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ORIGINAL ARTICLE

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A phase II and pharmacokinetic study of pegylated liposomal doxorubicin in patients with advanced hepatocellular carcinoma

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Abstract *Purpose*: Chemotherapy of advanced hepatocellular carcinoma (HCC) is frequently limited by unacceptable toxicity. Long-circulating polyethylene glycol-coated (PEGylated) liposomal doxorubicin (PLD) has low systemic toxicity. Its safety and efficacy in patients with advanced HCC and the relationship between hepatic function and pharmacokinetics were investigated in this phase II study. *Methods*: Patients were given 30 mg/m^2 PLD every 3 weeks and the dose was escalated to 45 mg/m^2 from the third course if the toxicity was deemed tolerable. The plasma level of doxorubicin was determined with fluorometry. *Results*: A total of 40 patients were recruited into this phase II study. The toxicities were usually mild but unexpectedly, three cirrhotic patients died of infection without neutropenia. Four had a partial response (response rate 10%, confidence interval 0–20%). The median duration of response was 5.6 months. The median time to tumor progression and the median survival of all patients was only 2 and 3 months, respectively. Patients with advanced HCC had lower initial serum concentration, larger volume of distribution and more rapid clearance than patients with other malignancies and normal liver function. However, the pharmacokinetic parameters correlated with neither toxicity nor response. *Conclusions*: The disposition of PLD in patients with liver dysfunction was not hampered, but it did not exhibit higher activity compared with free drug, and the risk of infection must be watched closely especially in patients with liver cirrhosis.

Keywords Inoperable hepatocellular carcinoma · Liposome · Doxorubicin · Liver function · Chemotherapy

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Introduction

The majority of hepatocellular carcinomas (HCCs) present at an advanced stage, when most are beyond curative treatment. The results of chemotherapy or other systemic treatment for disseminated HCC have been dismal [20]. Conventional chemotherapy, either as a single agent [4, 6, 7, 10, 19, 25, 29] or in some combinations [11, 14], has limited activity. Further effort to improve the treatment of inoperable HCC is urgently needed, but systemic chemotherapy of HCC is limited by (1) impaired liver function due to commonly coexisting liver cirrhosis or locally advanced tumors, (2) preexisting pancytopenia due to secondary hypersplenism, and (3) risk of reactivation of hepatitis by chemotherapy.

Doxorubicin provides the most consistent response rate in HCC. Long-circulating polyethylene glycol-coated (PEGylated) liposomal doxorubicin (PLD) circulates for prolonged periods with stable retention of its contents and in animal studies has been shown to lead to a passively preferential localization into tumors [12, 17]. In clinical trials of formulations of PLD, improved pharmacokinetic properties and reduced systemic toxicity have also been demonstrated [9, 12, 16]. PLD, with an altered metabolism and toxicity profile, may surpass the limitations encountered in systemic chemotherapy of HCC. We performed a phase II and pharmacokinetic study to investigate the safety and efficacy of PLD in the treatment of inoperable HCC.

Patients and methods

Eligibility criteria

Patients had pathologically or cytologically confirmed HCC or were HBV carriers with hepatic tumors of α-FP >400 ng/ml and an unequivocal clinical diagnosis of HCC. The tumors were considered inoperable and local treatments such as transarterial embolization were not considered feasible. Patients had to have disease measurable by radiography, sonography or CT scan. Other criteria for inclusion were: ECOG performance scale less than or equal to

2; ANC ≥1500/mm^3, platelets ≥75,000/mm^3; GOT and GPT less than ten times normal and bilirubin ≤ 3.0 mg/dl. For safety, inclusion criteria for the first 14 patients were more strict and confined to patients with ANC > 2000/mm^3, platelets > 100,000/mm^3, and GOT and GPT less than five times normal. Patients chosen had to have recovered from the effects of recent surgery, radiotherapy (at least 4 weeks apart), embolization or other regional therapies (at least 8 weeks apart) and be free of infection. Informed consent had to be signed. The study was performed after approval by the Human Investigation Committee.

