Phase I and Pharmacokinetic Study of the (6-Maleimidocaproyl)Hydrazone Derivative of Doxorubicin

Clemens Unger, Brigitte Häring, Michael Medinger, Joachim Drevs, Simone Steinbild
Felix Kratz, and Klaus Mross

Abstract

Purpose: The (6-maleimidocaproyl)hydrazone derivative of doxorubicin (DOXO-EMCH) is an albumin-binding prodrug of doxorubicin with acid-sensitive properties that shows superior antitumor efficacy in murine tumor models and a favorable toxicity profile in mice, rats, and dogs compared with doxorubicin. The purpose of the phase I study was to characterize the toxicity profile of DOXO-EMCH, establish a recommended dose for phase II studies, and assess potential anticancer activity.

Experimental Design: A starting dose of 20 mg/m^2 doxorubicin equivalents was chosen. Forty-one patients with advanced cancer disease were treated with an i.v. infusion of DOXO-EMCH once every 3 weeks at a dose level of 20 to 340 mg/m^2 doxorubicin equivalents.

Results: Treatment with DOXO-EMCH was well tolerated up to 200 mg/m^2 without manifestation of drug-related side effects. Myelosuppression (grade 1-2) and mucositis (grade 1-2) were the predominant adverse effects at dose levels of 260 mg/m^2 and myelosuppression (grade 1-3) as well as mucositis (grade 1-3) were dose limiting at 340 mg/m^2. No cardiac toxicity was observed. Of 30 of 41 evaluable patients, 12 patients (40%) had progressive disease, 15 patients (57%) had stable disease, and 3 patients (10%) had a partial remission.

Conclusions: DOXO-EMCH showed a good safety profile and was able to induce tumor regressions in tumor types known to be anthracycline-sensitive tumors, such as breast cancer, small cell lung cancer, and sarcoma. The recommended doxorubicin equivalent dose for phase II studies is 260 mg/m^2.

Doxorubicin is a highly potent antitumor agent that is among one of the most active antineoplastic drugs developed to date and is used in the treatment of hematologic and solid cancers, such as breast and ovarian carcinoma, sarcoma, and many other solid tumors. The clinical application of this anthracycline is, however, limited by its dose-related side effects that include bone marrow toxicity, gastrointestinal disorders, stomatitis, alopecia, acute and cumulative cardiotoxicity, as well as extravasation (1). Bone marrow suppression is generally dose limiting, and myelocytopenia and thrombocytopenia are most prominent after each course of treatment with maximum toxicity after 7 to 10 days and a rapid recovery thereafter. Of special concern is the cumulative cardiotoxicity (cardiomyopathy and congestive heart failure) that is irreversible, reaching >5% once cumulative doses of doxorubicin exceed 550 mg/m^2 (2, 3). This toxicity is of special concern in curative oncology when treating hematologic cancers as well as in adjuvant settings, such as in the treatment of breast cancer. Newer data have shown that the critical cumulative dose is even lower than the "old" threshold of 5% at 550 mg/m^2 (4).

To circumvent these limitations and to improve the therapeutic potential of anthracyclines, several drug delivery systems, such as liposomal formulations (5) or prodrugs (6), have been investigated. At our center, we have recently investigated a macromolecular prodrug strategy based on two features: (*a*) rapid and selective binding of thiol-reactive prodrugs to the Cys^{34} position of endogenous albumin after i.v. administration and (*b*) release of the albumin-bound drug at the tumor site due to the incorporation of an acid-sensitive or enzymatically cleavable bond between the drug and the carrier (7–9).

Endogenous albumin is a potential drug carrier because it accumulates in solid tumors due to the pathophysiology of tumor tissue, characterized by a high metabolic turnover, angiogenesis, hypervasculature, a defective vascular architecture, and an impaired lymphatic drainage (10). In addition, we discovered that the HS-group of Cys^{34} of albumin is a unique and accessible functional group of a plasma protein, considering that free thiol groups are not present in the majority of circulating serum proteins except for albumin, and that this group is the most abundant thiol group in human plasma.

The result of our preclinical research was an acid-sensitive prodrug of doxorubicin [i.e., the (6-maleimidocaproyl)hydrazone derivative of doxorubicin (DOXO-EMCH); see Fig. 1] that reacted ideally with the Cys^{34} of human serum albumin,

Authors' Affiliation: Department Medical Oncology, Tumor Biology Center, Freiburg, Germany

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Requests for reprints: Clemens Unger, Department Medical Oncology, Tumor Biology Center, Breisacherstrasse 117, D-79117 Freiburg, Germany. E-mail: unger@tumorbio.uni-freiburg.de.

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which is located in a hydrophobic crevice on the surface of the protein that is approximately 10 to 12 Å deep (7).

DOXO-EMCH is a prodrug of the anticancer agent doxorubicin in which doxorubicin is derivatized at its C-13 keto-position with a thiol-binding spacer molecule (i.e., 6-maleimidocaproic acid hydrazide). DOXO-EMCH contains an acid-sensitive hydrazone linker that allows doxorubicin to be released either extracellularly in the slightly acidic environment often present in tumor tissue or intracellularly in acidic endosomal or lysosomal compartments after cellular uptake of the albumin conjugate by the tumor cell.

DOXO-EMCH is quantitatively and selectively bound to the Cys^{34} position of endogenous albumin within a few minutes, the reaction following second-order kinetics. DOXO-EMCH was superior to free doxorubicin, the clinical standard, in a murine renal cell carcinoma model (RENCA) and in two breast carcinoma xenograft models in nude mice (MDA-MB 435 and MCF-7) with regard to antitumor efficacy and toxicity. Complete remissions were achieved with DOXO-EMCH in the RENCA and MDA-MB 435 model in contrast to therapy with doxorubicin (7). Subsequent toxicologic studies in mice rats and dogs showed a 3- to 5-fold increase in the maximum tolerated dose (MTD), moderate and reversible myelosuppression, no liver toxicity and immunotoxicity, and no new toxicity compared with doxorubicin (11). In addition, we have recently shown that DOXO-EMCH is significantly less cardiotoxic in a chronic rat model when compared with doxorubicin at equitoxic dose (12).

