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Phase I and pharmacokinetic study of MCC-465, a doxorubicin (DXR) encapsulated in PEG immunoliposome, in patients with metastatic stomach cancer

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Background: MCC-465 is an immunoliposome-encapsulated doxorubicin (DXR). The liposome is tagged with polyethylene glycol (PEG) and the $F(ab')_2$ fragment of human monoclonal antibody GAH, which positively reacts to >90% of cancerous stomach tissues, but negatively to all normal tissues. In preclinical studies, MCC-465 showed superior cytotoxic activity against several human stomach cancer cells compared with DXR or DXR-incorporated PEG liposomes. The main purpose of this trial was to define the maximum tolerated dose (MTD), dose limiting toxicity (DLT), recommended phase II dose and pharmacokinetics (PK) of MCC-465.

Patients and methods: Patients with metastatic or recurrent stomach cancer were eligible for entry. The initial dose was 6.5 mg/m^2. MCC-465 was administered as a 1-h infusion every 3 weeks and the treatment continued for up to six cycles.

Results: Twenty-three patients received a total of 62 cycles at the 6.5–45.5 mg/m^2 dose level. DLTs were myelosuppression and appetite loss at the 45.5 mg/m^2 dose level. Other toxicities were mild. Neither palmar–plantar erythrodysesthesia nor cardiotoxicity was observed. Acute reactions related to infusion were observed commonly in 16 patients over the entire dose range. While no antitumor response was observed, stable disease (SD) was observed in 10 out of 18 evaluable patients. The pharmacokinetic study showed a similar AUC and C_{max} to Doxil®.

Conclusion: MCC-465 was well tolerated. The recommended dose for a phase II study of MCC-465, for a 3-week schedule, is considered to be 32.5 mg/m^2 in an equivalent amount of DXR.

Key words: doxorubicin, drug delivery system, GAH, immunoliposome, MCC-465, pharmacokinetics

Introduction

There are two main concepts in any drug delivery system, namely active and passive targeting. The former involves monoclonal antibodies to tumor-related molecules that can target the tumor by utilizing a specific binding ability between the antibody and antigen. The latter can be achieved by the so-called enhanced permeability and retention (EPR) effect [1–3]. The EPR effect was named with reference to the pathophysiological characteristics of solid tumor tissue: hypervascularity, incomplete vascular architecture, secretion of vascular permeability factors stimulating extravasation within the cancer, and the absence of effective lymphatic drainage of macromolecules and nanoparticulates. Macromolecules and nanoparticulates have long plasma half-lives because they are too large to pass through the normal vessel walls unless they are trapped by the reticuloendothelial system (RES) in various organs. Such macromolecules can diffuse out of tumor blood vessels, reach the solid tumor tissue effectively and be retained for a long period due to the EPR effect.

PEG-coated liposomes are stable, long-circulating drug carriers useful for delivering doxorubicin (DXR) to the sites of solid tumors. Compared with conventional liposomes, pegylated liposomes are less extensively taken up by the RES and remain in circulation for a long time [4–6]. The long-term circulation and the ability of pegylated liposomes to extravasate through leaky tumor vasculature results in localization of DXR in tumor tissue, probably due to the EPR effect. In a number of animal and human tumors, PEG liposomal DXR produced higher intratumor drug concentrations and better therapeutic responses than equivalent doses of non-pegylated liposome-encapsulated DXR or free DXR [7, 8].

MCC-465 is a newly formulated immunoliposome-encapsulated DXR (Figure 1). This liposome is chemically conjugated to PEG and the $F(ab')_2$ fragment of the human monoclonal antibody,

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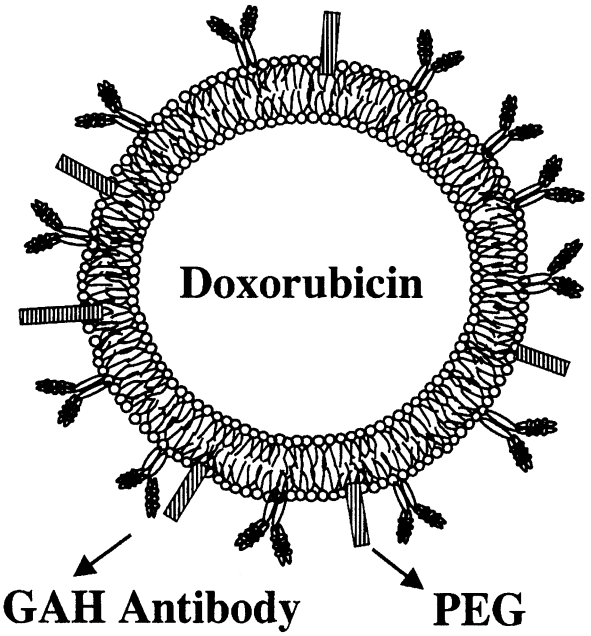

Figure 1. Schematic diagram of MCC-465.