Evaluation and follow-up

The prestudy work-up included a review of the patient's clinical history and a physical examination, biochemical profile (SMA 12), complete blood cell (CBC) count, chest radiograph, bone scintigraphy, abdominal sonography and/or CT scan. CBC, biochemistry, and clinical assessment of adverse events were performed weekly during the first two cycles of treatment. These examinations were repeated at least twice per cycle during subsequent courses. If there was evidence of disease stabilization after two cycles of therapy, treatment was continued with reevaluation for efficacy after each two courses.

All toxicities were evaluated at each course of PLD according to the Eastern Cooperative Oncology Group criteria except that the pretreatment levels (PTLs) rather than upper limit of normal of serum GPT and bilirubin were used as references to assess hepatotoxicity (PTL to 1.5×PTL, grade I; 1.5×PTL to 3.0×PTL, grade II; 3.0×PTL to 10.0×PTL, grade III; > 10.0×PTL, grade IV).

Evaluation for the primary end point (objective tumor response) was performed with a complete work-up 3 weeks after the second injection. Partial response (PR) was defined as a greater than 50% reduction in the sum of the products of the greatest perpendicular dimensions for all measurable lesions for at least 4 weeks, without the appearance of new lesions. Minor response was defined as a reduction of more than 25% but less than 50%. Progression of disease (PD) was defined as an increase of more than 25%, new lesions, or unequivocal worsening of other tumor indicators.

The prespecified secondary end points, time to progression, time to treatment failure, time to death (survival duration) and duration of response were calculated from the date of starting treatment. Time to progression represented the time to objective disease progression. Time to treatment failure was the time to earliest occurrence of progression, death or withdrawal. Time to death (survival duration) represented the number of days until death from any cause. Duration of response, which was recorded for those with either a complete or partial best response, was the time to objective progression or relapse.

Drug description and form of administration

The liposome components were distearoyl phosphatidylcholine, cholesterol, distearoyl-phosphatidyl-ethanolamine derived from PEG (average Mr 2000) with a molar ratio of 3:2:0.3 (Avanti Polar Lipids, Alabaster, Ala.). Small unilamellar vesicles (SUV, size 80–110 nm) were prepared by a combination of the ethanol injection method and repeated extrusion as described previously [16]. Doxorubicin was encapsulated by a remote loading method and its final concentration was 2.0 mg/ml. This PLD was prepared by TTY BioPharm (Taipei, Taiwan) authorized by the Taiwan Liposome Company (Taipei, Taiwan) and has been shown to have the pharmacokinetic properties of a second-generation liposomal drug [16].

Because patients with relatively poor performance and impaired liver function were allowed to enter this study, the dose of PLD for the first two cycles was 30 mg/m^2. For subsequent cycles, 45 mg/m^2 was used, if the toxicity was deemed acceptable. PLD was diluted in 200 ml 5% dextrose solution prior to administration and was given intravenously over a 1-h period. Premedication included cimetidine 200 mg, hydrocortisone 100 mg and diphenhydramine 30 mg.

The treatment was repeated every 3 weeks if toxicity allowed. If ANC was ≥1500/mm^3, platelets were ≥75,000/mm^3, GPT was less then twice PTL, bilirubin was less then twice PTL, and all other toxicities had resolved to grade 1 or less, the patient proceeded to the next course of chemotherapy. If grade 3 or higher toxicity occurred, the dose of subsequent courses was reduced by 25%. If treatment could not be started on day 43, the patient was taken off the study.

Pharmacokinetic study and analysis

Pharmacokinetic studies were performed after the first PLD treatment [16]. Blood samples (4 ml) were obtained before drug injection, and at 5 min and 2, 4, 10, 24, 48, 72 and 168 h following completion of PLD infusion. Pharmacokinetic analysis was done by nonlinear least-squares analysis using Pkanalyst software (MicroMath, Salt Lake City, Utah).

