Phase I Clinical Trial of Orally Administered 4-Demethoxydaunorubicin (Idarubicin) with Pharmacokinetic and in Vitro Drug Sensitivity Testing in Children with Refractory Leukemia

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ABSTRACT

Fifteen children with acute leukemia in relapse, refractory to conventional therapy, were treated with idarubicin administered orally for 3 consecutive days in dosages ranging from 30 to 50 mg/m² per day at 19- to 21-day intervals. Gastrointestinal complications, including nausea, vomiting, abdominal pain, diarrhea and stomatitis, were the major forms of dose-limiting toxicity, affecting the majority of patients at all levels of idarubicin dosage. Two patients who had received total-body irradiation for bone marrow transplantation developed life-threatening gastrointestinal toxicity suggestive of a radiation "recall" phenomenon. Echocardiographic evidence of depressed cardiac function, without clinical symptoms or signs, was noted in six of 11 patients, although the changes were judged to be significant in only one child. The maximal tolerated oral dose of idarubicin was 40 mg/m² per day. The medium terminal plasma half-life of idarubicin was 9.2 h (range, 6.4–25.5 h). Both idarubicin and its metabolite, idarubicinol, accumulated during the 3 days of therapy. Among the five patients with acute nonlymphoblastic leukemia whose cells were tested for drug sensitivity in vitro, the idarubicin concentration resulting in 50% inhibition (IC50) of cluster and colony formation ranged from 1.6 x 10⁻¹⁸ M to 5 x 10⁻¹⁷ M. There was no obvious relationship between the IC50 for idarubicin and that for epirubicin or daunorubicin. Oral idarubicin produced definite antileukemic effects, clearing blast cells from the circulation in 13 of the 14 evaluable patients. Future studies should define an optimal dose schedule to circumvent the limiting gastrointestinal complications associated with this agent.

INTRODUCTION

Idarubicin (4-demethoxydaunorubicin), an anthracycline analogue, was developed in an attempt to reduce the cardiotoxicity and enhance the therapeutic efficacy of the parent compound (1). It is 27 to 100 times more potent than daunorubicin in inhibiting the cloning efficiency of exponentially growing HeLa cells (2). Against the L1210 and P388 leukemia cell lines, it is more cytotoxic than either daunorubicin or doxorubicin (1–4). Unlike the parent compound which must be administered i.v., idarubicin can be given orally (5). In contrast to daunorubicin, it is retained selectively in tumor tissue relative to myocardium (6), and initial data suggest that it has a better therapeutic index with respect to cardiotoxicity (7, 8).

Given i.v., idarubicin has been shown to be an effective antileukemic agent in children and adults (9–15). Phase I and II studies of oral idarubicin in adults with solid tumors revealed that the degree of myelosuppression is comparable to or less than that of analogues or i.v. idarubicin (9, 16). However, nausea and vomiting were generally more severe in patients receiving oral idarubicin (16). As a single agent in adults, oral idarubicin has antileukemic activity at dosages of 45 to 90 mg/m² distributed over 3 consecutive days (9, 17, 18). Very little information is available for oral idarubicin treatment in children with leukemia. We report here the results of Phase I clinical, pharmacokinetic, and drug-sensitivity studies of oral idarubicin in 15 children with refractory acute leukemia.

MATERIALS AND METHODS

Fifteen children with refractory acute leukemia in hematological relapse were enrolled in the study after written informed consent had been obtained. These 10 boys and five girls ranged in age from 5 to 17 years (median, 9 years). Nine patients had ALL; three were in first relapse having failed remission reinduction attempts that included daunorubicin, two each in second or third relapse, and one each in fourth or fifth relapse. Six patients had ANLL including four with de novo refractory ANLL, one with lineage shift (19) at relapse of ALL and one with secondary leukemia after therapy for neuroblastoma. Of these six patients, four were in first relapse refractory to multiple reinduction attempts and two were in third relapse. A patient with B-cell ALL (positivity for surface immunoglobulins) had received five antileukemic drugs, and each of the remaining patients had been given 10 to 14 antileukemic agents. Two patients additionally received 12 Gy total-body irradiation for bone marrow transplantation 16 and 21 months before the study. All patients had received either doxorubicin or daunorubicin in total cumulative doses of 75–500 mg/m² (median, 150 mg/m²). They had recovered from the toxic effects of prior therapy, had adequate hepatic function (bilirubin ≤2 mg/dl; normal prothrombin time) and had normal kidney function (creatinine ≤1.5 mg/dl) as well as normal cardiac function as judged from results of electrocardiography, echocardiography, and isotope angiocardiography. M-mode echocardiograms were recorded with an Ekoline EK-05500D ultrasoundoscope interfaced to a strip-chart recorder (paper speed, 50 mm/s). The structures were viewed from the standard parasternal windows, using routine clinical measurements. The shortening fraction of the left ventricle was calculated as: (end-diastolic dimension — end-systolic dimension)/end-diastolic dimension.