GAH, which recognizes a cell surface molecule on various types of cancer cells [9]. Therefore, this formulation should possess the ability of both active and passive targeting. So far, the antigen recognized by this antibody has not been successfully purified, the reason for which is assumed to be that the antibody may have recognized the epitope as the conformation of the antigen(s). This occurs because of the characteristics of the antibody, which reacts only to viable cells and native protein and not to denatured protein, thus making analysis by conventional methods based on protein chemistry useless.

In reality, at the time of the initiation of this phase I study, it was already clear that the antigen exists on the cell surface, correlates to cytoskeletal protein and positively stains 90% of the various stomach cancer cells and tissues but is always negative in normal cells.

The antitumor activity of MCC-465 against GAH-positive human stomach cancer B37 cells was compared with that of GAH non-conjugated PEG liposomal DXR *in vitro*. The result clearly showed that MCC-465 was much more effective against B37 cells than GAH non-conjugated PEG liposomal DXR [9]. In nude mice, MCC-465 exhibited higher antitumor activity against several GAH-positive stomach cancers transplanted in the renal capsules of mice in comparison with free DXR or GAH non-conjugated PEG liposomal DXR [9]. Using a fluorescence-labeled liposome, it was revealed that GAH-tagged liposomes were extensively internalized, but GAH non-tagged liposomes were not [9]. Taking all the data together, we concluded that the GAH-conjugated immunoliposome was highly potent as a drug-targeting device, especially for human stomach cancer. Therefore, this phase I study was confined to patients with advanced gastric cancer.

Patients and methods

Eligibility criteria

Patients with cytologically or histologically confirmed advanced or recurrent gastric cancer refractory to conventional therapy were eligible for entry in this study. Patients with any serious infection, including hepatitis B and C viruses, and HIV, uncontrollable hypertension, symptomatic brain metastasis, allergy to anthracycline-type drugs, pre-existing cardiac disease including congestive heart failure, arrhythmia requiring treatment and myocardial infarction, vascular disordesr including a history of pulmonary embolism, deep venous thrombosis, and peripheral artery occlusive disease, were excluded. Patients were also excluded if they were pregnant or lactating, or showing gastrointestinal bleeding. In addition, any patient who the principal investigator or investigator considered ineligible was excluded. Eligibility criteria also included the following: (i) World Health Organization performance status ≤2; (ii) age ≥20 and <75 years; (iii) normal hematological (white blood cell count ≥4000/μl, platelet count ≥100 000/μl), hepatic [total bilirubin level ≤1.5 mg/dl; aspartate aminotransferase and alanine aminotransferase ≤2.5× the upper limit of normal (ULN), unless the elevation was a result of hepatic metastasis, in which case elevations ≤3× ULN were permitted], renal (serum creatinine within normal range), cardiac (classification of New York Heart Association ≤I) and pulmonary (PaO_2 ≥60 mmHg) function; (iv) no chemotherapy within 4 weeks (6 weeks for nitrosourea and mitomycin C) before administration of MCC-465; (v) a lifetime cumulative dose of DXR <100 mg/m^2 or that of epirubicin and pirarubicin <200 mg/m^2; (vi) no radiotherapy within 4 weeks before treatment; (vii) life expectancy >3 months; and (viii) full recovery from toxicity caused by any other test drug previously administered. The Institutional Review Boards for each hospital approved the protocol and informed consent brochures. Written informed consent was obtained from all patients.

Study drug and drug administration

MCC-465 was constructed from PEG (molecular weight 5 kDa on average), lipids, doxorubicin hydrochloride and F(ab′)$_2$/GAH. Lipids were consisted from dipalmitoylphosphatidylcholine, cholesterol and maleimidated cipalmitoylphosphatidyletahnolamine. The ratio of the conjugated F(ab′)$_2$/GAH, PEG, DXR and lipids was 1:4:5:50 (w/w/w/w), respectively. The mean size of MCC-465 was 143 nm [9].

MCC-465, which was manufactured under the good manufacturing practice regulations of the Ministry of Health, Labour and Welfare of Japan, was supplied by Mitsubishi Pharma Corporation (Osaka, Japan) in glass vials. Each vial contained lyophilized PEG immunoliposomes containing a total of 10 mg of doxorubicin hydrochloride. Appropriate amounts of MCC-465 dissolved in cold sterile saline for injection were diluted and adjusted with sterile saline up to 250 ml (or 500 ml when patients received >70 mg DXR equivalent dose/body). MCC-465 solution was infused intravenously for 60 min, or 120 min when the diluted volume was 500 ml, by an electric-driven pump with a fine filter F162 (Forte Grow Medical Co., Tochigi, Japan).

