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Phase I study of liposomal annamycin

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Abstract Annamycin is a highly lipophilic anthracycline with the ability to bypass the MDR-1 mechanism of cellular drug resistance. In this phase I study, annamycin entrapped in liposomes was administered by a 1- to 2-h intravenous infusion at 3-week intervals. Thirty-six patients with relapsed solid tumors were treated and 109 courses were administered at doses ranging from 3 to 240 mg/m^2. The dose-limiting toxicity was thrombocytopenia. Five patients had a probable allergic reaction, requiring discontinuation of treatment in one. Treatment was well tolerated otherwise. No cardiac toxicity was seen on endomyocardial biopsy of four patients studied. There was limited gastrointestinal toxicity and no alopecia. No objective tumor responses were observed. Pharmacokinetic studies at 24, 120 and 240 mg/m^2 showed a biexponential plasma concentration-versus-time profile. There was a linear relationship between the dose and the maximal plasma concentration with relatively constant plasma clearance values. The maximum tolerated dose (MTD) for liposomal annamycin defined in this study is 210 mg/m^2. Because of a subsequent change in the formulation of the drug, future studies will use 190 mg/m^2 as the MTD.

Key words Anthracycline · Phase I study · Pharmacokinetics

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Introduction

Anthracycline drugs are widely used in the treatment of many types of cancer. However, most responses are partial and temporary. In addition, many tumors do not respond at all, and others initially respond but acquire resistance. Acute and chronic toxicity, particularly cardiotoxicity, further limit the potential palliative effect of anthracyclines. Although acquired resistance to anthracyclines is thought to be multifactorial, attention has been focused on increased drug efflux from the intracellular space due to the overproduction of cell membrane transport proteins. The multidrug-resistance p-glycoprotein (pgp), or MDR-1, is the best-studied form of resistance. However, the clinical relevance of pgp-mediated resistance has not been precisely defined [1]. There have been some reports, nonetheless, of a possible role of pgp induction in some cases of refractory acute myelogenous leukemia and in breast carcinoma [2].

Annamycin (3′-deamino-4′-epi-3′-hydroxy-2′-iodo-4-demethoxydoxorubicin, Fig. 1) was developed as a potentially more effective anthracycline possessing a ''double-advantage'' [3]. This consists of an increase in its affinity for liposomes (to improve drug targeting to tumors and to reduce cardiac drug levels) and an ability to bypass the MDR-1 mechanism of cellular drug resistance.

In vitro studies have shown annamycin (AN) to be as cytotoxic as doxorubicin (DOX) against sensitive murine leukemia P388 cells and about 50 times more cytotoxic against P388/DOX multidrug-resistant cells. In addition, the efflux of AN was similar in both sensitive and resistant cells, suggesting the resistance was

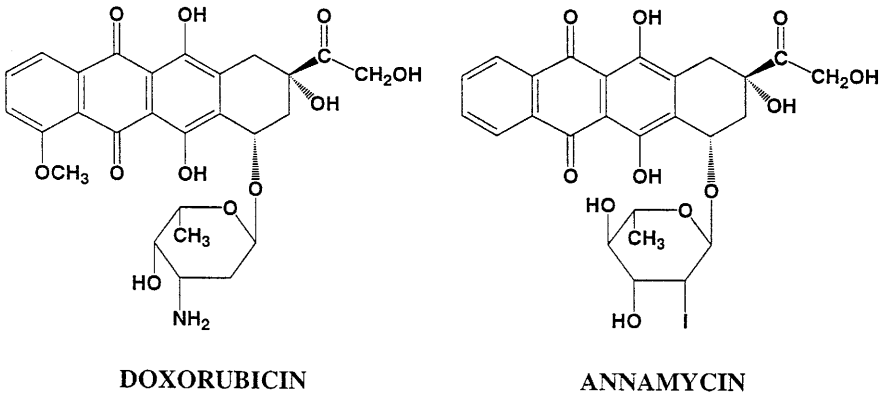

Fig. 1 Chemical structures of doxorubicin and annamycin

not mediated by pgp. AN was also more effective than DOX in inducing single-strand DNA breaks, double-strand DNA breaks, and DNA-protein cross-links in both sensitive and resistant cells [4, 5]. Further, liposomal AN (L-AN) induced three times more double- and single-strand DNA breaks than DOX in sensitive KB carcinoma cells. DOX, on the other hand, did not induce DNA damage in resistant KB-V1 cells, whereas the extent of DNA breaks induced by AN in these cells was similar to that induced by DOX in sensitive KB cells [6].