Idarubicin hydrochloride was provided by Adria Laboratories (Columbus, OH). It was administered orally for three consecutive mornings after a minimum 8-h fast. Patients took nothing by mouth for at least 2 h before each dose. To reduce the potential problem of medication loss from vomiting, we administered chlorpromazine, 25 mg/m², orally 1 h before the start of each dose and repeated the dose every 4 h if needed. Patients who tolerated a first course without unacceptable toxicity or progressive leukemia were eligible to receive a second course 16 to 18 days later. The starting dosage was 30 mg/m² per day, and dosages were escalated in subsequent patients to 40 and 50 mg/m² per day. No dosage escalation in the same patient was attempted. Toxicity and
response were assessed by a scalar grading system adopted by the Pediatric Oncology Group (20).

Pharmacokinetic Studies. Pharmacokinetic studies were performed for each patient during the first course of oral idarubicin. Heparinized blood samples, 2 to 3 ml each, were collected before the treatment and at 1, 2, 4, 6, 8, 12, and 24 h after the first dose and 24 h after the second and third doses. Each blood sample was centrifuged immediately, after which the plasma was separated and stored at -70°C until assayed.

Plasma concentrations of idarubicin and its metabolite, idarubicinol, were measured by reversed-phase high-performance liquid chromatography with fluorescence detection. Briefly, the system included a Nova-Pak Phenyl column (15 cm x 3.9 mm; particle size, 4 μm; Waters Associates, Milford, MA), a Spectroflow 980 fluorescence detector (ABO Analytical, Ramsey, NJ) with the excitation wavelength set at 250 nm and a 550-nm emission filter. A loop-column extraction procedure was used (21). The mobile phase for isocratic elution consisted of 0.2 M sodium phosphate monobasic and acetonitrile (73:27, vol:vol), pH 4.0, at a flow rate of 1.4 ml/min. Idarubicin and idarubicinol were quantitated with use of an external standard. The lower limit of sensitivity of this assay was 0.5 ng/ml. The coefficient of variation was 12.3% at 0.5 ng/ml and 3.4% at 25 ng/ml.

The AUC was calculated by use of the linear trapezoidal method. Terminal half-life was estimated for each course of chemotherapy from plasma concentrations measured at 12 and 24 h.

Clonogenic Assays for Drug-Sensitivity Testing. Leukemic blast cells from all six patients with ANLL were studied for growth properties and sensitivity to anthracyclines in clonogenic assays. Low-density marrow cells (<1.077 g/cc) from each patient were cultured in 0.3% agar in McCoy's medium with 20% fetal calf serum over leukocyte feeder layers, either without drug addition or with increasing concentrations of idarubicin, epirubicin, or daunorubicin. Cultures were incubated at 37°C in a 5% CO₂ atmosphere; clusters (3–19 or 20–50 cells) and colonies (>50 cells) were scored on Days 7, 10, and 14 as previously reported (22, 23). Cell growth was recorded on Day 7 except for one patient, for whom sufficient cell growth was noted only on Day 14. In control cultures, cluster and colony formation per 2 x 10⁵ plated cells ranged from 40 to 1760 (median, 250).