These results prompted us to select DOXO-EMCH as an investigational product for clinical evaluation. DOXO-EMCH is meanwhile the first albumin-binding prodrug that has been assessed clinically. The main purpose of the phase I study was to characterize the toxicity profile of DOXO-EMCH, establish a recommended dose for phase II studies, and assess potential anticancer activity.

Materials and Methods

The study was approved by the local ethics committee and carried out at the Tumor Biology Center in Freiburg, Germany in accordance with the requirements of the German Drug Law (Arzneimittelgesetz), the guidelines for good clinical practice (International Conference on Harmonisation-Good Clinical Practice), and the Declaration of Helsinki. All patients provided written informed consent before entering this study.

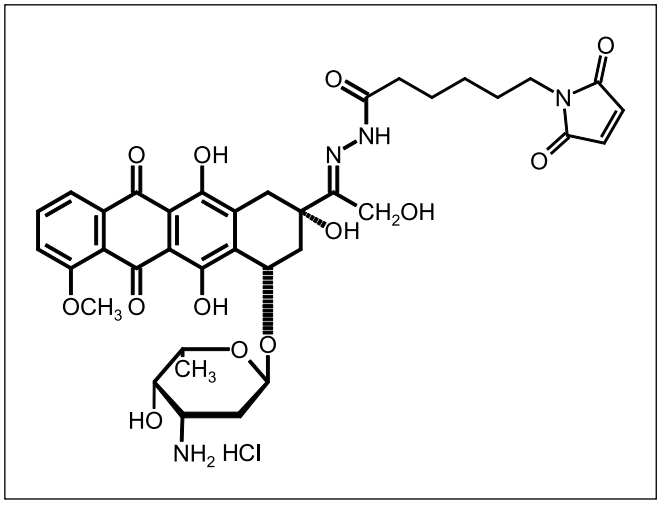

Fig. 1. Chemical structure of DOXO-EMCH.

Table 1. Tumor-related data of the 41 patients treated with DOXO-EMCH

	n	% of patients
Tumor localization		
Head and neck	7	17.1
Prostate	5	12.2
Kidney	5	12.2
Ovary	4	9.8
Sarcoma	4	9.8
Breast	3	7.3
Other	13	31.7
Metastatic sites (number)		
None	2	4.9
1	10	24.4
2	20	48.8
3	8	19.5
4	1	2.4
Metastatic sites (localization)		
Lymph nodes	20	48.8
Lung	16	39.0
Bone	14	34.1
Liver	11	26.8
Other	15	36.6
Grading		
Well differentiated	2	4.9
Moderately differentiated	12	29.3
Poorly differentiated	14	34.1
Undifferentiated	1	2.4
Unknown	12	29.3
Prior therapy		
None	1	2.4
Surgery		
0	6	14.6
1	19	46.3
2	8	19.5
≥3	8	19.5
Radiotherapy	28	68.3
Chemotherapy		
0	12	29.3
1	13	31.7
2	4	9.8
≥3	12	29.3

To be eligible for study enrollment, patients had to meet the following criteria: age at least 18 years, histologically confirmed malignancy beyond any hope of treatment by conventional therapeutic methods or for which no accepted therapeutic method existed, Eastern Cooperative Oncology Group performance status ≤2, an anticipated life expectancy of at least 3 months, adequate hematologic, renal, and hepatic function [WBC >3.5 × 10^9/L, absolute neutrophil count >1.5 × 10^9/L, platelets >100 × 10^9/L, hemoglobin >10 g/dL, bilirubin <1.5 mg/100 mL, transaminase levels <3× the upper limit of normal (in case of liver metastases <5× the upper limit of normal), albumin >2.0 g/dL, creatinine <1.5 mg/100 mL], no current pregnancy or breast feeding, no surgery within 2 weeks before start of study, no chemotherapy within 4 weeks before start of study, no radiotherapy with more than a 25% involvement of the red bone marrow within 6 weeks before start of study, no symptomatic brain metastases, no serious concomitant medical condition, no history of a cumulative doxorubicin dose >350 mg/m^2 (epirubicin >700 mg/m^2), no peripheral neuropathy common toxicity criteria (CTC) grade >2, and no persistent clinically relevant signs of toxicity associated with previous antitumor treatment. Informed consent was obtained from all patients before participation in the study.

Baseline evaluations within 2 weeks before study enrollment and interim evaluations after each treatment course included complete medical history, a physical and neurologic examination, vital signs, electrocardiogram, and laboratory investigations.

Tumor response was assessed after every other course of treatment whenever possible according to Response Evaluation Criteria in Solid Tumors criteria (13). Laboratory investigations were done on days 8 and 15 and at the end of each treatment course. Adverse events were monitored continuously throughout the study and graded according to CTC criteria (version 2.0). Additionally, from 135 mg/m^2 onwards, an echocardiographic evaluation was done at baseline and every other course (amendment 2) for safety reasons. For determination of plasma levels of total doxorubicin, blood and urine samples were collected up to 72 and 24 h, respectively, after the first administration of DOXO-EMCH.

Drug administration. Lyophilized DOXO-EMCH was supplied in 122 and 244 mg vials by ProPharma Ltd. DOXO-EMCH was reconstituted by the Clinical Pharmacy of the University of Freiburg, Germany by adding sterile 10 mmol/L sodium phosphate and 5% D-(+)-glucose (pH 5.8) and administered i.v. within 2 h after dissolution as a 30-min infusion. All doses of DOXO-EMCH administered in the study are stated in doxorubicin equivalents. Prophylactic antiemetics were allowed in patients with a history of nausea and/or vomiting after previous DOXO-EMCH infusions (from the second treatment course onwards). The molecular weight of doxorubicin is 580 g/mol and 787.22 g/mol for DOXO-EMCH, respectively. All doses were calculated as doxorubicin equivalents using the conversion factor 1.357. All doses given in this article are calculated in doxorubicin equivalent dosages.

Study design. The primary objectives of this prospective, open-label, dose-escalating phase I study were to determine the MTD and the dose-limiting toxicities (DLT) of DOXO-EMCH when administered as a 30-min infusion once every 3 weeks in adult patients with advanced cancer. Secondary objectives were the general toxicity, the pharmacokinetics, and the antitumor efficacy of DOXO-EMCH.