Dosage and dose escalation

The starting dose of MCC-465 was 6.5 mg/m^2, which is equivalent to one-tenth of the LD_{10} in rats. MCC-465 was administered once every 3 weeks and the treatment was continued up to six cycles unless any severe adverse event or disease progression was observed. Dose escalation proceeded according to a accelerated titration method described previously [10]. Toxicity was graded from 1 to 4 using the criteria of the Japan Clinical Oncology Group. If grade ≥2 toxicity occurred during the first 21 days, the dose of the next level should be increased according to the modified Fibonacci method and toxicity was to be confirmed in at least three patients. Intrapatient dose escalation was not permitted. At the level at which dose-limiting toxicity (DLT) was observed, toxicity was confirmed in up to six patients. The maximum tolerated dose (MTD) was then defined as one level below that level at which three out of six patients

Table 1. HPLC conditions

	HPLC condition 1	HPLC condition 2	HPLC condition 3
Column	CAPCELLPAK C18 UG120 (Shiseido)		
Column size (mm)	2.0 × 250	4.6 × 250	2.0 × 250
Column temperature	50°C		
Mobile phase	0.05 M phosphoric acid:acetonitrile:THF:PIC B-7 = 75:23:2:1.5 v/v	0.05 M phosphoric acid:acetonitrile (containing 7.5% THF):PIC B-7 = 75:23:2:1.5 v/v	0.05 M phosphoric acid:acetonitrile:THF:PIC B-7 = 70:28:2:2.5 v/v
Flow rate (ml/min)	0.2	1.2	0.18
Fluorescent detector wavelength (nm)	Ex = 475; Em = 554		

HPLC, high-performance liquid chromatography; THF, tetrahydrofuran; PIC B-7, ion-pair reagent (heptane sulfonic acid); Ex, excitatory; Em, emission.

experienced a DLT [11]. The recommended dose for a phase II trial was defined by the Efficacy and Safety Assessment Committee from the results of the safety and efficacy of this trial. DLT was defined as: (i) neutrophil count of <500/μl for >5 days or associated neutropenic fever of >38.5°C with infection; (ii) platelet count of <25 000/μl; and (iii) non-hematological toxicities except for nausea, vomiting and alopecia.

Pretreatment assessment and follow-up

At enrollment, patients were evaluated by a complete history and physical examination, performance status, complete blood cell count (CBC), blood chemistry, urinalysis, electrocardiogram (ECG), computed tomography or upper gastrointestinal series. Other exams were performed only in the presence of a clinical indication. Patients were monitored by physical examination every day up to the second administration, and at days 1, 2 and 3 and weekly thereafter by CBC and blood chemistry. ECG was recorded before and during treatment. Ultrasonic cardiography was repeated before every other administration. Human antihuman antibody (HAHA) was evaluated before every cycle. Tumor markers were also measured at the same time as HAHA.

Tumor response was evaluated according to the criteria of the Japan Society of Clinical Oncology. Complete response (CR) was defined as the disappearance of cancerous lesions and partial response (PR) required a >50% reduction in the sum of the bidimensional length of tumors on two points separated by at least 4 weeks. Stable disease (SD) was defined as a <50% reduction or <25% growth of lesions for at least 4 weeks. Progressive disease (PD) was defined as >25% of tumor growth, appearance of new malignant lesions or unequivocal worsening of other clinical evidence of malignancy. The Clinical Trial Coordinating Committee and the Efficacy and Safety Assessment Committee were organized to bridge between the three institutions at which the study was performed.

Sampling and storage

The measures recorded at the first cycle were as follows: (i) plasma concentrations of DXR (total DXR concentration); (ii) concentrations of DXR after gel filtration (liposomal-encapsulated DXR); (iii) concentration of DXR after ultrafiltration (free DXR); (iv) plasma concentrations of DXR metabolites; (v) urinary concentrations of DXR; and (vi) urinary concentrations of DXR metabolites. After the second cycle, only total DXR was measured. All the samples were chilled on ice during preparation and prepared using the appropriate method described below. Urine samples were stored in a refrigerator from the day before the drug administration to day 4. Prepared plasma and urinary samples were stored at –20°C except for encapsulated DXR samples, which were stored at –80°C.

Assay conditions

For the measurement of total DXR and metabolites [doxorubicinol (Dxol) and 7-deoxydoxorubicinol aglycon (7H-Dxol)] in plasma, 200 μl of boric acid buffer (pH 9.8) and 3 ml of chloroform/methanol (80:20 v/v) were added to 200 μl of human plasma and mixed. The separated organic phase was evaporated to dryness under a nitrogen stream. Residue was re-dissolved with the mobile phase and injected into a high-performance liquid chromatograph (HPLC) under the conditions described in 'HPLC conditions 1' in Table 1. For the measurement of encapsulated DXR by HPLC, plasma from each patient (60 μl) and the marker solution (60 μl) containing sufficient empty MCC-465 (no DXR), which is used as the marker for UV detection, were mixed. Then, 100 μl of the mixture was loaded onto a gel filtration column. The peak fraction of encapsulated DXR through gel filtration was collected, and 100 μl of the fractionated sample was mixed with 230 μl of methanol containing 0.15% trifluoroacetic acid. The mixture was injected into the HPLC under the condition described in 'HPLC conditions 2' in Table 1. For the free DXR measurement, 1 ml of freshly prepared plasma at each point was centrifuged immediately in Centrifree (Amicon Co., Ltd) at 2000 *g* at room temperature for 10 min. One hundred microliters of the collected sample were mixed with 250 μl of the mobile phase (described in Table 1) containing the internal standard. DXR and the metabolites in urine were measured using the same method as for plasma, except the urine volume was 400 μl.

