	50.0	174	1.60	57	33	26	+
28	M	51	79.0	177	1.96	85	44	56	+
29	F	47	60.6	170	1.70	83	79	53	+
30	F	50	60.5	173	1.72	74	17	10	–
31	M	50	76.0	175	1.91	62	17	26	–
32	F	51	73.0	164	1.80	56	50	27	+
33	M	54	68.0	192	1.95	63	29	26	+
34	M	61	76.0	168	1.86	86	30	33	+
35	M	53	90.6	186	2.20	107	19	21	–
36	M	66	78.0	180	2.00	73	50	41	+
37	M	46	62.8	175	1.76	70	20	19	–
38	M	58	72.5	183	1.90	82	25	20	–
39	M	57	78.0	183	2.00	90	19	37	–
40	F	34	63.0	179	1.77	68	24	24	–
41	M	53	91.0	183	2.14	97	13	23	–
42	M	58	66.0	174	1.80	75	26	19	+
43	F	54	59.0	172	1.70	67	23	14	–
Mean		52.5	72.1	178	1.89				
SD		8.7	13.5	8	0.20				

Table 2 Characteristics of the 11 patients in study 2 (*BSA* body surface area; *sCr* serum creatinine values day 1 of treatment, reference ranges 62–106 μmol/l and 0.6–1.3 mg/dl; *SGOT* serum glutamic-oxaloacetic transaminase day 1 of treatment, reference <25 U/l); *SGPT* serum glutamic-pyruvic transaminase day 1 of treatment, reference <30 U/l)

Patient no.	Sex	Age (years)	Weight (kg)	Height (cm)	BSA (m^2)	sCr (μmol/l)	sCr (mg/dl)	SGOT (U/l)	SGPT (U/l)	Liver metastases
7	M	70	82.0	177	2.00	–	1.3	15	13	–
8	F	61	60.0	158	1.60	75	–	22	9	–
9	M	69	70.0	173	1.83	83	–	22	14	–
13	M	50	66.6	173	1.78	–	1.0	16	15	–
14	M	65	82.0	169	1.96	–	1.2	36	38	+
15	F	45	74.5	158	1.76	–	1.3	18	22	–
22	M	70	97.7	177	2.00	–	1.6	22	24	–
23	M	55	100.0	173	2.13	–	0.8	25	29	–
19	F	66	58.0	167	1.65	62	–	37	39	–
20	M	74	70.5	171	1.82	–	0.8	21	22	–
21	F	53	52.0	143	1.41	–	0.8	17	24	+
Mean		62	73.9	167	1.81					
SD		10	15.4	10	0.21					

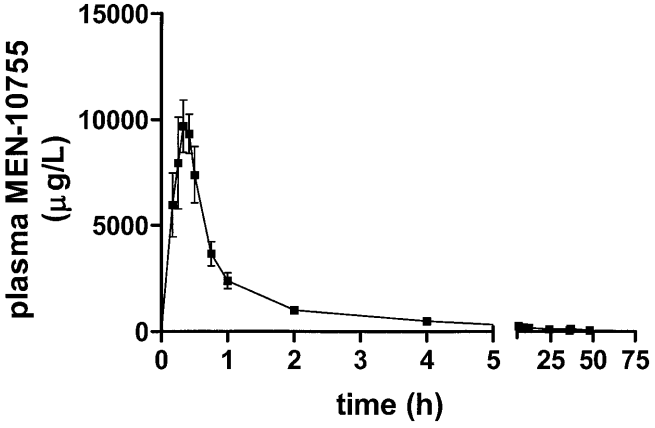

Fig. 2 Mean (±SEM) plasma concentration versus time curve of MEN-10755 from six patients treated at 80 mg/m^2 in study 1

respectively. All patients had normal total bilirubin levels with values <17 μmol/l or <0.5 mg/dl. The dose levels studied in the 3-weekly study were 4, 8, 16, 32, 55, 80, 110 and 100 mg/m^2 and in the weekly study with 1 week rest 15, 30, 45 and 40 mg/m^2 per infusion. In one-third of the administrations, the length of infusion was exactly 15 min, and in the others it varied between 10 and 45 min.