DOXO-EMCH was given as an infusion for 30 min at day 1 of a 21-treatment cycle. Dose escalation was done until the MTD was reached. Cohorts of at least three patients entered each dose level and could be expanded to six patients in case of toxicity. If no DLT was observed, the dose was escalated according to a modified Fibonacci-like strategy. If one DLT occurred in one of three patients, three additional patients were included at the same dose level. If two DLTs were observed at a dose level, that dose was declared the MTD and no further dose escalation occurred. The recommended phase II dose was defined as one dose level below the MTD.

Patients continued treatment until disease progression or until unacceptable toxicity occurred or until six treatment cycles were finished. No further treatment cycles beyond six cycles were done, as it was not intended to expose patients to excessive high cumulative doxorubicin doses. Intraindividual dose escalation was forbidden, and dose reductions were allowed in case of toxicity. A DLT was defined as the occurrence of any one of the following: grade 3 and/or 4 nonhematologic toxicity, excluding alopecia and nausea/vomiting, or platelet count <25/μL or absolute neutrophil count <500/μL lasting >7 days and/or associated with fever (>38.5°C).

Pharmacokinetic sampling and analysis. Blood and urine samples for determination of the pharmacokinetic values of doxorubicin were obtained from all patients during the first cycle. EDTA plasma samples (2 mL) were collected 15 min before treatment (t = 0 h) and 15, 30 (end of infusion), 5, and 30 min and 1, 2, 3, 4, 6, 24, 48, and 72 h after the end of the infusion. All samples were immediately centrifuged at 4°C on the ward and aliquots were frozen and stored until analysis at -70°C. Urine samples were collected before infusion and during 0 to 24 h after start of the infusion. The total urine volume was measured and an aliquot was frozen and stored until analysis at -70°C. Ten patients (24.4%) were not evaluable for urinary pharmacokinetics (patients 2, 3, 4, 5, 7, 11, 23, 24, 33, and 38). Reasons for this were missing baseline samples, missing sampling of 24-h urine, or missing data with regard to volume of urine.

DOXO-EMCH was determined in its albumin-bound form with a high-performance liquid chromatographic method with tandem mass spectrometry (MS/MS) detection by BioProof AG under Good Laboratory Practice. Validation was done with the pure albumin conjugate of DOXO-EMCH (DOXO-EMCH-human serum albumin) that was added to blank plasma. Daunorubicin was added as an internal standard. Plasma samples (100 μL) were diluted 1:50 and treated with acid to cleave the conjugate and to set free doxorubicin. Doxorubicin was isolated by solid-phase extraction. The eluate was evaporated to dryness and dissolved in mobile phase. After centrifugation, the supernatant of the sample was diluted with buffer and injected into the liquid chromograph. The monitoring of doxorubicin was based on mass transit (544.16→360.93 a.m.u.). Doxorubicin could be determined accurately and precisely in the range of 0.0025 to 1.003 μmol/L. The analyte concentrations were calculated from the peak area response ratios using a quadratic fit with 1/conc weighting. The intraday precision and accuracy of the method was determined from the relative SD and the absolute deviation from the spiked concentrations of five replicate assays on four quality control levels. High-performance liquid chromatography: alliance HPLC-System from Waters, D-65760 Eschborn; precolumn: Waters X Terra MS 18 3.5 μm, 3.9 × 20 mm;

Table 2. Evaluability of patients (individual data)

Patient no.	Dose (mg/m^2)	MTD*	PK*	PK urine*	Efficacy (RECIST)
1-4	20	3/4	4/4	1/4	1 SD, 1 PD, 2 NE
5-7	40	2/3	3/3	1/3	1 SD, 2 PD
8-10	80	3/3	3/3	3/3	2 PD, 1 NE
11-13	135	3/3	3/3	2/3	2 SD, 1 NE
14-19	200	5/6	6/6	6/6	3 PD, 3 NE
20-22	135	3/3	3/3	3/3	2 SD, 1 PD
23-25	150	3/3	3/3	1/3	3 SD
26-28	180	2/3	3/3	3/3	1 PR, 1 PD, 1 NE
29-31	260	3/3	3/3	3/3	1 PR, 2 SD
32-37	340	5/6	6/6	5/6	1 PR, 3 SD, 1 PD, 1 NE
38-41	260	3/4	4/4	3/4	1 SD, 1 PD, 1 NE
Total		35 (85.4%)	41 (100%)	31 (75.6%)	30 (73.2%)

Abbreviations: PK, pharmacokinetic; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; PD, progressive disease; NE, not evaluable; PR, partial response.
*Number of patients evaluable.

Table 3. Hematologic toxicity (number of patients)

Dose (mg/m^2)	No. patients	Anemia	Leucocytopenia			Neutropenia			Thrombocytopenia	
		CTC grade	CTC grade			CTC grade			CTC grade	
		1-2	1-2	3	4	1-2	3	4	1-2	3
20	4	0	1	0	0	0	0	0	0	0
40	3	0	1	0	0	0	0	0	1	0
80	3	1	0	0	0	0	0	0	0	0
135	6	3	1	0	0	0	0	0	2	0
150	3	0	0	0	0	1	0	0	0	0
180	3	1	0	0	0	0	0	0	1	0
200	6	2	0	2	0	1	0	1	0	0
260	7	2	2	2	1	0	5	1	2	0
340	6	5	1	2	3	0	2	4	0	2

analytic column: Waters X Terra MS 18 3.5 μm, 3.9 × 100 mm; data system: QuanLynx 3.5, Micromass, M23 9LZ Wythenshawe; MS system: Quattro Ultima, Micromass, M23 9LZ Wythenshawe; electrospray (ESP+); MS variables (source): capillary 3.42 V, cone 50 V; source block temperature: 130°C, desolvation temperature 400°C; MS variables (analyzer): ion energy 1.1 V, ion energy 2.2 V, entrance -17 V, exit 6 V, collision 31 keV. Gradient method: mobile phase A: 50 mmol/L ammonium formiate buffer (pH 3.0); mobile phase B: acetonitrile; gradient: 0-min 92% mobile phase A, 4-min mobile phase 10% A, 8-min 10% mobile phase A, 9-min mobile phase A; flow: 0.6 mL/min; injection volume: 30 μL.