Statistical considerations and data analysis

Simon's two-stage optimal design for phase II trials was used [23]. In the first stage, 14 patients were enrolled in the protocol. If none of the 14 patients responded, the probability that the true response rate was greater than 20% was less than 0.05. If there was any patient who responded to the drug, 26 more patients were enrolled to meet the requirement of registration. True response rate was determined after accrual of all 40 patients.

Response rate was evaluated in all enrolled patients (intent-to-treat analysis) and in evaluable patients (i.e., all patients who received at least two PLD doses). Time to event end points was estimated using the Kaplan-Meier survival methodology. The effect of baseline characteristics on response rates was evaluated by the χ^2 test and logistic regression model. The pharmacokinetic data of patients of a prior phase I study were reviewed and the difference between the two groups of patients was evaluated by the Mann-Whitney rank sum test. The patients of the phase I study had cancers other than HCC and received the same dosage (30 mg/m^2) of PLD [16]. The relationship between leukocyte counts at nadir and pharmacokinetic parameters was explored with Spearman's rank order coefficient. The point multiserial correlation coefficient was used to correlate stomatitis grade with pharmacokinetic parameters.

Results

Patient characteristics

Of the first 14 patients, 1 responded to PLD. A total of 40 patients entered this trial, and the characteristics of all patients are listed in Table 1. Of the 40 patients, 32 were HBV carriers and 9 were HCV carriers, 22 had liver cirrhosis (8 of whom were B and 2 were C in Child-Pugh's grading for hepatic reserve), and 11 had ascites.

Patients in this study tended to have big tumors. The median tumor diameter was 10.6 cm and 32 patients had a tumor larger than 5 cm in diameter. Of the 40 patients, 27 had metastatic lesions, of which lung was the most common site (21), 6 had multiorgan metastasis, 4 had been exposed to radiotherapy and 5 had received chemoembolization containing mitomycin C, doxorubicin and cisplatin.

Table 1 Patient characteristics ($n = 40$)

Age (years)	Median	50.3
	Range	17–75
Gender	Male:female	33:7
ECOG performance scale	0/1/2/3–4	6/19/15/0
HBV carrier	No/yes	8/32
Cirrhosis	No/yes	18/22
Ascites	No/yes	29/11
Child-Pugh grade	A/B/C	30/8/2
Largest tumor diameter (cm)	Mean	12 ± 8 (2.7–22)
	< 5	8
	5–22	32
Metastasis	Lung	21
	Bone	4
	Lymph node	3
	Others	3
Prior operation	No/yes	30/10
Prior transarterial embolization	No/yes	24/16
Prior radiotherapy	No/yes	36/4
Prior chemotherapy	No/yes	35/5

Toxicity

During the study period, a total of 120 courses were given. Among these courses, severe nausea and vomiting occurred in 2% (Table 2). Mild stomatitis occurred in 30% and grade 3 stomatitis in 2% of the courses. Mild hand and foot syndrome complicated 11% of the courses, but none was severe. Elevation of liver enzyme was one of the most common side effects. It occurred in 60% of the patients and 32% of courses. Of the 40 patients, 2 had grade 3 hepatotoxicity. Mild myelosuppression was also common, and severe leukopenia and thrombocytopenia occurred in 10% and 5% of courses, respectively. After dose escalation to 45 mg/m^2, most cycles were repeated every 4 weeks due to delayed recovery of white cell counts.

Although the treatment was generally well tolerated and few grade 3 and 4 toxicities occurred, three patients died of fulminant infection during chemotherapy in spite of intensive antibiotic treatment (Table 3). The occurrence of sepsis was not associated with neutropenia. Two of the three patients had disease in partial remission.