Analysis of the serum concentration-time curves after the first dose showed a triexponential profile for all patients. Curve fitting of the data to a different compartment model did not improve the fit. Figure 2 shows the mean (±SEM) plasma disappearance curve of MEN-10755 in six patients treated at 80 mg/m^2, which was the maximum tolerated dose level in study 1. In study 1, MEN-10755 was measurable at 48 h after the start of infusion in all patients. In the weekly study, the serum concentrations on days 8 and 15, just before the next administration of MEN-10755, were nearly zero in most of the patients. One patient treated at 30 mg/m^2 and another patient treated at 45 mg/m^2 had concentrations on day 8 up to 17 μg/l and 58 μg/l, respectively. The serum concentration in one patient treated at 30 mg/m^2 was 10 μg/l on day 15. The pharmacokinetic results of study 2 were combined with those of study 1 as there was no accumulation of MEN-10755 on day 8 and day 15 before the next infusion in nearly all patients. The administered dose levels on day 1 in study 2 were all in the low range compared with study 1. The pharmacokinetic results of both studies are therefore presented together.

The pharmacokinetic parameters of 43 patients treated at escalating dose levels in both studies are summarized in Table 3. The plasma peak levels (C_{max}) of MEN-10755 were reached at the end of the administration and showed a linear relationship with administered dose ($R = 0.91$, $P < 0.0001$; Fig. 3). Peak plasma MEN-10755 levels ranged between 474 and 21,587 μg/l. The mean (±SD) initial ($T_{1/2\alpha}$), intermediate ($T_{1/2\beta}$) and elimination half-life time ($T_{1/2\gamma}$) of MEN-10755 were 0.21±0.11 h, 1.49±0.64 h and 20.74±9.04 h, respectively. The $AUC_{0-\infty}$, which represents plasma exposure to drug, was proportional to the administered dose ($R = 0.94$, $P < 0.0001$; Fig. 4). The mean plasma clearance of MEN-10755 was 6.0±2.2 l/h per m^2 with a mean volume of distribution (V_{ss}) of 95.6±43.4 l/m^2. The MRT of MEN-10755 was about 17 h.

Regarding the eligibility criterion in this study for adequate hepatic function, there was no indication that liver function and/or liver metastases within this range influenced the pharmacokinetics of MEN-10755.

Urine collections were incomplete in two patients in the 3-weekly study and in one patient in the weekly study. Urinary concentrations of MEN-10755 were highest in the first collection period and decreased in the following periods. Concentrations in urine in the first 8 h ranged from 116 to 13,627 μg/l. The mean renal excretion of unchanged drug within the first 24 h was 4.3±1.8% (Table 4). The urinary creatinine concentrations over 24 h for all patients were within the normal ranges, indicating normal renal filtration function in all patients.

Table 3 Individual and mean pharmacokinetic parameters of 43 patients treated on escalating dose levels in the 3-weekly study (study 1) and in the weekly study with 1 week rest (study 2) (C_{max} peak plasma concentration, $AUC_{0-\infty}$ area under the curve, CL total body clearance, V_{ss} volume of distribution at steady state, $T_{1/2\alpha}$ initial half life, $T_{1/2\beta}$ intermediate half life, $T_{1/2\gamma}$ terminal elimination half life, T_{max} time to reach C_{max}, MRT mean residence time)