For doing the analyses, plasma samples were thawed in 5 min at 37°C and 800 min^{-1} (Eppendorf Thermomixer). Sample (50 μL) was diluted with 2.45 mL 0.01 mol/L PBS (pH 7.4) and mixed. Samples (100 μL) were transferred in a well of a deep-well plate (2 mL), and then 75 μL of diluted phosphoric acid (1:1,000) were added and incubated at room temperature for 1 h. For solid-phase extraction, 100 μL of internal standard solution III were added (concentration, ~173 ng/mL daunorubicin-HCl). Water (700 μL) was added and the plate was covered with foil and shaken for 5 min at 1,000 min^{-1}. Ninety-six–well plates Waters Oasis HLB (30 mg) were conditioned with 1 mL methanol or 1 mL water. The complete sample was transferred onto the 96-well plates. The sample was applied on the columns for 10 min at low vacuum and washed twice with 1 mL methanol/water (10:90, v/v). Finally, 96-well plates were dried for 15 min using maximal vacuum (~900 mbar).

The sample was eluted in two steps (each 0.75 mL) with methanol containing 0.1% ammonia (25%) into a deep-well plate. The elution plate was placed in the vacuum centrifuge (heating level 4, 180 min). The residue was dissolved in 100 μL 50 mmol/L ammonium formiate (pH 7.0), 5-min shaking at 1,000 min^{-1}, and then placed in an ultrasonic bath for 5 min. Samples were transferred in high-performance liquid chromatography vials with insert; each analytic batch started with a blank followed by the calibration curve. Then, all unknowns or quality control samples were placed in the autosampler and cooled to 4°C and 30 μL of each sample were injected.

Pharmacokinetic evaluation. All pharmacokinetic calculations were done by means of the pharmacokinetic software package WinNonlin 5.0 (Pharsight Corp.). The noncompartmental analysis was based on a model requiring a constant infusion (model 202). The associated statistics and charts were prepared by use of WinNonlin and Microsoft Excel. For the pharmacokinetic evaluation, all plasma samples (sample numbers 1-11, 0-72.5 h) were taken into account. C_{max} and T_{max} were defined from the concentration-time profile. The terminal elimination rate constant, λz, was estimated by linear regression of the log-transformed concentration-time data of the last three data points. The area under concentration/time curve (AUC) was computed by the WinNonlin program using the linear trapezoidal summation. The AUC_{inf} was determined by the linear trapezoidal rule integration areas from zero to the time of the last measurable plasma concentration and extrapolated as $AUC_{inf} = AUC_{last} + C_{last} / \lambda z$, where C_{last} is the last measurable plasma concentration. $t_{1/2}$ was calculated as (ln2) / λz for each dose group.

The validated high-performance liquid chromatography-MS/MS method showed a high reliability during the 15 runs. Comparing the data of all doxorubicin calibration functions, the mean coefficient of determination (r^2) was calculated with 0.998. All sequences fulfilled the acceptance criteria (at least six back-calculated standards <15% bias of <20% at limit of quantification). The calibration range for doxorubicin comprised 2.6 to 1,000 nmol/L. The limit of quantification was set to 2.6 according to the lowest point on the calibration curve. The % bias calculated for the individual calibration standards ranged between -4.7% and 17.5% for the lowest calibration standard Cal 1 and between -14.1% and 14.7% for the other calibrations standards (Cal 2-Cal 7). The results for the accuracy, described as % mean bias, ranged between -14.9% and 14.1%. All sequences fulfilled the acceptance criteria (<15% bias for at least four quality controls).

Results

Patient characteristics. The recruitment of patients started on August 28, 2003 and stopped on October 12, 2005 after enrollment of 41 Caucasian patients (22 females and 19 males). The age ranged from 32 to 72 years with a median of 62 years, the median height was 172 cm (range, 158-186 cm), and the median weight was 68.5 kg (range, 48.0-135.9 kg). Initial performance status (Eastern Cooperative Oncology Group) was 0 in 7 patients (17.1%), 1 in 26 patients (63.4%), and 2 in 7 patients (17.1%). One patient (2.4%) had an initial Eastern Cooperative Oncology Group performance status of 3. All patients presented with histologically confirmed malignant tumors that were either refractory to conventional therapy or for whom no established therapy existed.

The majority of patients had urological (24.4%), gynecologic (19.5%), or head and neck cancer (17.1%). The predominant metastatic sites were the lymph nodes (48.8%), the lung (39.0%), the bone (34.1%), and the liver (26.8%). Only two patients had no metastases before start of study. Most of the patients presented metastases at more than one location (70.7%).

All but one patient (patient 38 on the 260 mg/m^2 dose level) were pretreated for their cancer disease. Thirty-five patients (85.4%) had prior surgery (between 1 and 8), 29 patients (70.7%) had received previous chemotherapies (between 1 and 7 lines), and 28 patients (68.3%) had prior radiotherapy. Most

of the patients had combination therapies (85.4%). The median duration of illness was 27 months (range, 1-146 months). There were no relevant differences in the baseline demographic characteristics for the nine dose levels explored. Tumor-related data and an overview of the evaluability of patients at the different dose levels are summarized in Tables 1 and 2.

Dose escalation and DLT. A total number of 124 courses of DOXO-EMCH (median, 2; range, 1-6) were administered to 41 patients: 11 courses (8.9%) were given at a dose level of 20 mg/m^2, 9 courses (7.3%) at a dose level of 40 mg/m^2, 8 courses (6.5%) at a dose level of 80 mg/m^2, 21 courses (16.9%) at a dose level of 135 mg/m^2, 10 courses (8.1%) at a dose level of 150 mg/m^2, 9 courses (7.3%) at a dose level of 180 mg/m^2, 10 courses (8.1%) at a dose level of 200 mg/m^2, 26 courses (21.0%) at a dose level of 260 mg/m^2, and 20 courses (16.1%) at a dose level of 340 mg/m^2. The number of courses administered ranged from one to six.