Only 14 patients received more than two courses of treatment and 6 received more than four courses, including 1 who completed 24 courses of treatment during the study period with a total administered dose of 960 mg/m^2. No obvious accumulated toxicity was observed, except a slower recovery in hemogram after repeated treatment.

Antitumor activity and survival

After the first course of treatment, one patient died of infection, one refused further treatment and seven left the study due to bleeding of varices or a deteriorating clinical condition. Among the remaining 31 patients, 12 had stable diseases, and 1 had a minor response and 4 a partial response. The response rate was 13% (95% CI 2–24%) for patients who had received two or more courses and 10% (95% CI 0–20%) for all patients by intent-to-treat analysis. Three patients with liver tumors and one patient with lung metastasis responded. The median time to tumor progression and the median survival was

Table 2 Toxicity

Toxicity	% of first courses ($n = 40$)			% of all courses ($n = 99$)[a]		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
Nausea	20	0	0	11	2	0
Vomiting	13	0	0	9	2	0
Stomatitis	30	0	0	27	2	0
Alopecia	20	–	–	29	–	–
Skin	5	0	–	2	0	–
Leukopenia	43	5	3	48	6	3
Thrombocytopenia	15	3	3	11	2	1
Anemia	28	0	0	27	0	0
Hepatitis	43	3	0	24	2	0
Infection[b]	0	0	0	0	0	3

[a]Excluding patients who received only one course
[b]Three patients died of sepsis

Table 3 Data of patients who died of sepsis

UPN	Age (years)	Gender	Child-Pugh grade	ICG test (%)[a]	Cirrhosis	Courses given	WBC count (mm^3)		Blood culture growth
							Nadir	At time of infection	
8[b]	59	Male	A	14	No	4	6800	9800	*Staphylococcus aureus*
15	58	Female	B	55	Yes	3	3360	5600	*Klebsiella pneumoniae*
27	75	Female	A	33	Yes	1	5070	5070	*Escherichia coli*

[a]Indocyanine green test; normal $< 12\%$ at 15 min
[b]This patient had diabetes and a skin ulcer on the big toe

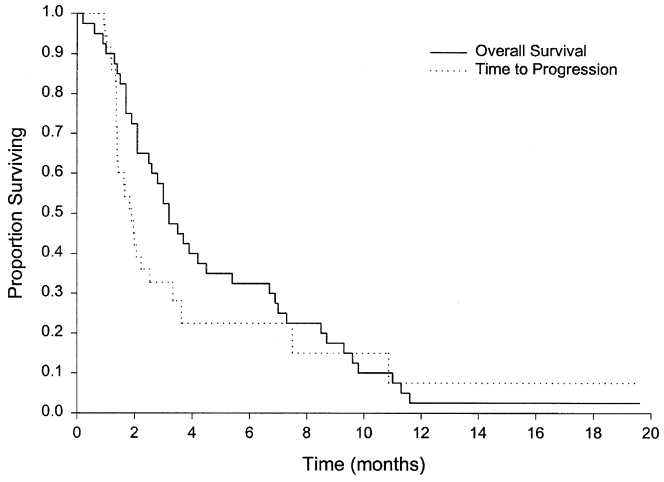

Fig. 1 Kaplan-Meier curves of time to tumor progression and overall survival of all patients ($n=40$)

