Phase 1 Clinical Study of Pegylated Liposomal Doxorubicin (JNS002) in Japanese Patients with Solid Tumors

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Received May 4, 2006; accepted August 5, 2006; published online November 9, 2006

Background: Pegylated liposomal doxorubicin (PLD, JNS002) is a formulation of doxorubicin encapsulated polyethylene-glycol coated liposomes with prolonged circulation time and unique toxicity profile. This phase 1 study aimed at investigating the maximum tolerated dose (MTD), recommended dose, toxicity, pharmacokinetics, and antitumor activity in Japanese patients with solid tumors.

Methods: Patients with solid tumors not amenable to standard forms of treatment were eligible. PLD was administered as an intravenous infusion every 4 weeks. Dose escalation of PLD was planned from 30 to 60 mg/m² in 10 mg/m² increments. The pharmacokinetics of total doxorubicin (encapsulated plus non-encapsulated) in plasma were examined for the first cycle of treatment.

Results: Fifteen patients, aged 49–69 (median; 56) years with advanced solid tumors were enrolled. The major non-hematological toxicities were hand–foot syndrome (HFS), rash and stomatitis. Myelosuppression, especially leukopenia and neutropenia were major hematological toxicities. Although HFS was not severe, a delay of doses for subsequent cycles was required with multiple dosing. The peak plasma concentration and the area under the concentration time curve of PLD increased proportionally to the dose. Objective response was observed in one patient and the normalization of tumor marker values in another. These two patients had been diagnosed with ovarian cancer.

Conclusion: The recommended dose for phase 2 clinical studies of PLD in Japanese patients was 50 mg/m² every 4 weeks. The encouraging results prompted us to plan a subsequent clinical study of PLD against ovarian cancer.

Key words: Phase 1 study – drug delivery system – Pegylated liposomal doxorubicin – JNS002

INTRODUCTION

Pegylated liposomal Doxorubicin (PLD) is a formulation of doxorubicin hydrochloride encapsulated in long circulating STEALTH® liposomes and formulated for intravenous administration. PLD was designed to enhance the efficacy and reduce the toxicities of doxorubicin such as myelosuppression, alopecia and cardiotoxicity by altering the plasma pharmacokinetics and tissue distribution of the drug. This pegylated-liposome system can evade non-specific capture by the reticuloendothelial system because the outer shell of the liposome is covered with a hydrophilic PEG. This character is the basis of the so-called 'stealth effect' (1). The diameter of the liposome is small (100 nm) but is still large enough to avoid renal secretion. Meanwhile, in the solid tumor tissues, it was found that solid tumors generally possess the pathophysiological characteristics: hypervasculature, secretion of vascular permeability factors stimulating extravasation of macromolecules within the cancer and absence of effective lymphatic drainage from tumors that impedes the efficient clearance of macromolecules accumulated in solid tumor tissues. These characteristics of solid tumors are the basis of the enhanced permeability and retention effect, the EPR effect (2,3). Taking these data together, conventional low-molecular-weight anticancer agents disappear before reaching the tumor tissues and exerting their cell-killing effect. However, macromolecules and nanoparticles including liposomal carrier should have time to reach, exit from tumor capillaries and stay for a long time in tumor tissue, by means of the EPR effect (2–5). Following intravenous injection of PLD into tumor-bearing mice,
doxorubicin levels measured in tumors are substantially higher than those seen in animals receiving comparable doses of non-encapsulated drug (6). It appears that PLD accumulates preferentially in tumor tissues with increased microvascular permeability, such as in the case of most tumors with active neoangiogenesis (7,8). At these tumor sites, the accumulating liposomes gradually break down releasing doxorubicin to the surrounding tumor cells (9,10). Antitumor efficacy of PLD has been evaluated in a variety of murine tumor models and human xenograft tumor models. In addition, it was also known to be effective against spontaneously arising malignancies in dogs (11).

Based on the previous clinical data, PLD is an active agent available for the treatment of AIDS-related Kaposi’s sarcoma (12,13) and has shown significant activity against some solid tumors, including ovarian and breast cancer, in phase 1 and 2 studies (14–16). Phase 1 study in the USA and Israel of PLD in patients with solid tumor pointed at a major change in the toxicity profile of doxorubicin, characterized by dominant and dose-limiting mucocutaneous toxicities in the form of palmar–plantar erythrodysesthesia (PPE, known also as hand–foot syndrome, HFS) (17) and stomatitis, mild myelosuppression, minimal alopecia and no apparent cardiac toxicity (14). With the aim of establishing an effective treatment against malignant solid tumors using this promising new formulation of doxorubicin hydrochloride, we initiated a clinical study of PLD in Japan. The objectives of this phase 1 study were (i) to determine the maximum tolerated dose (MTD) and recommended dose of PLD, (ii) to identify the toxicity profile, (iii) to assess its pharmacokinetic (PK) profile, and (iv) to observe any antitumor activities.

