Dose Escalation and Pharmacokinetics of Pegylated Liposomal Doxorubicin (Doxil) in Children with Solid Tumors: A Pediatric Oncology Group Study

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ABSTRACT

Purpose: To determine the maximum tolerated dose and pharmacokinetics of Doxil in children with recurrent or refractory solid tumors. Doxil is pegylated doxorubicin.

Experimental Design: Eligible patients were children with refractory tumors who had received cumulative anthracycline doses < 300 mg/m^2. Cohorts of at least three patients each received escalating doses of Doxil starting at 40 mg/m^2 at 4-week intervals. If no dose-limiting toxicity occurred, dosages were escalated by increments of 10 mg/m^2 in subsequent cohorts. Originally, Doxil was administered over 60 min, but significant infusion reactions prompted longer infusion times of 4 h. Patients also received premedication with dexamethasone, ranitidine, and diphenhydramine 24 h before infusion, with ranitidine continued 24 h after infusion. Periodic blood samples were collected and plasma doxorubicin concentrations were quantified to characterize the pharmacokinetics of Doxil.

Results: Between January 1997 and June 2000, 22 children ages 4–21 years with refractory tumors were treated with Doxil. Most patients had received one to five prior chemotherapy regimens, and all but five had prior radiotherapy (two had no prior therapy). Doxil was escalated to a dosage of 70 mg/m^2. At that level, dose-limiting mucositis was seen during the first cycle in two of six patients, thus defining dose-limiting toxicity, and in one additional patient during a subsequent cycle. Grade 4 neutropenia lasting less than 7 days was documented in two of six patients. The dose-limiting toxicity among two of six patients at 70 mg/m^2 was grade 3 mucositis during the first cycle of therapy. Painful desquamating dermatitis of the hands and feet, palmar-plantar erythrodysesthesia, occurred in six patients. In two of those patients, palmar-plantar erythrodysesthesia started as grade 1 and progressed to grade 2 during subsequent courses. Mean estimates of central volume of distribution, clearance, and elimination half-life were 1.45 liters/m^2, 0.03 liter/h/m^2, and 36.4 h, respectively.

Conclusion: The maximum tolerated dose of Doxil administered every 4 weeks to pediatric patients was 60 mg/m^2. The effect of Doxil on pediatric patients with malignancies remains to be determined and should be studied in pediatric Phase II trials.

INTRODUCTION

Doxorubicin, an anthracycline antibiotic, has significant activity against several pediatric malignancies (1, 2), and dose intensity appears to be related to outcome (3). Therefore, this drug has become a standard component of treatment for many pediatric malignancies. Acute DLTs, especially mucositis, limit its dosage escalation, and the total cumulative dose is limited by its cardiotoxic effects. The resultant cardiomyopathy can develop years after completion of treatment (4, 5). To improve outcomes for pediatric patients with malignancies, reduce toxicity, and maximize the dose intensity of doxorubicin, new anthracycline preparations with similar antitumor activity but reduced cardiotoxicity are being developed.

The encapsulation of anticancer drugs into liposomes appears to improve their therapeutic effects (6–8). However, increased uptake of the formulations by the reticuloendothelial system limits their usefulness (7, 8). Alterations in the liposomal surface charge and hydrophilicity produce “STEALTH liposomes” with increased circulation time and a reduced uptake by the reticuloendothelial system (9). Doxil is a novel formulation of doxorubicin encapsulated in polyethylene glycol-coated liposomes. Its PK are markedly different from those of doxorubicin, including a prolonged circulation time, reduced clearance, and a smaller volume of distribution (10, 11). Preclinical studies showed enhanced accumulation of doxorubicin in murine tu-

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3 The abbreviations used are: DLT, dose-limiting toxicity; MTD, maximum tolerated dose; PPE, palmar-plantar erythrodysesthesia; PK, pharmacokinetics; Klim, elimination rate constant; Vc, central compartment volume; Vss, steady-state volume; Cmax, maximum plasma concentration; AC, cyclophosphamide with doxorubicin; MC, cyclophosphamide with liposomal encapsulated doxorubicin.
mors when delivered as liposomal preparations (6, 7, 12), likely resulting from the prolonged circulation time and perhaps the preferential deposition of drug in tumor tissue. Pegylated liposomal doxorubicin also had substantial antitumor activity (superior to free doxorubicin) in several murine tumors and human tumor xenografts (7, 13–19).