From the standpoint of the subcellular distribution, DOX localized predominantly in the nucleus of sensitive KB-3-1 cells and in the membranes of resistant KB-V1 cells. In contrast, the subcellular distribution of AN was identical in both cell lines, with preferential localization in the perinuclear region, Golgi apparatus, endoplasmic reticulum, and endosomes. The partial ability of AN to circumvent multidrug resistance was associated with a comparable inhibition of DNA synthesis in the nuclear matrix of sensitive and resistant cells [7]. Although AN was less potent than DOX or idarubicin (IDA) in sensitive HL-60 leukemia cells lines, the coincubation of these agents with verapamil in resistant lines lowered the resistance index of DOX and IDA but not of AN, providing additional evidence that AN is not affected by MDR-1 [8].

The antitumor activity of L-AN was greater than that of free AN (in suspension) and DOX in several in vivo tumor models. In particular, L-AN showed significant in vivo antitumor activity against human xenografts with high levels of pgp (KB-V1), whereas DOX was inactive. In addition, C-57BL/6 mice with advanced subcutaneous B16 melanoma tumors showed a higher organ area under the curve (AUC) of L-AN compared with that of DOX in plasma and brain (×3), liver and kidney (×2), lung (×6), spleen (×9), and tumor (×10). The heart AUC was similar for both drugs but lower than that for free AN [9]. Granulocytopenia was the main toxic effect of AN in mice and was much more profound than that associated with an equitoxic dose of DOX. In chronic mouse studies, L-AN was remarkably less cardiotoxic than DOX. It also had a less vesicant effect after intradermal administration. Further, beagle dogs tolerated the mouse-equivalent LD_{10} dose of L-AN (1.4 mg/kg) with no side effects, including hematological effects and biochemical blood parameters or pathological changes [10]. On the basis of these in vivo findings, we undertook a phase I study of L-AN in patients with limited conventional treatment options.

Materials and methods

Patient selection

Thirty-six patients were entered into this phase I study (Table 1). All had histologically confirmed solid tumors refractory to conventional treatments and were between 37 and 74 years old (median, 51 years). Informed consent was obtained prior to drug administration and pharmacological studies. All patients had a Zubrod performance status of 2 or better and a life expectancy of at least 12 weeks. Other eligibility criteria included the presence of measurable or evaluable tumors and adequate bone marrow, hepatic, and renal function as evidenced by an absolute granulocyte count of ≥1500/mm^3, platelet count of ≥100,000/mm^3, bilirubin

Table 1 Characteristics of the 36 patients in the study

Characteristic	No. of patients
Sex	
Male	13
Female	23
Median age: 51 years (range, 37–74 years)	
Zubrod performance status	
0–1	25
2	11
Histology	
Breast carcinoma	14
Lung	
NSCLC	6
SCLC	1
Colon carcinoma	5
Sarcoma	3
Melanoma	2
Other	5
Number of prior chemotherapy regimens	
1	3
2	10
3	12
>3	11

level of ≤1.5 μmol/l, and creatinine level of ≤1.5 μmol/l. Patients were required to have undergone at least one chemotherapy regimen that was completed at least 3 weeks earlier. Those with reasonable standard second-line treatment options had to have received at least two different regimens of treatment for metastatic disease to be eligible for this phase I study. Those with cancer that was potentially responsive to anthracycline drugs had to have received a standard anthracycline or anthraquinone but were not required to have anthracycline-resistant disease, as defined by a relapse during or within 6 months after anthracycline treatment. The prior anthracycline was limited to a DOX-equivalent cumulative dose of 350 mg/m^2 if given by bolus or 450 mg/m^2 if given by prolonged (at least 48 h) infusion. An ejection fraction of ≥55%, as shown by two-dimensional echocardiography, was required, and patients with a history of heart failure were excluded.

All patients had a baseline history and physical examination; full blood count with differential; a biochemical profile including CEA or CA 27–29 measurement if appropriate; prothrombin and partial thromboplastin times; urine analysis; chest X-ray; computed tomography of the abdomen; bone scan and X-ray views of bones showing abnormal uptake; electro- and echocardiography. Hematological parameters were checked twice weekly beginning on day 8, and biochemical parameters were checked before each course. Radiological studies that showed abnormal findings were repeated after three courses of therapy unless there was an earlier suspicion of increasing disease in which case they were performed sooner. In the event of response, radiological tests were to be performed 1 month later to confirm the minimum duration of response. Echocardiography was repeated every three courses and more frequently if there was a drop in the ejection fraction to a value that was still above 50%. L-AN was to be discontinued if the ejection fraction declined below 50%. All concomitant medication was documented, and no other investigational drugs were administered.