PATIENTS AND METHODS

Patients

Patients with malignant solid tumors were eligible if they met the following criteria: (i) histologic or cytologic confirmation of malignant solid tumor; (ii) tumors resistant to standard therapies or for which there was no effective treatment; (iii) ≥20 years and ≤74 years of age; (iv) Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2; (v) life expectancy of at least 3 months; (vi) no chemotherapy, hormonal therapy, radiation therapy, or surgery within 4 weeks prior to the registration (in case of nitrosoureas or mitomycin for previous treatment: 6 weeks); (vii) adequate bone marrow activity (white blood cell count ≥4000/μl and ≤12 000/μl, absolute neutrophil count ≥2000/μl, platelet count ≥100 000/μl, and hemoglobin level ≥9.0 g/dl), adequate hepatic function (serum total bilirubin [Tbil] level ≤1.5 times the normal upper limit, transaminase ≤2.5 times the normal upper limit), adequate renal function (serum creatinine [Cr] level ≤1.5 times the normal upper limit), and adequate cardiac function (left ventricular ejection fraction [LVEF] ≥55%) by echocardiography; (viii) no severe complications such as uncontrollable infections, heart disease, diabetes and psychogenic disorders; (ix) written informed consent given. Patients with any one of the following conditions were excluded from the study: pregnancy or lactation; symptomatic brain metastasis; doxorubicin dose given prior to study ≥300 mg/m²; a history of hypersensitivity reactions to doxorubicin or ingredients of PLD; hepatic B or C virus or human immunodeficiency virus infection; prior extensive radiation therapy (>30% of bone marrow reserves), and others.

The protocol was approved by the institutional review board of the National Cancer Center and the study was performed in keeping the good clinical practice (GCP) regulations. The study was closed for accrual in March 2004.

DRUG ADMINISTRATION

PLD was supplied by Janssen Pharmaceutical K. K. (Tokyo, Japan) as a dispersion including 50 mg of doxorubicin hydrochloride in STEALTH® liposome per vial (2 mg/ml). An amount prescribed less than 90 mg was diluted in 250 ml of 5% glucose solution and that of 90 mg or more was diluted in 500 ml of 5% glucose solution prior to administration. Diluted PLD was infused intravenously at a rate of 1.0 mg/min from the start to the end of infusion to minimize the risk of infusion reactions.

Patients were administered PLD on day 1 of each 28-day cycle and they received two or more cycles in principle. All patients were admitted for the first cycle of treatment to be monitored carefully, giving consideration to unexpected adverse events. Subsequent cycles were performed in the outpatient setting. Although no standard premedication was given, infusion reaction, nausea and vomiting were treated as needed.

STUDY DESIGN

Based on the results of previously reported clinical study (14), the starting dose of PLD was 30 mg/m² (Level 1) and dose escalation in 10 mg/m² increments was planned up to 60 mg/m² (Level 4). At each dose level, three patients were scheduled for entry. Three additional patients were scheduled for treatment at the same dose level if any of the predefined dose limiting toxicities (DLTs) was observed in one of the initial three patients. The MTD was defined as the dose level at which any of the DLTs was observed in two or more of three to six patients. Intrapatient dose escalation was not allowed. The treatment was repeated every 4 weeks, unless patients developed progressive disease or DLTs. In this study the DLTs were defined as follows: (i) grade 3 or more non-hematological toxicity except for nausea/vomiting, anorexia and general malaise; (ii) grade 3 or more febrile neutropenia; (iii) grade 4 hematological toxicity except grade 4 neutropenia not lasting for 5 days, according to the Japanese version of NCI-Common Toxicity Criteria prepared by the Japan Clinical Oncology Group (JCOG). As multiple dosing is required for PLD to show the optimal antitumor effect, the
recommended dose was determined after an overall review of the results obtained for the following: status of manifestation of DLT in cycle 1; status of manifestation and disappearance of toxicity in cycle 2 and subsequent cycles; frequency and nature of treatment delay/discontinuation; pharmacokinetics; and antitumor effect. Tumor responses were evaluated according to RECIST (response evaluation criteria in solid tumors) criteria.