Doxil has been studied in adults with various malignancies, and preliminary results showed antitumor activity at dosages ≥40 mg/m² administered every 3–4 weeks (20). Infusion or hypersensitivity reactions might occur with the first dose. Those reactions can be ameliorated by slowing infusion time and/or including premedications such as hydrocortisone, cimetidine, and diphenhydramine. The single-dose DLT is mucositis and the most common cumulative toxicity is PPE (a painful desquamating dermatitis affecting the hands and feet). Myelosuppression is not dose-limiting (20). Patients exposed to relatively high cumulative doses, 540–840 mg/m², did not have evidence of acute congestive heart failure, which suggests that Doxil might be less cardiotoxic than doxorubicin (21).

On the basis of that information, the Pediatric Oncology Group developed a dosage escalation study of Doxil in children with refractory or recurrent solid tumors to determine the MTD and describe the PK of Doxil in children. This report describes those results.

PATIENTS AND METHODS

Patients. Twenty-two patients with recurrent or refractory solid tumors, or those for whom no standard therapy was available, were enrolled between January 1997 and June 2000. Eligibility requirements included age ≥21 years, adequate performance status ( Karnofsky scale ≥50%), and life expectancy of at least 8 weeks. Patients also must have completely recovered from effects of prior therapy, including: at least 2 weeks since prior chemotherapy; ≥1 week since growth factor administration; ≥6 weeks since substantial bone marrow irradiation; ≥6 months since craniospinal or total body irradiation, bone marrow transplant, or total abdominal, pelvic, chest, or mantle + Y port irradiation; ≥2 weeks since palliative radiation; and a cumulative anthracycline dose <300 mg/m². Other eligibility requirements included absolute neutrophil count ≥1.000/µL, platelet count ≥75,000/µL, hemoglobin ≥9 g/dl; normal creatinine for age or glomerular filtration rate ≥70 ml/min/1.73 m²; bilirubin <1.5 mg/dl; adequate hepatic function (alanine aminotransferase <5 times normal); and shortening fraction ≥27% or ejection fraction ≥50%. The protocol was approved by the institutional review board at each participating institution, and patients, parents, or guardians provided written informed consent as appropriate before participation.

Study Design. Doxil was administered at an initial dosage of 40 mg/m² i.v. over 60 min. Cohorts of at least three patients each were treated at each dosage level, with escalation by increments of 10 mg/m² in subsequent cohorts. There was no intrapatient dosage escalation, and all patients received at least two cycles of therapy (if they did not have progressive disease). Patients could receive the next cycles of therapy as soon as their absolute neutrophil counts were at least 1,000/µL and their platelet counts were at least 75,000/µL. Once MTD was established, the study was opened to patients who were receiving anticonvulsant therapy to determine whether toxicity and PK were different. The starting dosage was 50 mg/m², with escalation by increments of 10 mg/m². Only three patients were enrolled and the study was closed because of lack of interest.

Infusion-related reactions were observed early in the treatment period and required a premedication scheme. After the first two patients developed grade 2–3 hypersensitivity reactions, 1 mg/kg diphenhydramine was administered 30 min before infusion. Infusion reactions continued; therefore, the premedication scheme was amended to include dexamethasone (0.15 mg/kg p.o. four times a day for 24 h before Doxil), ranitidine (3 mg/kg p.o. twice a day 24 h before Doxil and 24 h after Doxil), and diphenhydramine (1 mg/kg p.o. four times a day for 24 h before Doxil). The Doxil infusion time was also lengthened from 1 h to 4 h and administered at 5 mg/m²/h for the first 10 min, then increased to 20 mg/m²/h over 230 min (total infusion of 4 h).

Toxicity was evaluated using version 1 of the National Cancer Institute Common Toxicity Criteria (22). Patients had at least weekly complete blood counts, but when neutropenia developed, most had blood counts at least twice weekly. Patients also had comprehensive skin examinations once a week, with liver and renal function studies every 4 weeks. The protocol also required that patients have serial echocardiograms performed before each cycle of Doxil. The MTD was defined as the dose level below that at which two patients among three to six at a given dosage level experienced DLT during the first treatment cycle. The DLT in this study was defined as one or more of the following: ≥grade 3 renal, cardiovascular, pulmonary or central nervous system toxicity; any grade 4 nonmyelotoxicity; grade 4 myelotoxicity longer than 1 week; and ≥grade 3 PPE or stomatitis persisting 2 weeks beyond the next scheduled dose.