Pharmacokinetic analysis

Pharmacokinetic (PK) parameters of total DXR, free DXR and metabolites in human plasma were calculated by a non-compartmental model using WinNonlin, version 2.1 (Pharsight Corporation). The parameters calculated were as follows: peak plasma concentration (C_{max}); time to reach the peak plasma concentration (T_{max}); half-life of terminal phase ($T_{1/2\lambda z}$); area under the concentration–time curve (AUC); total clearance (CL); volume of distribution at steady state (V_{dss}); and mean residence time (MRT). The total urinary excretion rates for DXR and metabolites were calculated for each subject, and mean urinary excretion rates of DXR and metabolites were calculated at each level. Microsoft Excel 97 was used for data management.

Results

Enrollment and dosing

Twenty-three patients were enrolled in this study. The details of each patient's background are shown in Table 2. Dose escalation was from 6.5 up to 45.5 mg/m^2. In total, 62 cycles of administra-

Table 2. Patient characteristics

	Dose (mg/m^2)					
	Level 1	Level 2	Level 3	Level 4	Level 5	
	6.5	13.0	21.0	32.5	45.5	Total
No. patients (male:female)	7 (6:1)	3 (3:0)	3 (3:0)	3 (2:1)	7 (5:2)	23 (19:4)
Age (years)						
Median	58	57	54	68	65	58
Range	40–68	57–58	30–60	56–72	52–69	30–72
Performance status						
0	1	2	1	1	3	8
1	5	1	2	2	4	14
2	1	0	0	0	0	1
Original lesion						
Yes	3	1	1	0	2	7
No	4	2	2	3	5	16
Metastatic lesion						
Lung	1	0	1	1	1	4
Liver	3	1	0	2	5	11
Adrenal gland	1	0	0	0	1	2
Bone	0	0	2	0	1	3
Peritonea	3	1	0	0	0	4
Esophagus	0	0	1	0	0	1
Lymph node	3	2	3	1	6	15
Prior therapy						
1	5	1	1	2	2	11
2	1	2	0	1	5	9
≥3	1	0	2	0	0	3

tion were performed. Sixteen patients received more than two cycles of administration. The maximum was six cycles at level 4 (32.5 mg/m^2) to one patient, and the average number of cycles over all levels was 2.7 (Table 2). The first patient, 1-101, developed obstructed jaundice caused by tumor progression. The Efficacy and Safety Assessment Committee suggested that the patient should not be a subject for PK and efficacy analysis, but was eligible for safety analysis, and recommended the enrollment of an additional patient at the first dose level. The second patient, 1-102, experienced grade 3 hypertension with grade 2 fever and shivering during infusion. At this time, administration was stopped immediately and restarted cautiously after the patient had recovered from all the symptoms induced by the medication, along with an anti-hypertensive drug. These symptoms were considered as an infusion-related reaction (IRR). The dose escalation method was switched to the modified Fibonacci method thereafter, and five more patients were enrolled at the first level.

IRR and other non-hematological toxicities

All the patients who received administration were assessed for safety. Major non-hematological toxicities are summarized in Table 3. IRRs were observed in 16 patients at the first exposure to the drug. Early reactions by infusion occurred 5–20 min after the start of the infusion, and chest discomfort, lumbago, back pain, itching and urticaria were observed as symptoms. All the symptoms disappeared shortly after chlorpheniramine maleate treatment or with no treatment. Late reactions to infusion developed usually at the end of infusion of MCC-465, and were characterized by chills and shivering. All the symptoms disappeared quickly, with no treatment. However, one patient at level 1 experienced grade 3 hypertension accompanying chills and shivering. Some patients developed grade 1 or 2 fever 30 or 60 min after the termination of the infusion; however, fever was transient in all cases. Patients who experienced such IRRs could be treated with MCC-465 repeatedly without pre-medications, and revealed no severe reactions thereafter. Skin toxicities such as mild rash, alopecia, erythema, pruritis and urticaria were observed in five patients. Palmar–plantar erythrodysesthesia (PPE) was not observed in this study. Grade 1 or 2 stomatitis was observed in four patients; grade 1–2 nausea and vomiting were observed in 11 patients; and grade 3 appetite loss was observed in one patient at level 5, which was defined as a DLT. There was no remarkable evidence of liver