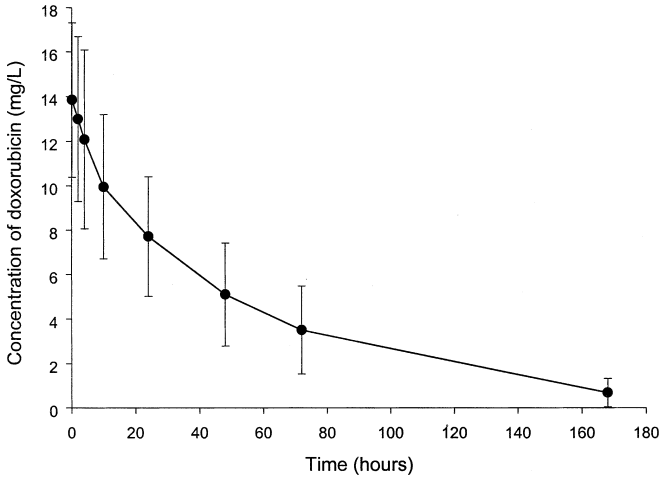

Fig. 2 Plasma doxorubicin concentrations (mean ± SD) determined after intravenous injection of 30 mg/m^2 ($n=40$) PLD into patients. Since the amount of free doxorubicin was small, the amount of liposomal doxorubicin was used to analyze the pharmacokinetic parameters

only 2 and 3 months, respectively, which reflected the advanced state and aggressive nature of the disease (Fig. 1). The median duration of response was 5.6 months (3.5 to 18+ months). The responders (including those with a minor response) had longer disease-specific survival than non-responders (median 12 months vs 4 months, $P=0.006$). There were no significant differences between responders and non-responders in terms of age, performance, tumor size etc.

Pharmacokinetic characteristics of PLD in patients with HCC

There were large variations among patients in the plasma doxorubicin concentrations and pharmacokinetic characteristics of PLD (Fig. 2, Table 4). The results of indocyanine green (ICG) testing in HCC patients did not correlate with these pharmacokinetic parameters (data not shown). Child class B and C had a shorter beta half-life than class A (30.5 vs 40.0 h, $P=0.047$), but the difference in initial plasma concentration, volume of distribution, and clearance did not reach a significant level.

We compared the pharmacokinetic parameters of HCC patients with those of nine normal liver function test patients recruited to the phase I study of this PLD (Table 4). At the same dose level of 30 mg/m^2, HCC patients had a lower initial plasma concentration (13.9 vs 17.0 mg/l, $P=0.025$, 95% CI for difference of means −5.860 to −0.411), a larger volume of distribution (2.3 vs 1.7 l, $P=0.016$) and more rapid clearance (0.069 vs 0.035 l/h, $P=0.026$).

The pharmacokinetic parameters did not correlate with the grade of either stomatitis or leukopenia. All pharmacokinetic parameters were compared between the responders and the non-responders, but there were no significant differences.

Discussion

Liver function and pharmacokinetics

Although the pharmacokinetics of PLD in patients with advanced HCC varied greatly, we still might conclude that, in contrast to the retarded metabolism of free doxorubicin in patients with liver dysfunction, clearance of PLD was not reduced (Table 4). On the contrary, due to a larger volume of distribution and more rapid clearance, the mean AUC in HCC patients was lower than that in patients with other cancers. The larger volume of distribution of PLD could be explained by the common presence of ascites. With regard to the clearance of PLD, the bottleneck was breakdown of liposomes, not hepatic metabolism nor excretion of free doxorubicin or its metabolites [13]. The higher serum level of bile acids due to either hepatocellular or obstructive jaundice may be destabilizing to the liposomes [1, 21] and lead to earlier release of doxorubicin from liposomes. Consistent with this reasoning, the beta half-life of PLD in patients with Child class B and C was shorter than that of patients with Child class A (data not shown).

Toxicity and response

PLD, as expected, had acceptable myelosuppression in patients with advanced HCC, even in the presence of impaired liver function. Stomatitis and dermatitis, which are common with the use of PLD, also seemed tolerable. Elevations of serum transaminases were frequently observed but were usually mild. Deterioration of liver function usually could be attributed to tumor progression in the terminal stage of the disease. Acute exacerbation of hepatitis was not observed in this study but it

Table 4 Comparison of pharmacokinetics between patients with advanced HCC and phase I study patients with non-hepato mamalignancies (C_0 initial concentration, AUC area under the concentration curve, Vss volume of the distribution at steady state)