The dose escalation scheme is shown in Table 2. No first-course DLT was noted at the starting dose (20 mg/m^2). Because one patient dropped out due to progressive disease within the first course (ileus), the patient was replaced. Until 200 mg/m^2, no DLT was observed. The third patient treated at this dose level experienced myelotoxicity (grade 4 neutropenia), and the cohort was expanded to six patients. No further myelotoxicity was seen but two of these additional patients had neurologic events (somnolence, confusion, and depressed level of consciousness). For safety reasons, another three patients were enrolled at the next lower dose level (135 mg/m^2). Moreover, for safety reasons, two additional lower dose steps (150 and 180 mg/m^2) were done. As no DLT was observed up to 180 mg/m^2, the next dose level was 260 mg/m^2 and thereafter 340 mg/m^2. At 340 mg/m^2, DLTs (mucositis and neutropenia) were observed and this dose level was judged as the MTD and the recommended dose level based on these results was 260 mg/m^2. To gain further experience with this dose level, four additional patients were treated at this dose level, expanding the patient number from three to seven. Two of these four additional patients developed a DLT (neutropenic fever and mucositis).

Deaths. There was no treatment-related death. Four patients died in the course of the study due to their cancer disease (two patients after cycle 1 at days 24 and 29, respectively, and one patient each after cycle 2, respectively 3).

Hematologic toxicity. Hematologic toxicity should be evaluated by weekly full blood counts and is summarized in Table 3. Mild (grade 1-2) hematologic toxicity occurred at all dose levels. Grade 3 or 4 hematologic toxicity was observed only at dose levels between 200 and 340 mg/m^2. No grade 3 or 4 anemia was observed and no patient experienced grade 4 thrombocytopenia.

Table 4. Common occurring treatment-related nonhematologic toxicities, >10% of treated patients, [number of patients (%)]

Symptom	All grades	Grade 3	Grade 4
Alopecia	22.0	0.0	0
Fatigue	43.9	7.3	0
Nausea	46.3	0.0	0
Vomiting	17.1	0.0	0
Mucositis	31.7	9.8	0
Stomatitis	12.2	2.4	0
Dyspepsia/dysphagia	17.1	2.4	0

Table 5. Grade 3 and 4 nonhematologic toxicities [number of patients (%)]

Symptom	All grades	Grade 3	Grade 4
Fatigue	43.9	7.3	0
Mucositis	31.7	9.8	0
Stomatitis	12.2	2.4	0
Dyspepsia/dysphagia	17.1	2.4	0
Somnolence/confusion	4.9	4.9	0
Dyspnoe	7.3	2.4	0
Balanitis	2.4	2.4	0
Diarrhea	4.9	2.4	0
Visual disturbances	4.9	2.4	0

A treatment delay due to prolonged neutropenia and/or leucopenia was necessary in 11 patients (26.8%) rsp. 25 courses (20.2%). The median time to nadir of grade 4 neutropenia was 15 days (range, 12-21 days) with a relatively short median duration of 7 days (range, 7-9 days). In two of six patients with grade 4 neutropenia, this was complicated by fever.

Nonhematologic toxicities. Thirty-five patients (85.4%) experienced at least one nonhematologic adverse event that was judged by the investigator as at least possibly related to study drug. The treatment-related nonhematologic toxicity profiles are listed in Tables 4 and 5. The majority of the symptoms were of mild (grade 1) or moderate (grade 2) intensity. Only five patients (12.2%) discontinued treatment due to toxicity as the primary reason for study withdrawal. The most commonly reported (>20% of patients) nonhematologic adverse events were nausea (19 patients, 46.3%), fatigue (18 patients, 43.9%), mucositis (13 patients, 31.7%), and alopecia (9 patients, 22.0%). Grade 3 fatigue was reported by three patients (7.3%), grade 3 mucositis by four patients (9.8%), and grade 3 stomatitis, dyspnoe, balanitis, and visual disturbances and grade 3 dysphagia by one patient (2.4%) each. Two patients (4.9%) at the 200 mg/m^2 dose level developed grade 3 somnolence, confusion, and depressed level of consciousness. Grade 3 or 4 toxicity was observed only in the upper dose levels (200-340 mg/m^2), with the exception of one grade 3 diarrhea that occurred in a patient at the 40 mg/m^2 dose level.

Gastrointestinal toxicities. Nausea and vomiting were controlled with the prophylactic use of antiemetics. DOXO-EMCH did not induce diarrhea. The DLTs were stomatitis/mucositis, which was observed in two of seven patients at 260 mg/m^2 and three of six patients at 340 mg/m^2.

One of the two patients who received 260 mg/m^2 DOXO-EMCH had a head and neck tumor, which was pretreated with radiation therapy. A recall radiation effect was observed during/after anthracycline treatment.

Biochemical toxicities. Biochemical laboratory investigations were done weekly. In general, there were no grade 3 or 4 toxicities observed in the biochemical variables tested; in a few exceptions, isolated modifications in the clotting factors, serum bilirubin, potassium, and alkaline phosphatase occurred. Other patients had minor deviations (generally grade 1) in some of the biochemical variables but were not regarded as clinically relevant.

Overall, 15 patients (36.6%) presented with elevations in liver enzyme in the course of study (1 patient at the 20 mg/m^2 dose level, 3 at the 40 mg/m^2 dose level, 2 at the 80 mg/m^2 dose level, 2 at the 135 mg/m^2 dose level, 1 at the 180 and 200 mg/m^2 dose level each, 2 at the 260 mg/m^2 dose level, and 1 at the 340 mg/m^2 dose level). Some of the patients had a metastatic spread in the liver.

Dermatologic toxicity. In one patient with metastatic oropharyngeal carcinoma, an extravasation was observed when treated with 150 mg/m^2 via a peripheral i.v. line. The amount of extravasation was estimated to be ~20 mg. Tissue swelling and erythema developed. DMSO (99%) was topically applied for 5 days with a change of the 10 × 10 cm swap four times daily. Additionally, dexrazoxane was administered on 3 days (1,500 mg at days 1 and 2 and 750 mg at day 3) i.v. as 15-min infusion. The first infusion was administered 4 h after the extravasation. No necrosis occurred and no sequelae remained. The patient received further DOXO-EMCH infusion without any problems (14).