Patient no.	Study no.	Dose (mg/m^2)	Dose (mg)	C_{max} (μg/l)	$AUC_{0-\infty}$ (μg·h/l)	CL ($l/h/m^2$)	V_{ss} (l/m^2)	$T_{1/2\alpha}$ (h)	$T_{1/2\beta}$ (h)	$T_{1/2\gamma}$ (h)	T_{max} (h)	MRT (h)
1	1	4	8	474	708	5.85	83.97	0.22	1.40	18.99	0.33	14.34
2	1	4	8	612	677	6.30	58.73	0.22	1.12	13.45	0.33	9.32
3	1	4	8	572	784	5.07	70.39	0.16	1.37	17.22	0.25	13.88
4	1	8	17	1,320	1,365	6.69	82.71	0.01	1.07	14.34	0.33	12.37
5	1	8	16	1,826	1,729	4.83	56.20	0.23	1.40	14.30	0.25	11.63
6	1	8	18	881	1,053	7.70	108.91	0.12	1.31	16.78	0.25	14.15
7	2	15	30	850	2,168	7.40	125.00	0.33	1.74	19.83	0.42	16.89
8	2	15	24	1,453	3,179	4.24	64.48	0.14	0.75	14.96	0.33	15.22
9	2	15	28	1,566	2,541	5.69	54.03	0.13	0.81	11.43	0.33	9.49
10	1	16	33	1,247	1,208	13.46	160.77	0.11	1.00	15.02	0.17	11.94
11	1	16	24	2,465	2,292	7.91	79.80	0.03	0.66	12.22	0.50	10.10
12	1	16	32	1,589	1,330	12.35	134.95	0.14	1.40	16.79	0.25	10.93
13	2	30	54	3,276	5,077	6.28	113.76	0.12	1.08	21.12	0.25	18.12
14	2	30	59	4,379	6,926	4.44	80.71	0.11	1.06	19.81	0.25	18.20
15	2	30	53	3,720	7,927	4.00	78.64	0.19	1.49	21.48	0.25	19.67
16	1	32	74	6,352	5,904	5.70	65.54	0.22	1.65	15.31	0.33	11.50
17	1	32	60	2,621	4,215	7.92	129.26	0.46	2.36	21.30	0.33	16.33
18	1	32	50	2,579	3,505	9.94	131.51	0.43	3.45	21.88	0.33	13.23
19	2	40	65	6,188	10,440	3.87	48.39	0.19	1.18	14.80	0.25	12.51
20	2	40	71	2,481	4,965	8.08	172.80	0.00	0.68	23.94	0.17	21.39
21	2	40	55	2,822	4,798	8.50	176.95	0.23	1.73	22.87	0.25	20.82
22	2	45	90	4,490	7,728	6.22	98.50	0.26	1.64	19.66	0.25	15.83
23	2	45	90	6,175	8,771	5.08	93.52	0.23	1.99	25.63	0.25	18.42
24	1	55	95	3,636	8,743	6.46	209.42	0.29	1.58	34.86	0.33	32.42
25	1	55	94	10,171	10,890	5.13	53.92	0.20	1.31	15.08	0.33	10.50
26	1	55	88	10,827	14,950	3.75	48.64	0.18	1.80	17.23	0.33	12.97
27	1	55	85	6,425	11,180	4.93	67.13	0.25	1.14	17.10	0.33	13.63
28	1	55	108	11,277	12,930	4.36	59.93	0.15	1.24	18.14	0.33	13.51
29	1	80	136	13,099	22,300	3.63	88.47	0.17	1.33	27.64	0.37	24.40
30	1	80	137	14,854	15,010	5.46	48.96	0.17	1.07	14.50	0.25	8.96
31	1	80	150	9,971	10,990	7.53	99.90	0.24	1.51	18.47	0.42	13.26
32	1	80	140	12,529	21,480	3.92	87.83	0.30	0.96	30.46	0.50	22.43
33	1	80	150	4,407	11,500	6.99	116.15	0.52	1.65	20.41	0.17	16.62
34	1	80	150	7,325	14,920	5.58	84.89	0.27	1.51	17.54	0.25	15.22
35	1	110	242	18,206	22,960	5.00	63.55	0.17	1.14	18.94	0.33	12.71
36	1	110	220	21,587	33,870	3.24	56.00	0.06	0.77	20.61	0.25	17.27
37	1	110	187	14,169	20,030	5.48	52.76	0.11	0.96	12.92	0.33	9.62
38	1	110	209	9,558	17,090	6.73	76.53	0.35	2.44	17.98	0.47	11.38
39	1	100	200	15,206	19,970	5.11	94.40	0.18	1.27	21.31	0.25	18.48
40	1	100	177	13,980	22,190	4.58	98.53	0.23	1.99	29.73	0.17	21.50
41	1	100	200	10,337	29,250	3.28	215.28	0.34	1.89	65.77	0.42	65.56
42	1	100	180	7,681	22,910	4.53	159.56	0.45	2.47	37.25	0.42	35.23
43	1	80	137	8,994	20,620	3.89	57.96	0.27	3.65	22.62	0.75	14.88
Mean						5.98	95.57	0.21	1.49	20.74	0.32	16.90
SD						2.19	43.39	0.11	0.64	9.04	0.11	9.42

Discussion

In preclinical studies the novel anthracycline MEN-10755 had a greater therapeutic efficacy than doxorubicin, especially in gynaecological and lung cancers [10, 11]. Therefore, this compound was selected for further clinical investigation in two phase I studies with short drug infusions but different administration schedules.