PHARMACOKINETICS

Pharmacokinetic (PK) evaluation was performed in all patients during the initial cycle of treatment, and in patients who could be administered repeatedly during the second cycle of treatment. Venous blood samples (5 ml, anticoagulant: EDTA) were taken before dosing, at the end of infusion and 1, 4, 8, 24, 34, 48, 96, 168 and 240 h after completion of infusion, and then before dosing, at the end of infusion in the second cycle. Blood samples were immediately placed in ice water and centrifuged at 4°C, 1000 × g for 10 min, and plasma was aliquoted and stored at −20°C or below in polyethylene tubes until analysis.

The concentrations of total (encapsulated plus non-encapsulated) doxorubicin and its major metabolite doxorubicinol in plasma were measured by validated reverse-phase high-performance liquid chromatography (HPLC) with fluorescence detection (excitation wavelength: 480 nm and emission wavelength: 560 nm) which is a modification of the measurement method previously reported (18).

The PK parameters ($C_{\text{max}}$, maximum plasma concentration; $t_{1/2}$, elimination half-life; AUC, area under the concentration–time curve; $V_c$, volume of distribution; CL, total clearance) were calculated by non-compartmental analysis using WinNonlin™ (Pharsight) software.

In addition, an assessment was made of the correlation between $C_{\text{max}}$ and AUC with the dose (mg/m²) of liposomal doxorubicin administered. Moreover, the presence or absence of accumulation was verified by comparing the individual plasma concentrations of doxorubicin and doxorubicinol determined before dosing and at the end of infusion in the second cycle with the corresponding measured values in the initial cycle.

RESULTS

PATIENTS’ CHARACTERISTICS

From April 2003 to January 2004, 15 patients were entered in this study. Their characteristics are listed in Table 1. There were five men and 10 women with good performance status and the median age was 56 (range, 49–69) years. The predominant types of tumor were ovarian cancer and non-small cell lung cancer. Seven patients had received surgical resection for primary tumors, all 15 patients had received prior chemotherapy and 11 had more than three prior regimens. Two patients had received anthracycline; one at a cumulative dose of 273 mg/m² and the other at 100 mg/m². A total of 67 cycles of PLD was administered, and the median number of cycles administered per patient was three (range, 1–15). All patients were included in the toxicity evaluation.

TOXICITY

The major toxicities in the first cycle and all cycles are listed in Table 2. The principal non-hematological toxicities were skin toxicities consisting of HFS and skin rash, and stomatitis. HFS and rash as major skin toxicities occurred in 12 (80.0%) and 10 (66.7%) patients, respectively. These toxicities were generally mild (≤ grade 2, Table 2) with clinical symptoms including erythema, swelling, itching, pain and desquamation. The median time to onset of HFS and grade 2 HFS were 39 days (cycle 2) and 96 days (cycle 3.5) after treatment initiation, and the median duration of grade 2 HFS was 7 days. The median time to onset of rash and grade 2 rash were 29 days (cycle 1.5) and 64.5 days (cycle 2.5) after treatment initiation and the median duration of grade 2 rash was 5 days. These skin toxicities increased in
frequency and severity at high dose or with multiple doses of PLD. In level 1 and 3 cohorts, treatment delays owing to skin toxicities were observed in six of 29 cycles and 10 of 32 cycles, respectively. However, these skin toxicities were manageable by delay of the next infusion and commonly used dermatologic medications including vitamin B2, B6 tablets, antihistamine and steroid tablets/ointment.

Stomatitis was observed in eight patients (53.3%) and was generally mild (≤grade 2, Table 2). The median times to onset of stomatitis and grade 2 stomatitis were 15 days (cycle 1) and 17 days (cycle 1) after treatment initiation, and the duration of grade 2 stomatitis was 7 days. This toxicity tended to occur after cycle 1, but resolved relatively promptly.

The principal hematological toxicities were leukopenia and neutropenia, and there was only one patient with grade 3 leukopenia in level 3 and there were 4 patients with grade 3 neutropenia in level 2 and 3 (1 and 3 patients, respectively, Table 2). The nadir time to leukopenia and neutropenia was approximately 3 weeks after treatment initiation. Although leukopenia and neutropenia increased in severity at high dose (50 mg/m²) compared with at low dose (30 mg/m²), they were manageable with just delay of subsequent treatment. No patient developed neutropenic fever, thrombocytopenia or grade 4 hematological toxicities in any dose levels. No patient required administration of granulocyte colony-stimulating factor or blood transfusion.

