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Phase I clinical trial and pharmacokinetic evaluation of 4'-0-tetrahydropyranyladriamycin (THP-adriamycin)*

M. N. Raber, R. A. Newman, K. Lu, S. Legha, C. Gorski, R. S. Benjamin, and I. H. Krakoff

Department of Medical Oncology, Division of Medicine, University of Texas M. D. Anderson Cancer Center, Houston, Texas, USA

Summary. Tetrahydropyranyladriamycin (THP-adriamycin) is an anthracycline analogue currently under development in Europe and Japan. Preclinical studies suggest that it may have greater activity and less cardiac toxicity than doxorubicin. We conducted a phase I clinical and pharmacologic study of THP-adriamycin given as a weekly 15-min infusion for 3 weeks, followed by 1 week of observation. Therapy was associated with minimal acute toxicity. The dose-limiting toxicity was neutropenia, usually maximal during the 4th week after treatment; alopecia was rare. The maximum tolerated dose was 25 mg/m^2; for phase II studies using this schedule, a dose of 20 mg/m^2 weekly for 3 weeks is recommended. Pharmacokinetic studies revealed a triphasic elimination of the parent compound with α, β, and γ half-lives of 5.6 min, 1.4 h, and 9.3 h, respectively. THP-adriamycin was rapidly taken up by blood cell components, with concentrations in red blood cells (RBCs), lymphocytes, and polymorphonuclear cells exceeding those in plasma. In all, $<10\%$ of the compound was eliminated in the urine within 24 h.

Introduction

Tetrahydropyranyladriamycin (THP-adriamycin) is a semisynthetic derivative of doxorubicin first synthesized by Umezawa and colleagues [14] that is part of a series of analogues selectively altered at the C-4' position (Fig. 1). Alterations at this position have resulted in several compounds with greater potency and antitumor efficacy than the parent compound. THP-adriamycin, the most promising of these compounds, exhibited considerable antitumor efficacy against the murine tumors L1210, P388 (including a line resistant to adriamycin), B16 melanoma, colon 38, and Lewis lung carcinoma [15].

In toxicity studies using hamsters and rats [3, 5], THP-adriamycin appeared to be less cardiotoxic than adriamycin. Preclinical pharmacokinetic studies of the former in mice revealed that this analogue's enhanced lipophilicity might have been responsible for a rapid uptake of drug into various tissues where drug levels were found to be much higher than those in plasma. However, in a direct comparison with adriamycin, tissue levels of THP-adriamycin in the heart were lower than those of adriamycin following a comparable dose. These data indicate an altered tissue distribution of THP-adriamycin that is relatively sparing of heart tissue [6]. Pharmacokinetic studies performed in conjunction with phase I studies in Japan and Germany confirmed this observation of higher tissue concentrations in blood cells [9, 12]. These and other preclinical pharmacokinetic and pharmacodynamic studies have recently led to the advancement of THP-adriamycin into clinical trials in Japan and Europe [11, 12]. The present report is the first description of phase I clinical and clinical pharmacologic experience with THP-adriamycin in the United States.

Materials and Methods

Patient population. A total of 23 patients with solid tumors refractory to standard therapy were entered in this phase I study (Table 1). All patients gave written informed consent in keeping with the policies of our institution. Eligibility requirements included normal renal and hepatic function as demonstrated by serum creatinine and bilirubin levels of <1.5 mg/dl and normal marrow reserve as demonstrated by $>1{,}500$ granulocytes/mm^3 and $>100{,}000$ platelets/mm^3. Normal cardiac function as measured by a nuclear cardiac scan was required in all patients. Patients with prior maximal exposure to adriamycin underwent cardiac biopsy before beginning treatment and were ineligible for the study if their biopsy result revealed abnormalities greater than grade 1 [2].

Treatment plan. THP-adriamycin was given as a weekly 15-min i.v. infusion for 3 weeks, followed by 1 week of observation. Patients were evaluated weekly during therapy and were followed semiweekly with complete blood, differential, and platelet counts and weekly with a biochemical profile (SMA 12). Patients received their therapy on a weekly schedule unless they had granulocyte counts of $<1{,}000$/mm^3 or platelet counts of $<100{,}000$/mm^3, in which case therapy was delayed for 1 week.