Table 3. Non-hematological toxicity

	6.5 mg/m^2		13.0 mg/m^2		21.0 mg/m^2		32.5 mg/m^2		45.5 mg/m^2		Total No. patients
	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	
General disorders											
Pyrexia	3		1		2		2		6		14
Rigors	2		1		2		2		2		9
Malaise							2		5		7
Shivering	2		1		1		1		1		6
Performance status decreased	1								3	2	6
Chest discomfort					1				1		2
Feeling hot									2		2
Gastrointestinal disorders											
Nausea	2		2				1		2		7
Anorexia			1		1				3	2	7
Vomiting					1		1		4		6
Stomatitis	1		1				2				4
Diarrhea	1								2		3
Skin and subcutaneous tissue disorders											
Urticaria NOS	1						1				2
Depilation									2		2
Others											
Blood pressure increased	1	1	1		1				1		5
Flushing	1						1		1		3
Back pain							1		1		2
Taste disturbance			1						1		2
Heart rate increased	1		1								2

NOS, not otherwise specified.

Table 4. Hematological toxicity

Dose (mg/m^2) (No. patients)	21.0 (3)				32.5 (3)				45.5 (7)			
Grade	1	2	3	4	1	2	3	4	1	2	3	4
Leukopenia	1	0	0	0	1	0	1	0	1	2	3	1
Neutropenia	1	0	0	0	1	0	1	0	0	1	2	3
Thrombocytopenia	0	0	0	0	0	0	0	0	1	1	0	0

function or kidney function abnormalities related to the treatment. No pain or local toxicity in the area of injection was observed.

No HAHA was detected throughout all treatment cycles.

Hematological toxicity

No significant myelosuppression was observed up to dose level 3. Grade 4 leucopenia was observed in one patient at level 5 (45.5 mg/m^2). Grade 4 neutropenia was observed in three patients at the same level (Table 4). One of these three patients experienced grade 4 neutropenia that lasted for 5 days, which was defined as a DLT. Leucopenia and neutropenia started from day 4–8, and the median time to the nadir was 15 days.

MTD and DLT

In total, three DLTs were observed through this trial, including grade 3 hypertension at level 1, grade 4 neutropenia lasting for

Table 5. Mean PK parameters at each level in the phase I clinical study

	n	Dose (mg/m^2)	C_{max} (μg/ml)	T_{max} (h)	$AUC_{0-^{-}}$ (μg·h/ml)	CL (ml/h/kg)	V_{dss} (×10^2) (ml/kg)	MRT (h)	$T_{1/2\lambda z}$ (h)
Total and free DXR									
Level 1			2.49 ± 1.45	1.01 ± 0.01	31.5 ± 23.7	(4.45 ± 1.78)[a] 20.4 ± 25.4	(0.413 ± 0.114)[a] 2.61 ± 3.43	10.9 ± 2.53	9.09 ± 4.30
Level 2	3	13.0	7.18 ± 0.847	1.28 ± 0.13	120 ± 44.6	3.36 ± 1.13	0.325 ± 0.0499	10.3 ± 3.11	7.66 ± 1.68
Level 3	3	21.0	11.4 ± 1.42	1.06 ± 0.10	177 ± 27.5	3.39 ± 0.509	0.282 ± 0.0190	8.39 ± 0.761	21.7 ± 12.0
Level 4	3	32.5	15.1 ± 1.47	1.39 ± 0.54	310 ± 139	4.00 ± 1.97	0.511 ± 0.140	14.5 ± 6.42	33.2 ± 17.7
Level 5	3[b]	45.5	30.3 ± 4.05	1.00 ± 0	637 ± 257	2.53 ± 1.16	0.345 ± 0.0257	16.2 ± 8.14	69.3 ± 58.9
	3[c]	45.5	21.3 ± 1.99	2.67 ± 0.58	397 ± 118	3.28 ± 1.07	0.325 ± 0.0170	10.6 ± 3.42	40.7 ± 26.8
Free DXR in level 4	3	32.5	0.325 ± 0.0990	0.67 ± 0.29	3.07	504	210	73.5	54.5
Free DXR in level 5	3[b]	45.5	0.251 ± 0.0874	0.50 ± 0	2.35	690	279	48.6	36.8
	3[c]	45.5	0.131 ± 0.0795	2.00 ± 0	3.02 ± 1.57	473 ± 197	234 ± 26.3	57.1 ± 28.2	41.2 ± 21.6

	n	Dose (mg/m^2)	C_{max} (μg/ml)	T_{max} (h)	AUC_{0-t} (μg·h/ml)	$AUC_{0-^{-}}$ (μg·h/ml)	CL (×10^2) (ml/h/kg)	V_{dss} (×10^3) (ml/kg)	MRT (h)	$T_{1/2\lambda z}$ (h)
Metabolites										
Dxol in level 5	3[b]	45.5	0.0099 ± 0.0026	19.67 ± 9.24	1.13 ± 0.274	1.52 ± 0.155	9.22 ± 0.730	145 ± 28.0	156 ± 18.7	109 ± 25.8
	3[c]	45.5	0.0074 ± 0.0068	50.00 ± 24.00	1.10 ± 1.17	1.30 ± 1.21	15.2 ± 9.39	216 ± 134	142 ± 1.73	90.4 ± 4.65
7H-Dxol in level 5	3[b]	45.5	0.0036 ± 0.0013	1.33 ± 0.577	0.104 ± 0.0956	0.204	108	354	44.8	29.0
	3[c]	45.5	0.0079 ± 0.0071	2.67 ± 0.577	0.160 ± 0.143	0.272	44.3	126	27.4	19.2

PK parameters are shown as mean ± SD, but in case of $n = 2$, PK parameters are shown as means.