Parameter	HCC (n = 40)			Phase I (n = 9)			P value[a]
	Median	Mean	95% CI	Median	Mean	95% CI	
C_0 (mg/l)	13.7	13.9	7.1–20.7	17.5	17.0	9.9–24.1	0.025
$\alpha_{t1/2}$ (h)	3.7	4.6	−3.7–12.8	5.7	6.3	−3.5–16.0	0.317
$\beta_{t1/2}$ (h)	36.2	43.7	2.8–84.5	61.8	59.5	26.9–92.0	0.017
AUC (mg · h/l)	638.0	753.5	−34.5–1541.5	984.2	1123.9	249.9–1997.8	0.032
Residence time (h)	51.1	61.6	5.0–118.2	81.8	82.1	36.9–127.3	0.022
Clearance (l/h)	0.050	0.069	−0.116–0.254	0.033	0.035	0.0–0.1	0.026
Vss (l)	2.1	2.3	1.0–3.5	1.7	1.7	1.2–2.2	0.016

[a]Mann-Whitney rank sum test

was still a potential risk [8, 15, 28]. The previously reported favorable toxicity profile of PLD was essentially confirmed even in patients with impaired liver function, and this was consistent with the results of the pharmacokinetic study.

However, three patients died of infection, which occurred in the absence of neutropenia. Two of them had liver cirrhosis and the blood culture grew enteric gram-negative bacilli. This was not observed in our phase I pharmacokinetic study [16] or other clinical studies of liposomal doxorubicin [2]. The reticuloendothelial system plays an important role in preventing infection in patients with cirrhosis, and impaired function of the reticuloendothelial system is correlated with a higher risk of spontaneous bacterial peritonitis and mortality [3]. Liposomal drug has been reported to impair the function of the reticuloendothelial system. In rats, doxorubicin-containing liposomes can exert major toxic effects on the liver macrophage population for a considerable period of time: a strong impairment of phagocytic function and even a substantial depletion of the liver macrophage population has been observed [24]. Therefore, the use of PLD in patients with liver cirrhosis may involve a higher risk of infection. It is important to take care of spontaneous bacterial peritonitis or systemic infection in further trials or clinical use of PLD in HCC patients with liver cirrhosis.

The response rate to this PLD was not superior to that of free doxorubicin. Due to the common presence of hepatic dysfunction in patients with HCC, a starting dose of 30 mg/m^2 was used. This dose was lower than the recommended dose for phase II studies (45 mg/m^2) concluded from a prior phase I study [16]. The majority of patients progressed rapidly within the first two courses and did not have a chance to receive the maximal tolerated dose. Thus, the response rate might have been underestimated. However, viewing the considerable overall toxicities, patients with advanced HCC might have a lower maximal tolerated dose than patients with other solid tumors.

Role of PLD in HCC

In a meta-analysis [20], compared to the control, there was no survival benefit with doxorubicin, percutaneous ethanol injection, or transarterial chemotherapy. The therapeutic benefit of interferon and tamoxifen also is doubtful. As to the recently developed drugs, paclitaxel is not effective [5], while both gemcitabine and topotecan show very limited activity [26, 27]. Further intensive basic and clinical research is obviously needed to improve the ominous outcome of patients with advanced HCC.

In this study we demonstrated that the disposition of PLD in advanced HCC patients was not retarded. Correspondingly, hand and foot syndrome and stomatitis, the characteristic limiting toxicities of PLD, were mild and not frequent. Unfortunately, this PLD, similar to Doxil, a more commonly used second-generation liposomal doxorubicin, is not more active than conventional chemotherapy [15, 22]. However, before the advent of better therapy, PLD is a reasonable choice for locally advanced or metastatic HCC, especially in the presence of liver dysfunction [18]. In addition, PLD, with its low myelotoxicity and hepatotoxicity and advantageous pharmacokinetic characteristics, may act as a base for the study of combinational chemotherapy. However, the use of PLD in patients with liver cirrhosis should be avoided because of the potential risk of life-threatening infection.

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