Pharmacokinetic Studies. Patients had blood samples drawn during their first cycles of therapy to determine the PK of Doxil. Blood samples (5 ml) were collected to measure total doxorubicin concentration before infusion, at the end of infusion, and at 1, 2, 4, 24, and 48 h after infusion. Samples were collected in EDTA-containing tubes and centrifuged immediately at 1000 g for 10 min. The plasma fraction was collected and frozen at or below −70°C until analysis.

Plasma samples were analyzed for total doxorubicin levels using reverse-phase high-performance liquid chromatography with fluorescence detection (PHARMout Laboratories, Sunnyvale, CA). A 1.0-ml aliquot of plasma was used for analysis by addition of 50 µl of 50:50 methanol:water and 100 µl of internal standard (2.5 µg/ml daunomycin), followed by the addition of 200 µl of 1% H₃PO₄ and 7.0 ml of isopropyl alcohol; this processing step caused destruction of the intact liposomes. Sample tubes were capped and shaken for 10 min at 1 rps on a horizontal shaker. The resulting mixture was centrifuged for 7 min at 3000 rpm. The supernatant solution was decanted and evaporated to dryness under N₂ at 40°C. The concentrated samples were reconstituted in 500 µl mobile phase [0.055 m ammonia acetate (pH 4.0):acetonitrile (73:27) and 5 ml/liter triethylamine], vortexed for 1 min, and centrifuged at 3000 rpm for 7 min. Portions of supernatant (50 µl) were injected on the high-performance liquid chromatography system equipped with a fluorescence detector. Separation was on a Phenomenex IB-Sil CN 150 mm × 4.6 mm column eluted with a water:acetonitrile mixture (60:40) at 1.0 ml/min. The analytes were detected by
fluorescence at 233-nm excitation and 550-nm emission wavelengths. The linear range of the assay for doxorubicin was established between 0.005 and 1.00 μg/ml, with a lower limit of quantitation of 0.010 μg/ml. The within-day coefficient of variation of the assay ranged from 5.2% at a concentration of 0.015 μg/ml to 3.6% at 0.30 μg/ml. The overall deviation (bias) from the nominal values was less than 6.0%. Doxorubicin and the internal standard (daunorubicin) were well separated, and there was no interference from concomitant medications, endogenous compounds, or any components of the pegylated liposomes.

**Pharmacokinetic Analysis.** NONMEM (23) version V (University of California, San Francisco, CA), a nonlinear regression program, and mixed-effect modeling were used to analyze the data. The parameters of one- and two-compartment, linear and nonlinear models were fitted to the data. The final model was selected by comparing the objective function, bias, and precision of the models. (Only the results from the final one-compartment linear model are presented in this paper.) The PK parameters that were estimated included rate constants ($K_{10}$, $K_{12}$, and $K_{21}$), volumes ($V_1$ and $V_{ss}$), maximum rate ($V_{max}$; nonlinear model), and the Michaelis-Menten constant ($K$; nonlinear model). Intraindividual variability was calculated using a constant coefficient of variation model; interindividual variability was estimated using a log-normal distribution model. The doxorubicin $C_{max}$ values were obtained by direct observation of the data.

**Statistical Analysis.** At each dose level, at least three patients were enrolled and the study was temporarily closed until complete toxicity data were available. If two of three patients developed DLT, the MTD was considered exceeded and three additional patients were accrued at the previous dosage level. If no patient developed DLT, escalation proceeded to the next dosage level. If one patient developed DLT, three more assessable patients were accrued at that dosage level, and dosage would be escalated only if none of those patients developed DLT. If one or more of those patients had DLT, the MTD was considered exceeded and three patients were accrued at the previous dosage.

**RESULTS**

Twenty-two treated patients with refractory or recurrent solid tumors, or for whom no standard therapy existed, were enrolled (Table 1). The median age of patients was 9.3 years (range 4–21 years). They received a median of one cycle of Doxil (range 1–6); 13 patients received one cycle, 5 patients received two, 3 patients received three, and 1 patient received six cycles. Three patients were receiving concomitant anticancer drugs and were enrolled and treated at 50 mg/m² after the MTD was reached.

**Pharmacokinetics.** Data from 10 patients (57 total observations) were included in the pharmacokinetic analyses. Unfortunately, although blood samples were obtained in three patients receiving anticancer therapy, as a result of a clerical error, they were unavailable for evaluation. The median number of plasma samples collected per patient was six (range five to seven). $C_{max}$ concentrations ranged between 36.4 and 48.2 μg/liter at different dosage levels (Table 2).