[a]$n = 4$ (exclusion of patients 1-105 and 1-107).

[b]60 min infusion.

[c]120 min infusion.

C_{max}, peak plasma concentration; T_{max}, time to reach the peak plasma concentration; $T_{1/2\lambda z}$, half-life of terminal phase; AUC, area under the concentration–time curve; CL, total clearance; V_{dss}, volume of distribution at steady state; MRT, mean residence time; DXR, doxorubicin; PK, pharmacokinetic; SD, standard deviation.

Table 6. The efficacy and number of doses at each dose level

Level	Dose (mg/m^2)	No. patients	NE	Excluded	PD	SD	No. cycles administered	
							Range	Average
1	6.5	7	2	1	3	1	1–2	1.4
2	13	3			1	2	2–5	4
3	21	3			1	2	2–5	4
4	32.5	3			1	2	3–6	4
5	45.5	7	1	1	2	3	1–4	2.3

NE, not evaluable; PD, progressive disease; SD, stable disease.

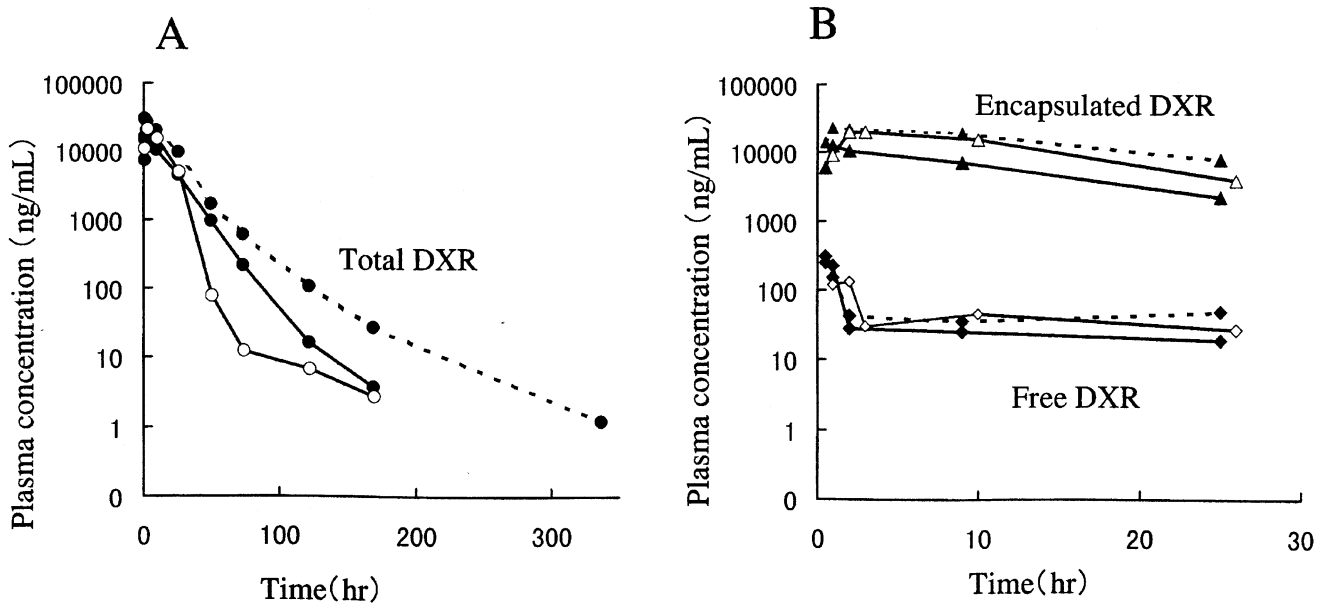

Figure 2. Mean plasma concentrations of doxorubicin (DXR) in the phase I clinical study (level 4 and level 5 of 60 or 120 min infusion). (**A**) Total DXR in plasma: filled circles, solid line, level 4; filled circles, dotted line, level 5 (60 min); open circles, level 5 (120 min). (**B**) Free (open or filled diamonds) and encapsulated (open or filled triangles) DXR in plasma: filled triangles or diamonds, solid line, level 4; filled triangles or diamonds, dotted line, level 5 (60 min); open triangles or diamonds, level 5 (120 min). Time in the figures was expressed as scheduled time. $n = 3$, except level 5 (*$n = 2$, patient 1-122 excluded).