Cardiac toxicity. Globally, the electrocardiogram evaluations showed normal variability in all variables and no time effect. There were four patients who presented clinically asymptomatic electrocardiogram changes. Patient 6 at the 40 mg/m^2 dose level showed an isolated ventricular arrhythmia on day 1 of cycle 1. Patient 8 at the 80 mg/m^2 dose level showed after two courses of treatment a bundle block. One patient (patient 27, 180 mg/m^2 dose level) presented a negative T wave on day 1 of cycle 1.

No clinical signs of congestive heart failure were observed. Of special relevance are those patients that received courses with 180 mg/m^2 (n = 1), 260 mg/m^2 (n = 5), and 340 mg/m^2 (n = 2) DOXO-EMCH, which corresponds to >2,000 mg doxorubicin equivalent; >1,200 mg/m^2 doxorubicin represents a cumulative doxorubicin dose with a risk for congestive heart failure of >50% according to the literature (15). The absolute amount of doxorubicin varied in these patients between 2,122 and 3,316 mg with a mean of 2,837 mg (~1,650 mg/m^2). In all patients, sonographic heart examinations before and after the last DOXO-EMCH administration showed no drop of the ejection fraction or shortening fraction. During the follow-up period after the last treatment cycle, there was no clinical indication for a deterioration of heart function. No clinical relevant cardiovascular events, such as blood pressure or heart rhythm problems, occurred during the study or follow-up period.

Evaluation of tumor response. Although tumor response was not the primary objective of this study, patients were evaluated for response (according to Response Evaluation Criteria in Solid Tumors criteria) every other course by clinical evaluation and imaging whenever possible. All patients who received at least two courses of treatment without major protocol violations were considered evaluable for response (see Table 2). Patients who discontinued from the study before completing the second course of treatment because of disease progression were also regarded as evaluable for response. Best tumor response was defined as the best response achieved during the study.

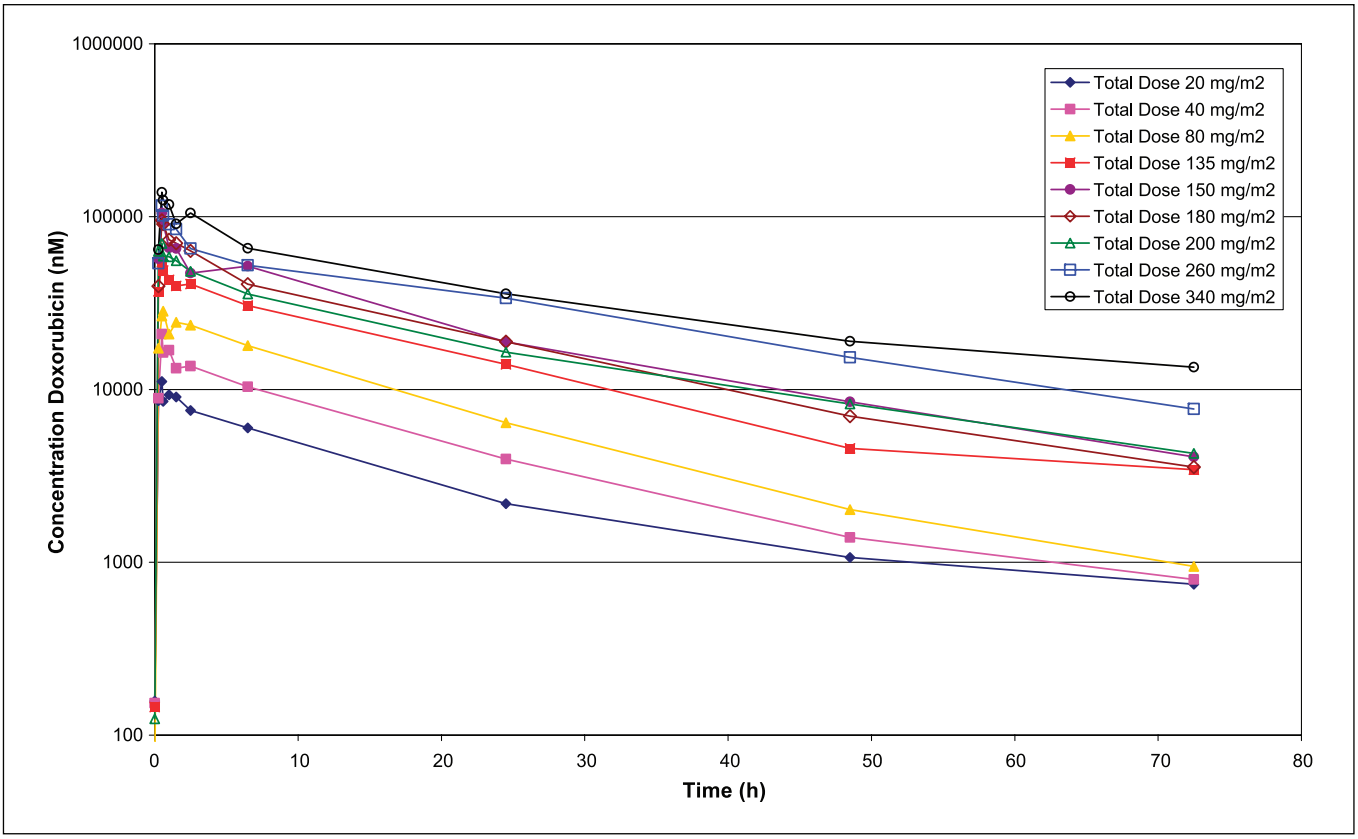

Fig. 2. Mean plasma concentration versus time curves of total doxorubicin for all treatment groups (semilogarithmic plot).