During the study, all patients were seen every 3 weeks in the outpatient clinic by the physician and research nurse to record all toxicities and to evaluate abnormalities detected on physical examination. Toxicities were graded according to the National Cancer Institute common toxicity criteria, and standard response criteria were used for assessment. L-AN was stopped if a patient's cancer increased, an unacceptable toxicity developed, or the patient requested it.

Administration

L-AN was prepared as a preliposomal lyophilized powder in vials containing 10 mg of AN, 500 mg of phospholipids, and 17 mg of Tween 20, or it was prepared in a 50-mg vial with 2500 mg of phospholipids and 85 mg of Tween 20. On the day of use, the vials were reconstituted with 10 ml or 50 ml of saline at 37 °C and hand-shaken for 1 min. This resulted in a red milky suspension formed of liposomes measuring around 150 nm in diameter. The drug was given as a 1- to 2-h intravenous infusion, usually through a peripheral vein, at 3-week intervals.

Dose-escalation scheme

The first three patients received 3 mg/m^2 L-AN intravenously over 30 min. This dose was determined on the basis of the LD-10 observed in CD-1 Swiss mice and in anticipation of a maximum tolerated dose (MTD) of about 40 mg/m^2. We also took into consideration the fact that because epirubicin (AN is an epirubicin derivative) is partially metabolized through the formation of glucuronides in humans but not in mice [11], the safe dose in humans could be underestimated.

If one dose level was associated with no toxicity in three patients, a 100% dose escalation was recommended for the next three new patients as well as for patients continuing on study treatment. A 50% escalation was recommended if a grade 2 toxicity was observed in one new patient. The last dose escalation of 33% proved to be too toxic, and so an additional intermediate dose level of 210 mg/m^2 was studied to establish the MTD. At least three patients received at least three courses at the MTD to ascertain whether there was cumulative toxicity.

Pharmacokinetic studies

Pharmacokinetic studies were performed in one patient each at 24, 120, and 240 mg/m^2. Blood samples (5 ml) were collected in heparin-coated tubes at 15 and 30 min and at 1, 2, 4, 8, 12, and 24 h after the start of the L-AN infusion. The blood samples were kept on ice before separation of the plasma by centrifugation at 4000 *g* for 10 min at 4 °C. The plasma samples were kept at −20 °C until assayed. The AN present in the plasma samples was extracted by the method previously described [12]. Briefly, 1 ml of plasma was mixed with 5 ml of chloroform/methanol (9:1 v/v) and shaken in a mechanical shaker at 100 rpm for 10 min. The mixture was then centrifuged at 4000 *g* for 10 min at 4 °C. A 3-ml aliquot of the organic layer was collected and dried at room temperature in a vacuum centrifuge. The dried plasma extracts were dissolved in 1 ml of methanol for quantitative determination by high performance liquid chromatography (HPLC). In this assay, AN elutes at 4.3 min.

The pharmacokinetic parameters were calculated by non-compartmental analysis of the plasma level-versus-time data (Winwonlin, Pharsight, N.C.).

Results

Toxicity

Thirty-six patients received a total of 109 courses of L-AN, and all were evaluable for toxicity.

Hematological effects

Hematological toxicity was not seen in the 20 patients who received 66 courses of L-AN at doses ranging from between 3 to 120 mg/m^2, except in one patient who developed grade 2 thrombocytopenia and a bilirubin level that rose as her liver metastases worsened rapidly after a single dose of 24 mg/m^2.

Sixteen new patients were given potentially myelosuppressive L-AN doses of 180 to 240 mg/m^2. Doses in three other patients were increased from 120 to 180 mg/m^2 when they showed no myelosuppression at the lower dose, but the dose had to be reduced to 150 mg/m^2 in one of these patients after neutropenic pneumonia developed. Thrombocytopenia was the dose-limiting toxicity. Grade 4 thrombocytopenia developed in two patients given 240 mg/m^2 (although 5 of 7 courses were given with minimal myelosuppression). Their platelet counts were 20 and 11×10^3/mm^3 on days 19 and 23 of course 1; and 17×10^3/mm^3 on day 11 of course 2, respectively. Platelet transfusions were not required. Six patients were studied at 210 mg/m^2 to establish this as the MTD (Table 2). Four patients had grade 2 or 3 thrombocytopenia at day 22, and one had prolonged thrombocytopenia following a cumulative dose of 780 mg/m^2. One patient was given erythropoietin for a gradually worsening anemia, and six received blood transfusions.