Estimates of pharmacokinetic values for each individual patient were obtained from a traditional data set of more than ten postinfusion plasma samples drawn over 24 h after the start of a 15-min infusion of MEN-10755. A population approach was chosen for the analysis of the individual parameters. This has the advantage over analysis of mean curves that a proper weighting for curve fitting is implemented, based on the inherent variability of pharmacokinetic parameters in the population. According to protocol the prescribed infusion time was 15 min, but in daily practice the actual infusion time was highly variable, ranging between 10 and 45 min. This may have been due to the method of administration, as MEN-10755 was administered via a free-flowing peripheral venous infusion without the use of an infusion pump.

The evaluation of kinetic data in preclinical studies showed that there was no difference in C_{max} or AUC following single or repeated doses, and therefore it could

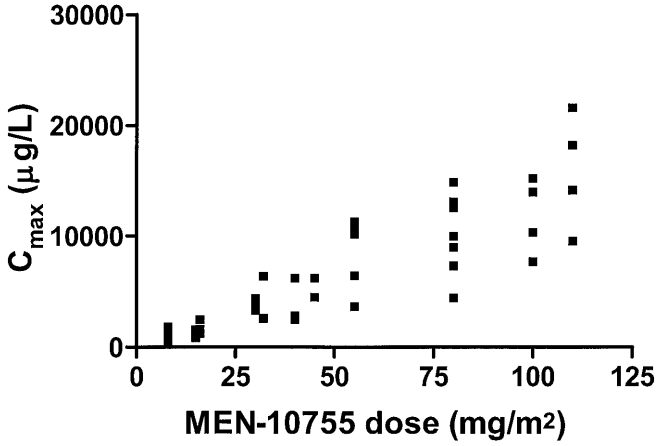

Fig. 3 Linear relationship between the peak plasma levels (C_{max}) of MEN-10755 and administered drug dose ($R = 0.91$, $P < 0.0001$)

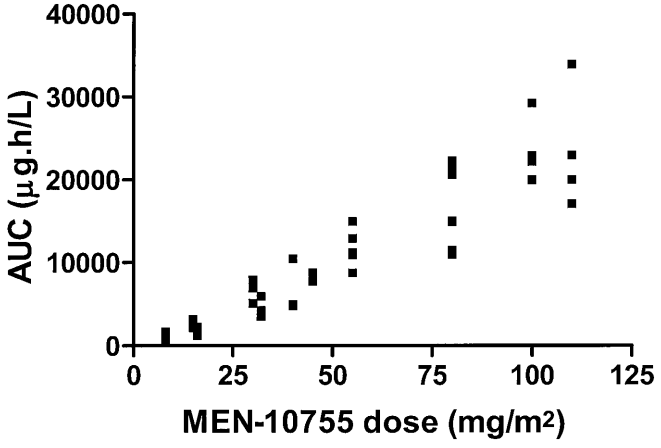

Fig. 4 Linear relationship between the AUC $_{0-\infty}$ of MEN-10755 and administered drug dose ($R = 0.94$, $P < 0.0001$)

be concluded that there is no accumulation of MEN-10755 [19]. At the start of the second and third administrations in the weekly study, the plasma concentrations of MEN-10755 were nearly zero for most of the patients. Therefore, it is clear that the pharmacokinetics of MEN-10755 administered in two different schedules did not show significant differences. The disposition of MEN-10755 in rats and mice is characterized by a large volume of distribution, a low plasma clearance and a half-life of 30 h (rats) and 8 h (mice) [20, 21].