Table 6. Pharmacokinetic variables of total doxorubicin per patient and dose group

Total dose (mg/m^2)	Mean (n)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (μmol/L)	AUC_{all} (h μmol/L)	AUC_{inf_obs} (h μmol/LL)	V_{z_obs} (L/m^2)	Cl_{obs} (L/h/m^2)	V_{ss_obs} (L/m^2)
20	Mean (4)	24.8	0.4	11.3	169.6	200.4	4.45	2.27	3.48
40	Mean (3)	20.8	0.7	21.5	301.7	326.6	4.77	2.68	3.67
80	Mean (3)	17.6	0.6	28.4	494.5	518.9	5.15	3.33	4.06
135	Mean (3)	18.2	0.5	60.3	962.6	1,046	4.58	2.83	4.17
150	Mean (3)	21.2	0.5	104.9	1,470	1,602	3.78	2.15	3.13
180	Mean (3)	20.2	0.5	96.1	1,347	1,453	5.01	2.86	4.21
200	Mean (6)	25.3	0.5	73.4	1,161	1,392	7.28	3.37	6.11
260	Mean (6)	20.3	0.6	234.6	2,299	2,534	4.70	2.60	4.60
340	Mean (6)	38.2	0.9	145.3	2,547	3,341	2.14	2.14	1.93

Abbreviations: $t_{1/2}$, apparent terminal half-life (ln2 / λz); T_{max}, time of maximum concentration; C_{max}, maximum concentration; AUC_{all}, area under concentration/time curve up to the last quantifiable concentration; AUC_{inf_obs}, AUC calculated using the trapezoidal rule from 0 h to infinity according to the formula AUC_{∞} = AUCz + Cz / λz; V_{z_obs}, apparent volume of distribution (V_z = Cl / λz); Cl_{obs}, total body clearance calculated according to the formula dose / AUC_{inf} ($AUC_{(0-\infty)}$).

Altogether, 30 patients (73.2%) were assessable for analysis of response. No complete response was observed. A partial response was observed in three (10%) patients (patient 28, 180 mg/m^2; patient 29, 260 mg/m^2; and patient 33, 340 mg/m^2). Fifteen patients (50%) showed transient stable disease, and for 12 patients (40%), evidence of progression was observed (Table 2).

Of the 30 patients evaluable for response, 3 anthracycline-naive patients (small cell lung cancer, breast cancer, and liposarcoma) had a partial response lasting for 80, 24, and 17 weeks, respectively. All patients entered the study with evidence of disease progression. One patient had a small cell lung cancer pretreated with etoposide and cisplatin. He progressed after this treatment, developing pleural effusions and clinical deterioration (dyspnea and cough), and was included into the study receiving six courses with 180 mg/m^2 DOXO-EMCH. Thereafter, he was followed in regular time intervals until progressive disease was observed with computed tomography scan 14 months after discontinuation of DOXO-EMCH. An excellent tumor control was achieved and time to progression was 18 months. A patient with liposarcoma (260 mg/m^2, pretreated with an angiogenesis inhibitor) reached a partial remission and had a time to progression period of 17 weeks. A patient with metastatic breast cancer (340 mg/m^2) pretreated with adjuvant cyclophosphamide, methotrexate, and 5-fluorouracil and different hormonal treatments (Trenantone, letrozole, exemestan, tamoxifen, and trofosfamide) reached a partial remission and time to progression was 24 weeks. Fifteen patients reached a stable disease at different dose levels for different times ranging from 2 to 15 weeks.

Pharmacokinetics. The objective of the pharmacokinetic study was the determination of albumin-bound doxorubicin levels in human plasma samples and urine. The overall mean plasma concentration versus time profiles, overall arithmetic means per group, obtained for the different dose groups treated with DOXO-EMCH are depicted in Fig. 2 (semilogarithmic plot). Plasma concentrations obtained for total doxorubicin in plasma samples for the individual patients and dose groups are shown in Table 6 together with pharmacokinetic variables.

The plasma concentrations of total doxorubicin were well quantifiable up to the end of the collection interval of 72.5 h. The evaluation of the terminal half-lives was done with the last three time points (in general 24.5 to 72.5 h after start of infusion), except for subject 22, where the last time point was excluded due to an increase in the plasma concentration of doxorubicin (therefore 6 to 48 h used).

The mean half-lives ranged between 17.6 h (subjects treated with 80 mg/m^2 DOXO-EMCH) and 38.2 h (subjects treated with 340 mg/m^2 DOXO-EMCH) for all nine treatment groups. The half-life for DOXO-EMCH is in the same order as the terminal half-life reported for doxorubicin (~15-40 h; ref. 16). There was no clear correlation between the terminal half-lives and the dose of DOXO-EMCH applied, except that the longest terminal half-lives were observed in the group receiving the highest dose (340 mg/m^2 DOXO-EMCH).

After administration of DOXO-EMCH, the mean peak concentrations (C_{max}) ranged from 11.3 μmol/L in subjects receiving 20 mg/m^2 DOXO-EMCH (lowest dose) to 234.5 μmol/L in subjects receiving 260 mg/m^2 DOXO-EMCH (second highest dose). The mean AUC_{all} results ranged from 169.6 h μmol/L in subjects receiving 20 mg/m^2 DOXO-EMCH (lowest dose) to 2,547 h μmol/L in subjects receiving 340 mg/m^2 DOXO-EMCH (highest dose). The large AUC values are representative for a long circulating drug delivery system; reported AUC values for conventional doxorubicin are ~3 h μmol/L at the dose of 60 mg/m^2 (17). There was a correlation between the dose of DOXO-EMCH administered and the obtained C_{max} and AUC_{all} values for total doxorubicin. Values for C_{max} and AUC_{all} increased proportionally with the dose (i.e., a dose dependency for C_{max} and AUC_{all} was observed except for dose groups 150 and 180 mg/m^2).

The maximum plasma concentrations (C_{max}) of total doxorubicin were measured between 0.25 and 1 h (T_{max}) after the beginning of the respective i.v. infusion for subjects receiving 20 to 200 mg/m^2 DOXO-EMCH. No increase of the time at maximum concentration was observed within these dosing groups. Maximum plasma concentrations in subjects receiving 260 and 340 mg/m^2 were reached later between 0.5 and 2.5 h after beginning of their infusion.

Mean percentage of extrapolation for AUC_{inf} of total doxorubicin ranged between 4.9% and 22.3% for the different dosing groups. The mean values obtained for clearance of doxorubicin (Cl_{obs}) ranged between 2.15 and 3.37 mL/min/m^2, which is significantly lower than for doxorubicin (~580 mL/min/m^2; refs. 18, 19). There was no correlation between the clearance and

the dose of DOXO-EMCH applied. The corresponding mean volume of distribution of total doxorubicin was in the range of 2.14 to 7.28 L/m^2 in contrast to approximately 700 to 1,100 L/m^2 reported for doxorubicin (17, 18).