Table 2 Hematological toxicity

Dose mg/m^2	No. of patients		No. of courses	Toxicity by no. of courses (grade)	
	New	Total		Granulocytopenia	Thrombocytopenia
150	0	1	1	1 (3)	1 (3)
180	6	9	19	5 (3)	1[a] (3)
				3 (4)	0 (4)
210	6	6	15	6 (3)	4[b] (3)
				1 (4)	0 (4)
240	4	5	7	1 (3)	0 (3)
				2 (4)	2[b] (4)

[a] One patient had prolonged thrombocytopenia following a cumulative dose of 780 mg/m^2

[b] A total of six patients had grade 3/4 thrombocytopenia with one treatment course. Four patients still had grade 2/3 thrombocytopenia on day 22 following a dose of 210–240 mg/m^2

Three patients had febrile episodes requiring four hospitalizations, one for non-neutropenic (absolute neutrophil count > 1000 μl) pneumonia; two for neutropenic (absolute neutrophil count < 500 μl) pneumonia (dose levels, 180 and 240 mg/m^2); and one for non-neutropenic *Staphylococcus aureus* venous catheter sepsis. Neutropenia was otherwise brief and asymptomatic.

Gastrointestinal effects

Nausea and vomiting were minimal, with only seven patients experiencing vomiting and an additional five experiencing nausea.

Hypersensitivity

Five patients experienced flushing and bronchospasm shortly after the drug infusions began; three of the episodes occurring during the first course. This generally cleared in response to treatment with dexamethasone and diphenhydramine and to slowing the drug infusion. However, one patient had a grade 4 allergic reaction during the third course at a dose of 180 mg/m^2 despite premedication and was removed from the study. It is suspected the reaction may have been to the Tween 20 used in the formulation, but this was not established.

Cardiotoxicity

Heart failure developed in one patient with extensive pulmonary metastases and hypoxia from metastatic lung cancer after a cumulative L-AN dose of 60 mg/m^2 over five treatments. This patient had previously received a cumulative dose of DOX of 250 mg/m^2 administered by bolus injection. Subsequent patients were therefore required to have a cardiac biopsy at designated cumulative anthracycline doses. No changes were observed on electron micrographs in three patients at cumulative L-AN doses of 360 mg/m^2 (two patients; one had previously received a cumulative dose of DOX of 300 mg/m^2 given as bolus injections) and 780 mg/m^2. This third patient suffered intermittent atrial fibrillation thought to be related to his esophageal carcinoma and previous cardiac irradiation. A fourth patient, who had previously received a cumulative DOX dose of 300 mg/m^2 by 48-h infusion, had grade 0.5 cardiac changes [13] after a cumulative L-AN dose of 240 mg/m^2, but subsequent findings on biopsy specimens obtained at cumulative doses of 1050 and 1680 mg/m^2 were normal. No patients required discontinuation of L-AN treatment because of a decreased ejection fraction.

Other toxicity

Hair, skin, and mucosal toxicities were minimal. Scalp hair grew during L-AN treatment in patients with alopecia resulting from previous chemotherapies. Neural, renal, and hepatic tonicities were not observed. One patient asked to have treatment discontinued after two courses because of fatigue and depression. There were no toxic deaths.

Responses

No tumor regression was noted in the 20 patients treated with potentially myelosuppressive doses (180–240 mg/m^2) of L-AN. One patient had a previous supraclavicular metastasis from breast cancer that was probably overlooked at study entry but noted again after one course. Her disease was stable throughout nine courses of L-AN treatment before lung metastases increased.

Pharmacokinetics

Plasma concentration-versus-time profiles appeared to follow a biexponential pattern in patients receiving any of the dose levels of L-AN, with a rapid first phase of distribution followed by a slower phase of elimination (data not shown). Table 3 shows the pharmacokinetic properties of AN in patients receiving 24, 120, or 240 mg/m^2. As shown in Fig. 2, there was a linear relationship between the dose and the maximal plasma concentration with the levels for the 120- and 240-mg/m^2 doses, which correspond to the cytotoxic in vitro concentrations reported previously [4, 5]. There was also a linear relationship between the dose and the AUC values for the doses ranging from 24 to 240 mg/m^2, and this was reflected in the relatively constant plasma clearance values. There were no meaningful trends in the volume of distribution over the dose range examined, or in the terminal plasma half-life, which ranged from 1.1 to 2.5 h.