RESULTS

Twenty-one courses of oral idarubicin were administered to the 15 patients, six of whom received two courses. Gastrointestinal complications were the major forms of dose-limiting toxicity in this study; however, no Grade 3 or 4 complications were observed at the 30 mg/m² dosage level (Table 1). All but two courses of therapy were complicated by nausea and vomiting that developed within 1–14 h (median, 3.5 h). Grade 3 vomiting occurred in two of 10 courses at a drug dosage of 40 mg/m² and in two of five courses at 50 mg/m². Loss of oral idarubicin as a result of vomiting was noted in at least six courses; the drug was identified in vomitus by its characteristic orange-red color. Diarrhea, appearing on days 2–6 of treatment, was common at or above the 40 mg/m² level and persisted from 1 to 33 days (median, 2 days). Therapy (at the 40 mg/m² level) was complicated in one patient by Grade 4 diarrhea. Abdominal pain beginning on Days 1–10 was also common. If the pain developed after a week, it was uniformly associated with diarrhea. Although none of the courses at the 30 mg/m²-dose level was complicated by mucositis, half of those at levels ≥40 mg/m² resulted in mucositis, including one episode of Grade 4 toxicity. In summary, at a dosage of 40 mg/m², three patients developed Grade 3 or 4 toxicity after the first course of treatment, and none of them was given a second course of treatment. One child had Grade 4 mucositis with hematemesis and endoscopic findings of extensive esophageal, gastric and duodenal ulcers; one had Grade 3 nausea, vomiting and abdominal pain; and one had Grade 4 diarrhea and Grade 3 nausea and vomiting with gastritis proven by endoscopic examination. Interestingly, the first two of the three patients with Grade 3 or 4 gastrointestinal complications had received total-body irradiation for bone marrow transplantation. At the 50 mg/m²-dose level, two of the four children had Grade 3 nausea and vomiting.

Deterioration in cardiac function was noted in the 11 patients tested by serial echocardiogram (P = 0.04 by paired t test). Six of the patients had 12 to 30% decreases in shortening fraction (median, 15%) of the baseline value obtained before idarubicin treatment. However, only one child had a decrease to below normal values (29 → 20% in shortening fraction by echocardiography and 54 → 47% in left ventricular ejection fraction by radionuclide cineangiography) after receiving 120 mg/m² of idarubicin. This child had congenital heart disease (endocardial cushion defect) and had previously received daunorubicin and doxorubicin (total cumulative dose, 300 mg/m²).

Hepatic toxicity (Grade 2) manifested by hyperbilirubinemia (peak, 2.7 mg/dl) occurred in one case at the 50 mg/m² dose level. No renal toxicity was observed. Two of nine evaluable patients had hair loss after treatment. Hematopoietic toxicity was not evaluable since all patients had leukemia in relapse. All patients but one required hospitalization and antibiotic therapy for documented infections or fever developing after treatment. One child who did not have circulating leukemic blast cells was not evaluable for treatment response because bone marrow examination was not performed after the treatment. The remaining 14 evaluable patients had oncolytic responses characterized by clearance of circulating leukemic cells in 13. Bone marrow was hypocellular 3 weeks after treatment in seven of the 10 patients examined; however, none attained a complete remission.

Pharmacokinetic Results. A representative example of the concentration versus time course of idarubicin and its metabolite, idarubicinol, in a patient receiving 50 mg/m² of idarubicin is shown in Fig. 1. In all patients the plasma concentration of idarubicinol exceeded the concentration of the parent drug at all sampling times; clearance of the metabolite was slower than that of the parent drug. The AUC for idarubicinol was consistently greater than that for idarubicin.

Pharmacokinetic parameters are summarized in Table 2. Calculation of the terminal plasma half-life of idarubicin from plasma concentrations at 12 and 24 h following the first dose of oral idarubicin was based on the assumption that drug

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* G, toxicity grade (see Reference 20).
absorption is completed by 12 h after drug administration. Elimination rate for the metabolite and total-body clearance for both the parent compound and the metabolite cannot be reliably estimated when patients only receive drugs orally.

Table 3 shows the median plasma concentrations of drug measured 24 h after each of the three daily doses. It is apparent that both the parent drug and the metabolite accumulated in plasma when they were given orally on a daily schedule. The AUC was calculated for 24 h after the first dose of idarubicin. The median ratio of the AUC for parent drug and metabolite was 3.8 (range, 2.2–6.1). We were unable to demonstrate an association between any of the pharmacokinetic parameters and the degree of toxicity.

Results of Drug-Sensitivity Assay. Cultures of leukemic blast cells produced sufficient growth for evaluation of cluster and colony formation in five of six patients with ANLL. The IC50 value or concentration of idarubicin inhibiting cluster and colony formation by 50% ranged from 1.6 x 10^-10 to 5 x 10^-7 M (Table 4). In three patients (nos. 1, 3, and 5), the blast cells were judged to have intermediate sensitivity to idarubicin with IC50 values of 2.7, 3.2, and 3.0 x 10^-9 M. The blast cells of Patient 2 were highly sensitive to idarubicin, as demonstrated by an IC50 value of 1.6 x 10^-10 M. The peak plasma concentration of idarubicin and idarubicinol after only one dose of the drug was above the IC50 in all four patients. At 10^-5 M idarubicin, two patients had no growth of clonogenic cells and two others had less than 3% growth compared with control cultures; the remaining child (Patient 4), in third relapse, had fewer idarubicin-sensitive blast cells. Blast cells from four patients were also studied for their sensitivity to epirubicin and daunorubicin. In a comparison of the IC50 results for the three drugs, only Patient 4 had blast cells that were less sensitive to idarubicin than to epirubicin or daunorubicin. The blast cells from the remaining three patients were more (or approximately equally) sensitive to idarubicin than to the other two agents.