The plasma doxorubicin concentration-time data were best described by a one-compartment linear model. The population PK parameters are displayed in Table 3. $K_{10}$ was 0.019 h⁻¹ with an interindividual variability of 29%. $V_1$ correlated with body surface area of the patients and was 1.42 liters/m² with an interindividual variability of 20%. Intraindividual variability was estimated at 13%. The post hoc Bayesian PK parameters are displayed in Table 4. The clearance of Doxil ranged from 0.02 to 0.05 liter/h/m² (mean of 0.03 liter/h/m²), and the central volume of distribution ranged between 1.02 and 2.01 liter/m² (mean of 1.45 liter/m²). Elimination half-life was between 21.5 and 55.2 h, with a mean of 36.4 h. Fig. 1 displays the individual model-predicted plasma doxorubicin concentrations compared with observed concentrations and shows the goodness of fit of the PK model.

**Toxicity.** Hypersensitivity infusion-related reactions complicated Doxil infusions and required premedication with diphenhydramine, hydrocortisone, and ranitidine. The final premedication scheme included dexamethasone (0.15 mg/kg p.o. four times a day for 24 h before Doxil), ranitidine (3 mg/kg p.o. four times a day for 24 h before Doxil), and diphenhydramine and hydrocortisone (0.15 mg/kg p.o. four times a day for 24 h before Doxil).
received Doxil at a starting dose of 50 mg/m². One of them was treated for 4 weeks. At the third dosage level (60 mg/m²), five of five assessable patients had minimal hematological toxicity with short-lived grade 3 neutropenia. At dosage level 1, two patients had minimal hematological toxicity only at weekly intervals. Among those patients, one developed severe neutropenia. Thus, four patients were not assessable for hematological toxicity. There was one patient in each of the first two dosage levels that had minimal hematological toxicity (one had no blood counts and the second received granulocyte colony-stimulating factor), whereas three other patients (all at dosage level 3) had evaluations for hematological toxicity only at weekly intervals. Among those patients, one developed severe neutropenia. Thus, four patients were not assessable for hematological toxicity. There was one patient in each of the first two dosage levels that had minimal hematological toxicity with short-lived grade 3 neutropenia. At the third dosage level (60 mg/m²), five of five assessable patients had grade 3 to 4 neutropenia during the first cycle (one grade 4 that lasted 10 days), and four had grade 3 thrombocytopenia. One of those patients had prolonged grade 4 neutropenia during subsequent cycles. At the fourth dosage level, three of six patients developed grade 3 to 4 neutropenia (two grade 4, <7 days) and two developed transient grade 4 thrombocytopenia.

Table 5 displays nonhematological toxicities during the study. Those included infusion reactions ranging from grades 1 to 3, which were ameliorated by the described premedication scheme. Serial echocardiograms were performed on five patients receiving Doxil. Two of these patients received three cycles of Doxil and one patient received six cycles. None of the five patients had any changes evident on serial echocardiograms, including the patient who received six courses of the drug. Other nonhematological toxicities included mucositis, PPE, and other skin reactions. At the first dose level, there was no evidence of significant (grade 3–4) nonhematological noninfusion-related toxicities. At dosage level 2, one patient had PPE (grades 1 and 2 in successive cycles). At dosage level 3, one of seven patients had grade 3 mucositis, whereas at dosage level 4, three of six patients had grade 3 mucositis (two with the first cycle). Interestingly, at this dosage level one patient received six cycles of Doxil and developed worsening mucositis with subsequent cycles despite dosage adjustments. During the last two cycles, the interval between cycles was increased to approximately 6 weeks. Thus, the DLT was grade 3 mucositis in three of six patients (one after cycle 2) at 70 mg/m², which caused significant delays. Therefore, MTD was 60 mg/m² every 4 weeks.

Three patients receiving concomitant anticonvulsants received Doxil at a starting dose of 50 mg/m². One of them was unassessable for toxicity because he received concurrent radiotherapy. One of the other two developed grade 3 mucositis and both had transient grade 3–4 neutropenia and grade 1 PPE.

**Responses.** Response was evaluated using standard criteria. Three of 21 assessable patients had stable disease as best response, whereas the other 18 patients developed progressive disease. One of the three with stable disease, a child with a nonrenal rhabdoid tumor, received six cycles of Doxil.

**DISCUSSION**

Doxorubicin has become a standard component of therapy for many pediatric malignancies. However, mucositis and potential cardiotoxicity that can occur years after treatment limit dosage escalation and cumulative dose (4, 5). To improve outcomes of patients with pediatric malignancies, reduce toxicity, and maximize the dose intensity of doxorubicin, new anthracycline preparations with similar antitumor activity but reduced cardiotoxicity are desirable.