The left ventricular ejection fraction (LVEF) was determined at baseline and serially by heart ultrasonography. There was one patient each with grade 1 LVEF decrease after the administration of PLD cumulative dose of 150 mg/m² (total doxorubicin dose of 250 mg/m²). No patient required treatment for cardiotoxicity.

Grade 1 or 2 infusion reactions developed in 4 patients and they appeared within 10 min after initiation of infusion. All symptoms caused by infusion reaction disappeared within 60 min without any medication, interruption of infusion or infusion rate adjustment.

Three DLTs were recognized in one patient administered 30 mg/m² of PLD with grade 3 diarrhea, grade 3 infection not accompanied by neutropenia, and grade 3 hypoxia. Diarrhea and infection were recovered and improved at the end of the observation period, respectively, while hypoxia lasted. There was no DLT at the level of 40 or 50 mg/m². There were no treatment-related deaths in this study.

**ANTITUMOR ACTIVITY**

All of 15 patients were evaluable for antitumor response. One and eight out of 15 evaluable patients had achieved

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**Table 2. Major toxicities**

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>No. of patients</th>
<th>CTC grade</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>HFS</td>
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<tr>
<td></td>
<td></td>
<td>1</td>
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<td>1st cycle</td>
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<tr>
<td>30</td>
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<td>40</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>50</td>
<td>6</td>
<td>4</td>
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</tbody>
</table>

HFS, hand–foot syndrome; Leu, leukemia; Neu, neutropenia; anemia, hemoglobin decrease.
partial response and stable disease, respectively. The patient who achieved partial response (PR) was a 53-year-old female diagnosed as ovarian cancer with three lesions in peritoneum and one instance of pelvic lymph node metastasis. The duration of response was 441 days. In the case of the other patient with ovarian cancer who was evaluated as not evaluable (NE), the elevated tumor marker CA125 (241 U/ml) prior to the study entry was normalized (11 U/ml) after the second cycle of PLD.

**PHARMACOKINETICS**

Pharmacokinetic study was performed using plasma samples obtained from all 15 patients during the initial cycle of treatment, and for 11 patients during the second cycle of treatment. Pharmacokinetic parameters are summarized in Table 4 and the mean plasma doxorubicin concentration–time profiles are illustrated in Fig. 1. Plasma doxorubicin concentrations after administration of PLD showed a monophasic decline, consistent with a one-compartment model. Total doxorubicin exhibited a long $t_{1/2}$ (range of mean values: 86.3–95.3 h), slow clearance (range of mean values: 11.0–13.1 ml/h/m$^2$), and small volume of distribution (range of mean values: 1.47–1.57 l/m$^2$) that was similar to the plasma volume.

The plasma $C_{\text{max}}$ and AUC values increased proportionally with the dose of PLD ($P < 0.0001$ respectively, Fig. 2), suggesting linear pharmacokinetics in this dose range. Moreover, PLD did not significantly accumulate in plasma when administered at intervals of 4 weeks or longer. Plasma concentrations of doxorubicinol, the major metabolite of doxorubicin, were lower than the lower limit of quantitation in most samples (data not shown).

**DISCUSSION**

We report a phase 1 study of pegylated liposomal doxorubicin (PLD) given every 4 weeks in Japanese patients with solid tumors. The major non-hematological toxicities were HFS, rash and stomatitis. Myelosuppression especially, leukopenia and neutropenia were the most common hematological toxicities. HFS is rarely seen with standard doses of conventional doxorubicin to reduce the risk of cardiotoxicity (21). Our study result and previous clinical studies suggest that PLD can be used in place of conventional doxorubicin therapy showed that the risk of cardiotoxicity with PLD was significantly lower than that with conventional doxorubicin (21). Our study result and previous clinical studies suggest that PLD can be used in place of conventional doxorubicin to reduce the risk of cardiotoxicity without reducing the efficacy of therapy.

All infusion reactions appeared within 10 min after initiation of PLD infusion at rate of 1 mg/min and all of them were generally mild. However some cases that required discontinuation of treatment were reported (21). So it is very important to monitor the patients’ condition carefully during the initial 10–15 min after start of PLD infusion. Infusion reaction was correlated with the initial PLD infusion rate—a lower infusion rate reduces the risk of infusion reaction (25).