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Abbreviations used: THP-adriamycin, 4'0-tetrahydropyranyladriamycin; HPLC, high-pressure liquid chromatography; AUC, area under the curve; Vd, volume of distribution; PMN, polymorphonuclear neutrophil lymphocytes; RBC, red blood cell

Offprint requests to: Department of Medical Oncology, Box 92, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas 77030, USA

THP-Adriamycin

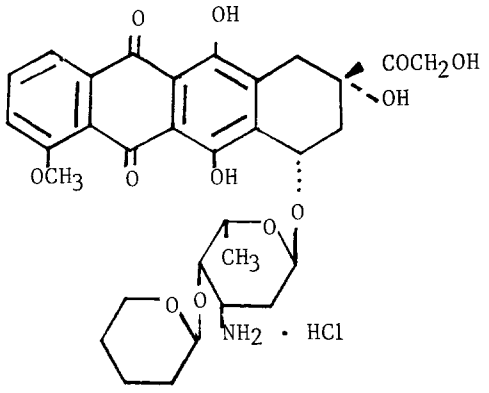

Fig. 1. Chemical structure of THP-adriamycin

Table 1. Patient characteristics

Number of patients		23
Median age (range)		55
Performance status:	0	2
	1	18
	2	3
Male : female		8:15
Prior therapy:		
none		1
chemotherapy[a]		22
immunotherapy		8
radiation		14
Tumor types:		
head and neck		8
sarcomas		5
colorectal		4
lung		2
melanoma		2
lymphoma		1
adnexal		1

[a] Eight patients had received adriamycin prior to entering this study

All patients were evaluable for toxicity, which was recorded for each course. Nonhematologic toxicity was graded using the WHO criteria [16]; patients who experienced less than grade 2 toxicity during a course of treatment were eligible to receive subsequent courses at escalated doses. Patients were followed with repeated nuclear cardiac scans and were required to undergo cardiac biopsy after four courses of therapy.

The starting dose in this study was 5 mg/m^2 weekly for 3 weeks, which represents approximately one-third of the phase II dose established in the Japanese trials [12]. Doses were escalated in increments of 5 mg/m^2 per week. The maximum tolerated dose was defined as that causing grade 3 or 4 toxicity in more than half of the patients treated at that dose level.

Pharmaceutical information. THP-adriamycin hydrochloride for injection was obtained from Bristol Myers Company (New York, NY) as a freeze-dried powder (20 mg) with lactose (180 mg); it was prepared for i.v. injection by dissolving the contents of the vial in sterile water. The resulting solution was further mixed with 100 ml 5% dextrose solution immediately prior to administration. For analytical purposes, standards of adriamycin aglycone, daunomycin, and adriamycinol were obtained courtesy of Adria Laboratories, Inc. (Columbus, Ohio) and Farmitalia-Carlo Erba (Milan, Italy). Reagents of analytical grade and solvents of HPLC grade were purchased from Fisher Scientific Company (Fair Lawn, NJ) and Burdick and Jackson Laboratories, Inc. (Muskegon, Mich), respectively.

Pharmacologic studies. Pharmacologic data were obtained from ten patients, including five at the maximum tolerated dose. Blood samples (10 ml) were drawn from a catheter separate from that used for administration of the drug and placed into heparinized tubes. Samples were drawn before and immediately after injection as well as 5, 10, 15, 30, 45, 60, and 90 min and 2, 3, 4, 6, 8, 24, 48, and 72 h after drug infusion; they were immediately put on ice in a dark container. Plasma was obtained as soon as possible after blood collection (1500 *g* for 10 min at 4° C) and stored at −70° C for subsequent assay. Blood cell components were separated using Ficoll-hypaque (Histopaque 1077, Sigma Diagnostics, St. Louis, Mo) and differential centrifugation as previously described [1]. A baseline urine sample was collected prior to drug administration, and subsequent 6-h collections were obtained throughout the initial 24 h following the end of drug infusion.