DISCUSSION

In this study, we found the maximally tolerated dose of oral idarubicin in children to be 40 mg/m² per day for 3 consecutive days. Gastrointestinal complications were frequent, representing the acute dose-limiting toxicity. Of the 21 courses of idarubicin, 19 were complicated by nausea and vomiting, 14 by abdominal pain, 13 by diarrhea, and seven by mucositis. In at least six courses, the early onset of vomiting resulted in the loss of drug from patients, despite use of antiemetic therapy. Notably, the two children who had received total-body irradiation for bone marrow transplantation developed unacceptable gastrointestinal toxicities. Conceivably, the increased toxicity in them was caused by "recall" of the radiation effect, as has been reported for other anthracycline compounds (24–26).

Animal studies have indicated that idarubicin is relatively less cardiotoxic than the other anthracyclines (8, 27), especially when given orally (6). In most clinical trials, the patients have had refractory malignancies with multiple organ involvement, have received anthracyclines or other cardiotoxic agents, and have had limited survival times. Hence, it is very difficult to assess the cumulative cardiotoxic effect of idarubicin. In one study of oral idarubicin in 50 adults with advanced breast cancer who had not been treated with anthracyclines, none developed congestive heart failure, including 12 patients with cumulative doses ≥360 mg/m²; changes in cardiac function by isotope angiocardiography were minimal (28). In this study, we were unable to evaluate the chronic cardiotoxic effect of oral idarubicin. With respect to acute toxic effects, six of 11 children had a substantial decrease (≥12% reduction) in shortening fraction. Only one of them had a significant deterioration in cardiac function such that the shortening fraction was below normal, but this child had underlying heart disease and prior therapy with daunorubicin at a cumulative dose of 300 mg/m². The relative cardiotoxicity of this agent will need to be addressed in a larger prospective randomized trial.

Previous pharmacokinetic data for idarubicin in children has been limited to only seven patients who received the intravenous form of the drug (11). The terminal plasma elimination half-life of idarubicin in this study did not differ substantially from that reported after intravenous administration. However, longer half-lives have been reported for adults after either oral (mean, 34.8 h) (29) or i.v. (mean, 13.9–27 h) (11, 16, 29–33) drug administration, suggesting that clearance is faster in children.

During the 24 h after the first dose of oral idarubicin, the AUC for the metabolite idarubicinol averaged 3.8 times that of the parent compound. This ratio contrasts with the 1.9 calculated from the i.v. data provided in the study by Tan et al. (11). First-pass hepatic metabolism is a possible factor contributing to this difference. A higher metabolite:parent compound AUC ratio following oral versus i.v. administration in adults has been reported (31). This observation has clinical relevance in view of
the cytotoxic effect reported for the metabolite (34).

Carella et al. (14) reported that two of eight patients with ANLL refractory to cytarabine, amsacrine, and other anthracyclines achieved a complete remission after i.v. idarubicin treatment, suggesting a lack of cross-resistance between these drugs and idarubicin. However, in an in vitro study to determine the drug sensitivity of myeloblasts using clonogenic cells from six patients, Schözel et al. (35) found no difference in sensitivity to mitoxantrone, 4'-deoxydoxorubicin, idarubicin, and daunorubicin. Moreover, cross-resistance was demonstrated in one patient refractory to daunorubicin in that study. We found clonogenic cells to be sensitive to idarubicin in vitro at concentrations achievable in vivo. The relative sensitivity to idarubicin, epirubicin, and daunorubicin varied widely among the four cases studied. Thus, an in vitro clonogenic assay may be useful in selecting the optimal anthracycline for individual patients.

Although we were unable to induce a complete remission in any of the 15 children, oral idarubicin had definite antileukemic activity. The relative sensitivity to idarubicin, epirubicin, and daunorubicin varied widely among the four cases studied. Thus, an in vitro clonogenic assay may be useful in selecting the optimal anthracycline for individual patients.

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REFERENCES


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