Only one patient treated at level 1 developed DLTs and no patients developed DLT in level 2 and 3. However, the

<table>
<thead>
<tr>
<th>Dose (mg/m$^2$)</th>
<th>No. of patients</th>
<th>$C_{\text{max}}$ ($\mu$g/ml)</th>
<th>AUC ($\mu$g h/ml)</th>
<th>$t_{1/2}$ (h)</th>
<th>CL (ml/h/m$^2$)</th>
<th>Vd (l/m$^2$)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
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<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>19.312 (2.502)</td>
<td>2512.7 (783.5)</td>
<td>89.50 (24.05)</td>
<td>13.14 (4.84)</td>
<td>1.569 (0.187)</td>
</tr>
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<td>40</td>
<td>3</td>
<td>25.605 (2.866)</td>
<td>3228.0 (789.6)</td>
<td>86.30 (14.72)</td>
<td>12.99 (3.70)</td>
<td>1.568 (0.174)</td>
</tr>
<tr>
<td>50</td>
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<td>34.057 (3.293)</td>
<td>4663.3 (1061.8)</td>
<td>95.33 (25.32)</td>
<td>11.10 (2.05)</td>
<td>1.471 (0.130)</td>
</tr>
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</table>
independent data monitoring committee did not recommend further dose escalation beyond level 3. We accepted this recommendation for the following reasons. First, the repeated dosing toxicity of PLD in level 3, which was the approved dosage established in Europe and the USA, was acceptable. Second, among six patients treated at level 3, delay of therapy was required in three patients because of leukopenia in the present phase 1 study. Of these three, two patients also developed HFS leading to delay of therapy. In the level 3 cohort, HFS causing delay of therapy was observed in 10 of 32 cycles in total. Based on these findings, further dose escalation over level 3 seemed to be difficult as PLD requires multiple dosing to show antitumor activity. Third, from the results of PK analysis, PLD did not significantly accumulate in plasma when administered at intervals of 4 weeks or longer by level 3. Fourth, antitumor effect was already obtained in patients with ovarian cancer in the present study. From the above-mentioned facts, we concluded that level 3 (50 mg/m²) was the recommended dose for subsequent phase 2 study. HFS showed an aggravating trend with repeated JNS002 treatment in our study, but did not lead to a severe toxicity. However, repeated JNS002 treatment in the previous phase 1 study in USA and Israel resulted in a severe dose-limiting toxicity. Therefore, further studies should be carefully conducted in a greater number of patients paying attention to the severity of HFS.

Regarding pharmacokinetics of PLD, the profile clarified in our study is largely consistent with previous findings in overseas studies indicating that PLD has an extremely long circulation time with a slow clearance and a small volume of distribution (22,26,27). Lyass et al. provided the results of correlation analysis that dose and Cmax are strongly correlated with stomatitis and nadir leukocyte count, whereas plasma t1/2 is significantly correlated with HFS which is one of the important cause for prolongation of dosing interval leading to delay of treatment for consequent cycle (22). The half-life values in the present study (86–95 h) are comparable to those reported previously (80–84 h, Hamilton et al. (26); 62–86 h, Lyass et al. (22); 75–91 h, Hubert et al. (27)).

PLD is already approved for the treatment of AIDS-KS and ovarian cancer in Europe and the USA, and breast cancer in Europe. Also in our study of six patients with ovarian cancer, one had achieved partial response and one had achieved normalization of the tumor marker CA125. This result is very encouraging in planning for further clinical studies in Japanese patients with ovarian cancer.

In conclusion, we confirmed the tolerance of the recommended dose (50 mg/m²) in Europe and the USA, which was intravenous infusion of PLD every 4 weeks in Japanese patients, and one partial response and one normalization of CA125 were observed in patients with ovarian cancer. We concluded that the recommended dose in phase 2 clinical study was 50 mg/m² every 4 weeks. At present, a phase 2 clinical study in Japanese patients with ovarian cancer is ongoing.

Acknowledgments

This work was supported by Janssen Pharmaceutical K. K. (Tokyo, Japan). We thank Dr N. Saijo (National Cancer Center Hospital East, Chiba) for careful review of the protocol in this study. We thank Dr S. Tsukagoshi (Representative of Clinical Pharmacology Development Study Group, Tokyo), Dr N. Yamamoto (Shizuoka Cancer Center, Shizuoka), Dr M. Nishio (Cancer Institute Ariake Hospital, Tokyo) and Dr Y. Matsumura (National Cancer Center Research Institute East, Chiba) for their critical review of the clinical data as members of the independent data monitoring committee. We thank Dr Y. Ohashi (University of Tokyo, Tokyo) for his support and suggestions.
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