>5 days at level 5, and grade 3 appetite loss at level 5. At level 5, two out of six patients experienced grade 4 neutropenia, in addition to two patients with DLT. Since it was suggested that >50% of the patients would develop DLT at the next level (level 6), the dose escalation was stopped at level 5. The MTD was considered to be 45.5 mg/m^2 (level 5).

Antitumor activity

Although the antitumor activity was not the primary end point, 18 out of 23 patients were evaluable. No responses were observed definitely in these evaluable 18 patients. However, 10 patients had SD (median duration was 92.5 days, range 48–135) (Table 6). Seven of these 10 received more than four cycles of treatment within at least 16 weeks, while these patients have received multiple (once as many as 5 cycles; median 2 cycles) prior chemotherapy cycles (Tables 2–5).

Pharmacokinetics

PKs were evaluated in 21 patients. The mean plasma concentrations of total DXR, free DXR and encapsulated DXR at levels 4 and 5 (60 and 120 min infusion, respectively) are shown in Figure 2. Most of the total DXR existed in the circulating blood in an encapsulated form, because the plasma concentration of encapsulated DXR was very close to that of total DXR. The peak plasma concentration of total DXR at each level reached C_{max} at the end of the infusion or 1 h after the infusion. While the plasma concentration profiles of total DXR showed a biphasic elimination pattern at levels 1–3, a monophasic elimination pattern was observed at

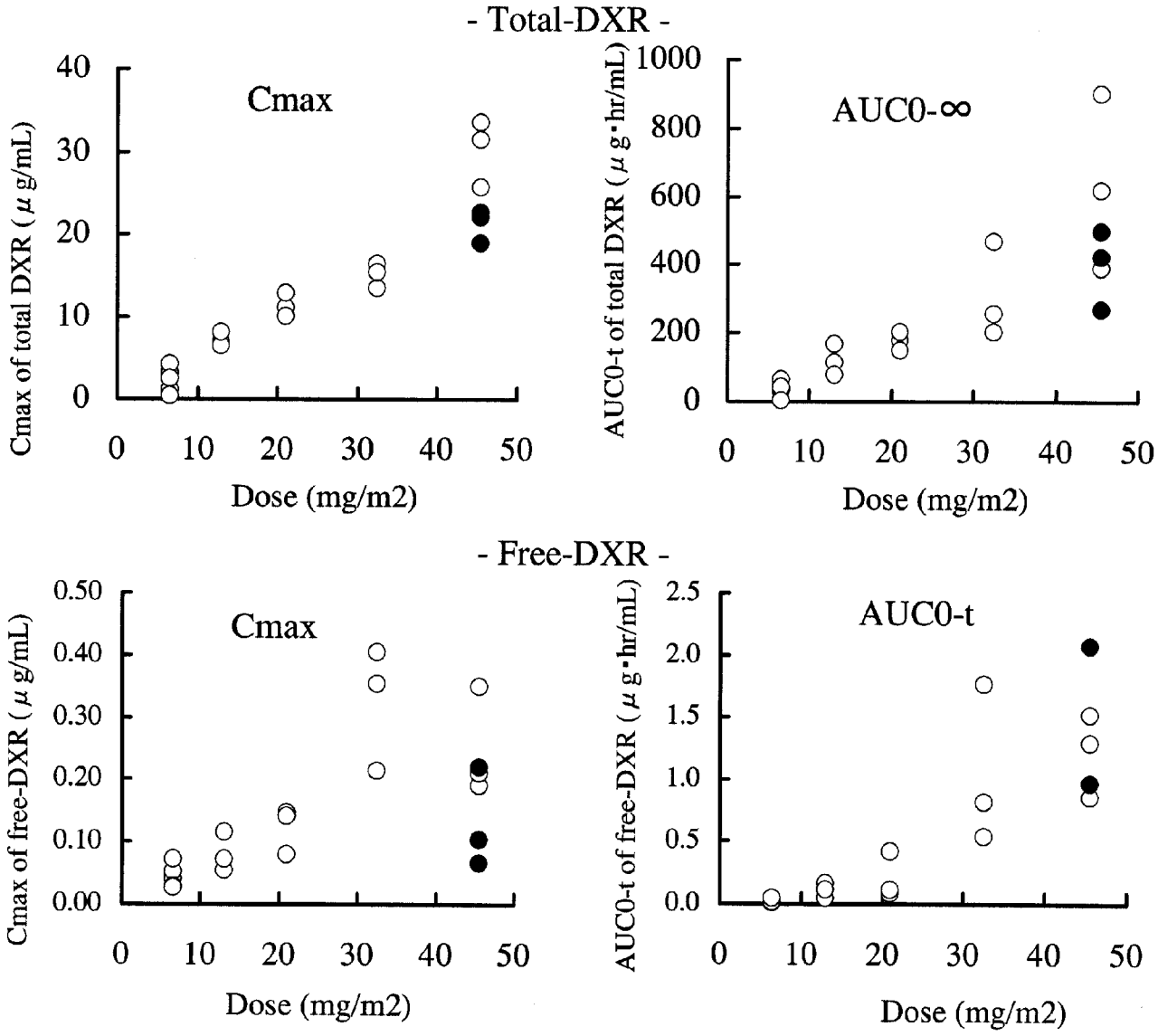

Figure 3. Relationship between dose and C_{max} or AUC. Open circles, 60 min infusion; filled circles, 120 min infusion.