A three-compartment linear model was the best fit for the current MEN-10755 plasma concentration-time data from these two phase I studies. The kinetics appeared to be linear, as the C_{max} and AUC increased with dose in both studies. The plasma half-life, plasma clearance and volume of distribution were not dependent on dose. These clinical pharmacokinetic findings are consistent with the preclinical observations described above.

The pharmacokinetics of epirubicin and doxorubicin have been evaluated in a number of studies [22, 23, 24, 25, 26, 27, 28]. The plasma elimination of epirubicin and doxorubicin appears to be triphasic after an i.v. bolus injection of 50 mg/m^2 [24]. The terminal elimination half-life of epirubicin is approximately 30 h, which is slightly shorter than the half-life of doxorubicin in crossover studies [24]. The total plasma clearance of epirubicin is approximately 50–100% higher than that of doxorubicin (50 versus 30 l/h per m^2) and the volume of distribution of epirubicin compared with that of doxorubicin is 1000 versus 500 l/m^2 [24].

Compared to these pharmacokinetic data of epirubicin and doxorubicin, the pharmacokinetics of MEN-10755 were characterized by an approximately twofold shorter terminal half-life, a much lower total plasma clearance and a much smaller volume of distribution. The pharmacokinetic profile of epirubicin is not affected

Table 4 Renal excretion of unchanged MEN-10755 during the first 24 h following a single i.v. administration

Patient no.	Study no.	Dose (mg/m^2)	Dose (mg)	Renal excretion (%)
1	1	4	8	4.2
2	1	4	8	2.4
3	1	4	8	4.4
4	1	8	17	1.4
5	1	8	16	6.7
6	1	8	18	5.8
7	2	15	30	4.3
8	2	15	24	7.8
9	2	15	28	5.8
10	1	16	33	2.8
11	1	16	24	1.9
12	1	16	32	6.9
13	2	30	54	3.5
14	2	30	59	?
15	2	30	53	2.3
16	1	32	74	4.2
17	1	32	60	5.3
18	1	32	50	3.2
19	2	40	65	6.2
20	2	40	71	4.0
21	2	40	55	2.3
22	2	45	90	1.7
23	2	45	90	5.5
24	1	55	95	4.2
25	1	55	94	2.5
26	1	55	88	7.8
27	1	55	85	5.9
28	1	55	108	6.0
29	1	80	136	3.5
30	1	80	137	4.8
31	1	80	150	4.4
32	1	80	140	6.5
33	1	80	150	1.8
34	1	80	150	3.4
35	1	110	242	1.7
36	1	110	220	5.5
37	1	110	187	4.0
38	1	110	209	6.5
39	1	100	200	6.2
40	1	100	177	4.5
41	1	100	200	3.4
42	1	100	180	1.5
43	1	80	137	?
Mean				4.3
SD				1.8

by i.v. administration interval in the range 1–3 weeks [27]. As already mentioned, the pharmacokinetics of MEN-10755 in preclinical studies were the same with single and repeated doses. The pharmacokinetic parameters of MEN-10755 did show large interpatient variation. This is consistent with previous studies, in which exceptionally large interindividual variations for doxorubicin and epirubicin were noted [22, 29], although Mross et al. did not find such large interindividual variations [23].

The mean renal excretion in 24 h ranged from an average of 1.4% to 7.8% of the total administered amount of MEN-10755. As the total plasma half-life is about 17 h, it could be expected that after 24 h about 65% will be excreted. The pattern of urinary excretion suggests a large non-renal clearance, which is in agreement with the findings with epirubicin and doxorubicin [27, 28]. About 6 to 7% of epirubicin is eliminated renally as unchanged drug, and approximately 35% of the administered dose undergoes biliary excretion [27]. Biliary excretion is the major route of elimination for doxorubicin, whereas urinary excretion accounts for about 13% of total clearance [28].

In conclusion, the pharmacokinetics of MEN-10755 administered in two different regimens did not show significant differences and could be analysed together. Compared to epirubicin and doxorubicin, the pharmacokinetics of MEN-10755 were characterized by about a twofold shorter terminal half-life, a much lower total plasma clearance and a much smaller volume of distribution.

The 3-weekly regimen of MEN-10755 has been selected for phase II clinical studies in patients with advanced or metastatic ovarian cancer, non-small-cell lung carcinoma and soft tissue sarcomas.

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