At the highest level (240 mg/m^2) for which pharmacokinetic data are available in this study, HPLC

Table 3 Pharmacokinetic properties of annamycin in patients. *Cmax* maximal plasma concentration, *AUC* area under the curve, *Cl* clearance, *Vss* volume distribution steady state

Property	Dose[a]		
	24 mg/m^2	120 mg/m^2	240 mg/m^2
Cmax (ng/ml)	500	1770	4000
$AUC_{0\rightarrow\infty}$ (ng · h/ml)	422	1893	5231
Cl (ml/h/m^2)	53	63	46
Vss (ml/m^2)	102	120	44
Terminal $t1/2$ (h)	2.5	1.9	1.1

[a] Measurements were carried out in one patient at each dose

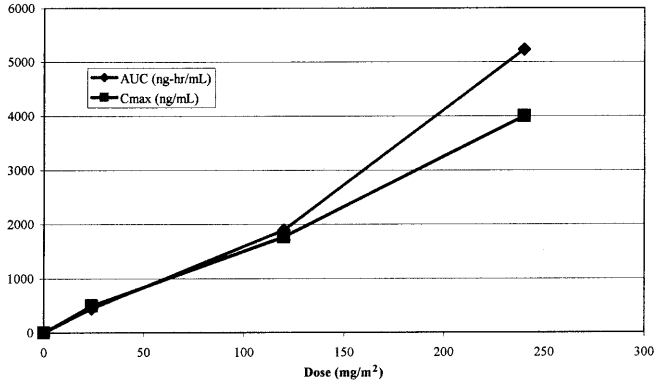

Fig. 2 Relationship between increasing dose and plasma area under the curve (AUC) and increasing dose and maximal plasma concentration (Cmax) in patients receiving annamycin intravenously as a 1–2 h infusion

studies indicated that a small amount of intact AN remained in plasma for 8 to 12 h after dosing. In addition, two metabolites were formed: one at an earlier eluting peak and one at a later eluting peak than annamycin. The later eluting peak was still present 24 h after dosing. Preliminary studies indicate that the earlier eluting metabolite may be annamycinol. Attempts to definitively identify these two metabolites are continuing.

Discussion

The MTD (determined by thrombocytopenia) of L-AN is 210 mg/m^2 in comparison with an MTD of about 90 mg/m^2 for DOX. (Because of a later change in formulation of the drug, subsequent studies will use 190 mg/m^2 as the MTD.) The four patients with thrombocytopenia at day 22 after treatment indicate the need to watch for cumulative myelotoxicity in future studies. Nausea and vomiting were minimal, mucositis was not a significant problem, and hair grew during treatment. Cardiac toxicity was not seen at cumulative doses of up to 1890 mg/m^2, even in patients who had previously received a cumulative dose of DOX of 300 mg/m^2 (with the exception of one hypoxic patient who developed heart failure after a cumulative dose of DOX of 250 mg/m^2 given by bolus injections followed by a cumulative dose of L-AN of 60 mg/m^2 given over five courses).

No antitumor activity was seen in 19 patients treated at myelosuppressive doses of at least 180 mg/m^2. This included eight patients with breast cancer whose tumors were potentially sensitive to anthracyclines as defined by a relapse occurring more than 6 months after DOX chemotherapy.

AN was cleared relatively quickly from plasma over the dosing range employed in this study although small amounts of intact drug remained for extended periods (8–12 h) following the higher doses. The parent compound undergoes metabolism, as reflected by the presence of at least two metabolites in the plasma. One metabolite appears to be annamycinol, the alcohol produced by reductive metabolism at the 13-carbon site. Although this compound has some cytotoxic activity in vitro against mdr+ tumor cells with MDR-1 (data not shown), its resistance index is higher than that of AN. Studies are planned to examine the metabolism of AN in more detail and to try to determine the role of the OH group at the 4′ position. Epirubicin, which like AN has an epi-OH at the 4′ position, is partly metabolized by glucuronidation and the plasma glucuronide is eliminated rapidly. Our preliminary results do not indicate that a glucuronide of AN at the 4′ position is present in plasma.

L-AN has antitumor activity in vitro and in animal models, including tumors overproducing pgp. No tumors were biopsied in the study to determine potential mechanisms of drug resistance or to measure drug uptake after administration. It could be important to undertake this in order to clarify the therapeutic potential of AN in patients with cancer.

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