level 4 and 5, where one subject at each level (one at level 4 and one at level 5) showed a monophasic elimination pattern with a long half-life. The mean pharmacokinetic parameters of total DXR, free DXR and metabolites in plasma at levels 1–5 are shown in Table 5. The CL, V_{dss} and MRT of total DXR were almost the same for all the dose levels. The $T_{1/2\lambda z}$ of total DXR was prolonged by the dose escalation because the concentration of the terminal phase was detected as the dose increased. There was no difference in terms of total DXR concentration during repeated administrations (data not shown). It was suggested that the PK profile of total DXR in plasma was not affected by repeated administration of MCC-465 every 3 weeks. Plasma concentration profiles of free DXR showed slow elimination at levels 4 and 5. The CL and V_{dss} of free DXR were almost identical among the dose levels tested. The plasma concentration of Dxol (60 min infusion) reached C_{max} at 19.67–25.26 h (mean value at each level), and was eliminated more slowly than DXR. The plasma concentration of 7H-Dxol was detected at level 4 and 5, but was close to the lower limit of quantification.

The C_{max} and AUC of total DXR and free DXR increased with dose escalation (Figure 3).

Discussion

Goren et al. [12] prepared immunoliposomes conjugated with whole monoclonal antibody against Her-2 and studied the tumor targeting efficacy of the immunoliposomes in comparison with plain liposomes. They suggested that the antitumor efficacy of the drug-containing liposomes depended on the drug delivery to the tumor, and that the rate-limiting factor of liposome accumulation in tumors was the liposome extravasation process, irrespective of

liposome affinity to, or targeting of, tumor cells. In addition, it was reported that immunoliposomes conjugated with whole IgG had a shorter plasma half-life because the immunoliposomes were entrapped by the RES. In order to overcome this problem, Maruyama et al. [13] prepared an Fc-removed antibody for immunoliposomes and succeeded in reducing the RES entrapment of the immunoliposomes. Taking these reports into consideration, $F(ab')_2$ of GAH was conjugated to MCC-465.

The present clinical data indicate that the PK parameters of MCC-465 differ from those of free doxorubicin, but were very similar to those of Doxil in humans [6]. These data show the stability of MCC-465 in the blood circulation. They also indicate that the conjugation of the $F(ab')_2$ of GAH does not interfere with the stealth effect of the PEG liposomes.

The DLTs of MCC-465 were neutropenia and appetite loss. Unlike the findings from previous reports of phase I trials for a similar drug, Doxil, we did not experience any severe skin toxicity such as PPE or mucositis. The reason for the difference in toxicity between MCC-465 and Doxil is not known; however, it is speculated that the accumulation of MCC-465 in the skin is different from Doxil, or that Caucasian patients are more prone to skin damage by such stealth liposomal DXR in terms of skin toxicity compared with Japanese patients.

IRR was the most common adverse effect. Sixteen out of 23 treated patients showed a variety of symptoms. The major symptoms were fever, rigors, shivering, flushing, chest discomfort, back pain, red eye, itching, vomiting, feeling hot and numbness, which developed at the beginning of the infusion, while fever, rigors, shivering and hypertension were observed at the end of the infusion. Some patients showed two or more symptoms at the same time, but these symptoms were mild, and they disappeared during the infusion or within a few hours. Eight out of nine patients who had chill or shivering also experienced fever. Among these patients, four also had hypertension, and one developed grade 3 hypertension. Chlorpheniramine maleate was administered to four patients for shivering, itching or flushing, and an antihypertensive drug was administered to one patient. The other patients had no medication for IRR.

Other non-hematological toxicities were mild, and no malfunctioning was observed in the organs such as the liver and kidney. Although cardiac safety was not addressed specifically, since the maximum cumulative dose of doxorubicin in this phase I study was 195 mg/m^2, no patients presented with cardiac toxicity symptoms or loss of function in terms of the left ventricular ejection fraction.

In this study, we needed a 3-week interval schedule in order to determine the safety, tolerability and PKs. No objective tumor response was seen. It is, however, worth noting that 10 SD patients were observed among our 18 evaluable patients, who belonged to a population with a very low probability of response because of extensive prior chemotherapy for gastric cancer.

Our study shows that the MTD of MCC-465 using the 3-week schedule (45.5 mg/m^2) is slightly lower than the MTD of Doxil (50 mg/m^2). This result may be due in part to the unstable condition of heavily treated patients with advanced gastric cancer. These data warrant further investigation of MCC-465, and the recommended dose for a phase II study with a 3-week administration protocol is considered to be 32.5 mg/m^2.

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