Of the 44 accumulative urine samples, 41 showed between 0.004 and 26 μmol/L doxorubicin. From the 88 one-time samples, 14 were below the limit of quantification and the 74 yielded values ranging from 0.017 to 30.7 μmol/L doxorubicin. The fraction of doxorubicin that was excreted in the urine with respect to the amount of administered DOXO-EMCH was in the range of 1.4% to 7.7%.

Discussion

The antitumor potency and toxicologic profile of anthracyclines has been the impetus for developing more effective and less toxic anthracycline analogues and prodrugs during the past 20 years (20–23). Initial work concentrated on strict analogues of doxorubicin and daunorubicin, and although epirubicin, idarubicin, pirarubicin, zorubicin, aclarubicin, and carminomycin are meanwhile registered drugs, they have not significantly improved the therapeutic index of anthracyclines.

Tumor-targeted delivery with low and high molecular weight prodrugs aims at realizing less toxic derivatives of the parent drug that are activated within the tumor or that carry an additional ligand with tumor-targeting properties that transports the payload to the tumor where the drug is then released, either intracellularly or extracellularly (24). Anthracyclines probably represent the class of anticancer agents that has been most widely used for the development of respective prodrugs (e.g., with antibodies, peptides, carbohydrates, serum proteins, or synthetic polymers; reviewed in ref. 6).

Over the past 5 years, we have investigated a prodrug concept that exploits endogenous albumin as the drug carrier in which thiol-binding prodrugs are selectively bound to the Cys^{34} position of circulating albumin. The plasma protein albumin is a potential carrier of anticancer drugs, considering that numerous preclinical studies show an accumulation of albumin in experimental solid tumors (reviewed in ref. 10). The mechanism by which macromolecules, such as serum proteins, accumulate in tumor tissue has been termed enhanced permeability and retention of macromolecules (“EPR effect”) and serves as a working model for explaining the targeting potential of macromolecules for solid tumors (25)

As a result of our preclinical work, DOXO-EMCH emerged as a clinical candidate due to superior efficacy of DOXO-EMCH compared with free doxorubicin in three murine tumor models, an approximate 4-fold increase in the MTD in mice when compared with doxorubicin, rapid and selective binding to circulating albumin, high plasma stability, and high water solubility.

The substantial increase in the MTD allowed high doses of DOXO-EMCH to be administered to tumor-bearing mice with a concomitant increase in antitumor activity compared with free doxorubicin. Generally, a high degree of protein binding, especially to albumin, is considered a disadvantage because only the free drug can exert its pharmacologic effect. *In situ* binding of DOXO-EMCH to albumin turned this potential disadvantage into a therapeutic benefit by incorporating an acid-sensitive bond between the drug and the albumin-binding moiety that ensures a specific release of doxorubicin at its site of action. By distributing the drug in its albumin-bound form in the body, it seemed that the natural detoxifying function of endogenous albumin is temporarily exploited for improving the safety and efficacy of doxorubicin.

In this clinical phase I study, the dose of doxorubicin could be increased by a factor of 4.5 to 340 mg/m^2 when 75 mg/m^2 free doxorubicin is considered the dose, which can be administered as a single agent concomitant with the typical spectrum of side effects (i.e., myelotoxicity and stomatitis/mucositis). This shift in the MTD is in good agreement with the results of our toxicologic studies showing that a 3- to 5-fold dose of DOXO-EMCH can be given to mice, rats, and dogs compared with the MTD of doxorubicin in these species (11).

Because the frequency of DLTs and adverse events grade 3 at 340 mg/m^2 approached 50%, this dose level was considered to define the MTD so that the next lower dose level is recommended for further phase II studies. The typical spectrum of toxicities known from doxorubicin was observed as DLT: neutropenia, neutropenic fever, and mucositis/stomatitis.

No clinical signs of congestive heart failure were observed. Sonographic examination of heart function, although not done in each patient completely, showed no significant deterioration of left cardiac function in patients who had cumulative dosages of >1,000 mg/m^2 DOXO-EMCH. It should be remembered that patients receiving six treatment courses with 180 mg/m^2 ($n = 1$), 260 mg/m^2 ($n = 3$), and 340 mg/m^2 ($n = 1$) had actually received a cumulative dose of 1,080, 1,560, and 1,600 mg/m^2 doxorubicin, respectively. Although there were only eight patients with very high cumulative dosages, none of these patients had any clinical signs of congestive heart failure. As these patients are still alive and no cardiac problems were noted in the follow-up period after stopping treatment, an important issue for further clinical trials will be to monitor acute and chronic cardiac toxicity in greater depth and over longer periods.

Although not a primary end point of this study, it was shown that DOXO-EMCH is able to induce tumor regression in tumor types known to be anthracycline-sensitive tumors. In breast cancer, small cell lung cancer, and sarcoma, a clinical significant tumor control was observed. Tumors known as being not sensitive to anthracyclines, such as prostate and kidney cancer, showed no significant tumor regression or tumor control.

A potential advantage of DOXO-EMCH is the long half-life of the drug carrier albumin. The pharmacokinetics of DOXO-EMCH are typical for a long circulating drug delivery system with a low clearance, a small volume of distribution, and a high plasma AUC. As a consequence, extracellular regions of solid tumor will be exposed to the albumin-bound form of DOXO-EMCH for longer periods. If the pH value in these regions is slightly acidic, then small amounts of doxorubicin would be released, mimicking a constant presence of doxorubicin in small concentrations. Thus, a treatment cycle of DOXO-EMCH can be judged as an intermittent cytotoxic and metronomic cytostatic therapy modus combining two application modes within one schedule.

In summary, the antitumor efficacy seen in this phase I study is encouraging and needs to be confirmed in phase II studies, preferably in a direct comparison with doxorubicin. With respect to toxicity, DOXO-EMCH has the potential for producing less patient-felt toxicities and a lower rate of cardiac toxicity than doxorubicin, which are important aspects in curative, adjuvant, and palliative treatment options where anthracyclines play a crucial role.

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