This dose escalation study evaluated the use of doxorubicin encapsulated in polyethylene glycol-coated liposomes (Doxil) in pediatric patients with recurrent or refractory malignancies. Doxil was escalated to a dose of 70 mg/m², at which point two of six patients developed grade 3 mucositis requiring dosage adjustments (during the first cycle of therapy). Thus, the MTD for this study was not different from the MTD for adult patients (20), although in two subsequent phase II trials in adults, Doxil was given at a dosage of 50 mg/m² every 4 weeks, which appears to be the recommended phase II dosing (24, 25). The DLT in this trial was similar to that in previous adult trials, primarily consisting of mucositis (20). Although PPE occurred in approximately 20% of the children enrolled in this study, unlike the adult experience (20), it was not severe.

Although this medication was generally tolerated, we were disappointed to see no objective responses in this group. This might not be surprising because 60–70% of patients had received doxorubicin previously. However, it suggests cross-resistance between doxorubicin and Doxil and potentially limits use of this medication in children who have been treated with doxorubicin. Similar results were seen in two adult studies for
patients with soft tissue sarcomas, in which investigators reported no objective responses in 31 patients treated previously with doxorubicin (24, 25).

A one-compartment linear model best described the plasma concentration-time profile of Doxil in pediatric patients. The low clearance (mean 0.03 liter/h/m²) and long half-life (mean 36.4 h) of Doxil are typical of drugs encapsulated in pegylated STEALTH liposomes as reported in clinical and preclinical studies (10, 11, 26). Doxorubicin PK in pediatric patients have been characterized by only a few researchers (27–29). The steady-state volume of distribution for conventional doxorubicin in children was 632.5 liters/m² (27), which is approximately 436 times larger than for Doxil (mean 1.45 liters/m²). The small volume of distribution estimated for Doxil suggests that it is restricted largely to the plasma-intravascular space. Clearance of conventional doxorubicin in children was approximately 87 liters/h/m² (27), much faster than that of Doxil (mean of 0.03 liter/h/m²).

The range of Cmax obtained in children in the present study (36.4–48.2 mg/liter) corresponds well with the range in adults after similar dosage levels (40–60 mg/m²) of Doxil (28.9–31.8 mg/liter). This is probably caused by similarity in estimates of central volumes of distribution for Doxil in children (mean 1.45 liters/m²; present study) and adults (mean 1.93 liters/m²). The estimated elimination half-life of Doxil in adults (mean 73.9 h) is approximately twice the estimate in children (mean 36.4 h; present study).

In a recent trial, women with metastatic breast cancer were randomized to receive AC versus MC (30). The authors reported similar activity (43% objective responses in both arms), less grade 4 neutropenia (61% MC versus 75% AC; log-rank, P = 0.017), and markedly decreased cardiotoxicity. Only 6% of patients treated with MC developed cardiotoxicity compared with 21% of controls (AC; log-rank, P = 0.0001). The median time of onset of cardiotoxicity was also different: 22 months for MC versus 10 months for AC (log-rank, P = 0.0003) (30). If this also proves to be the case for pediatric patients, Doxil might be an attractive alternative to doxorubicin. Although other drugs such as dextroxazone have prevented cardiotoxicity in adult and pediatric trials (31–33), that particular agent is associated with significant myelotoxicity, which potentially compromises the delivered dose intensity. Therefore, if liposomally encapsulated doxorubicin is less cardiotoxic than the parent compound, it is a potentially attractive alternative, especially for pediatric patients whose diseases are curable and who have prolonged life spans.

In conclusion, Doxil was tolerated in pediatric patients. Its DLT was mucositis, which occurred at a dose of 70 mg/m². The recommended dose for future phase II trials is 60 mg/m² every 4 weeks. This dose is higher than the dose administered in Phase II trials for adults with soft tissue sarcomas. If Doxil proves to be as efficacious as native doxorubicin with reduced cardiotoxicity in pediatric patients, the liposome-encapsulated drug might be a valuable substitute treatment for the many pediatric cancers that are currently treated with doxorubicin. Doxil might be investigated in Phase II trials to further evaluate its cardiotoxicity, using serial echocardiograms. However, because dextroxazone appears to provide protection from doxorubicin cardiotoxicity without an impact on antitumor activity, the role of Doxil in pediatric malignancies remains to be determined.

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