An isocratic, reversed-phase HPLC method adapted from that reported by Matsushita et al. [10] was used to separate and quantitate THP-adriamycin and its metabolites THP-adriamycinol, adriamycin, adriamycinol, and adriamycinone. Briefly, 1 ml plasma or urine was added to 3 ml 0.01 *M* ammonium chloride buffer (pH 9.0) and the pH was adjusted to 9.0 with NaOH. Drug and metabolites were extracted by the addition of 6 ml chloroform:methanol (2:1, v/v) to this mixture. Samples were shaken for 5 min and centrifuged (12,000 *g* for 10 min at 4° C). The organic phase was removed and saved prior to readjustment of the pH and reextraction; the combined organic phases were dried under a stream of nitrogen, after which they were reconstituted with 500 µl mobile phase. The extraction efficiency for THP-adriamycin was 85%–90%. The chromatographic system consisted of a model 510 pump and 740 data module from Millipore/Waters (Milford, Mass) as well as a Spectraflow 980 programmable fluorescence detector (Kratos Division, ABI Analytical, Ramsey, NJ), which was used at an excitation wavelength of 254 nm and an emission wavelength of 550 nm. A Nova-Pak C18 phenyl column was used (Millipore/Waters, Milford, Mass). The mobile phase consisted of 0.035 *M* ammonium formate buffer (pH 3.0):acetonitrile (65:35, v/v), which was used at a flow rate of 0.8 ml/min. The peak areas of interest were automatically integrated by means of the Waters 740 data module. Data were analyzed using a nonlinear kinetic analysis program (PCNONLIN; Statistical Consultants, Edgewood, Ky).

Results

Toxicity. In all, 23 patients received a total of 55 courses of THP-adriamycin. The major toxicity of THP-adriamycin was myelosuppression (Table 2), which consisted primarily of granulocytopenia and occurred during the 4th week

Table 2. Significant hematologic toxicity (grade III or IV)

Dose[a]	Number of patients	Number of courses	Neutropenia	Thrombocytopenia
5	4	4	–	–
10	6	6	–	–
15	7	10	1	–
20	7	11	3	1
25[b]	10	24	8	1

[a] In mg/m^2 per week
[b] Therapy was delayed in four patients due to myelosuppression

of each course. At a dose of 20 mg/m^2, three of seven patients experienced grade 3 or 4 granulocytopenia (< 1,000 granulocytes/mm^3); one also experienced grade 3 thrombocytopenia (< 50,000 granulocytes/mm^3). At 25 mg/m^2, eight patients experienced grade 3 or 4 granulocytopenia; one also experienced grade 3 thrombocytopenia. At this dose level, prolonged myelosuppression caused therapy to be delayed in 4 of the 24 courses given.

Nonmyelosuppressive toxicity was minimal: five patients experienced grade 1 or 2 nausea and vomiting, two suffered grade 1 stomatitis, one complained of grade 2 diarrhea, and six experienced mild phlebitis. There were no cases of extravasation. Mild alopecia was noted in two of ten patients treated at the highest dose level. Of the 23 patients entered, only 7 received more than 2 courses of treatment and could thus be assessed for cumulative toxicity. There was no evidence of cumulative myelosuppression.

All patients who received more than four courses of therapy were required to undergo cardiac biopsy. Two patients who had received prior maximal doses of adriamycin (450 mg/m^2 bolus or its infusion equivalent) underwent repeated courses of therapy and repeated cardiac biopsies: in one case, the ejection fraction and biopsy score remained unchanged after 300 mg/m^2 THP-adriamycin; in the second, the patient's ejection fraction fell from 65% to 49%, concomitant with an increased biopsy score, after 585 mg/m^2 THP-adriamycin.

One patient with metastatic squamous carcinoma from a head and neck primary tumor achieved a brief partial remission. One patient with metastatic leiomyosarcoma underwent a prolonged period of stabilization of disease, and one with Ewing's sarcoma had a minor response.

Pharmacology. The clinical pharmacology of THP-adriamycin was examined in ten patients who received one of three doses (10, 15, or 25 mg/m^2) as a short i.v. infusion. Analyses of the plasma and urinary levels of THP-adriamycin and its metabolites were carried out in all ten patients. Figure 2 depicts a chromatogram from the analysis of a plasma sample obtained 5 min after the end of drug infusion. The early appearance of a small adriamycin peak, which is clearly evident, was a consistent finding in all samples. Chromatograms obtained at later times showed that peaks coincident and consistent with adriamycinol, adriamycinone, and THP-adriamycinol also appeared, in addition to adriamycin and THP-adriamycin peaks. THP-adriamycin was rapidly taken up into blood cell elements. As shown in Fig. 3, THP-adriamycin levels measured in RBCs, lymphocytes, and polymorphonuclear cells exceeded those observed in the plasma at corresponding times. No other tissues were sampled.

THP-adriamycin pharmacokinetic parameters and urinary excretion data are presented in Table 3. Plasma elimination of this drug was best described by a triphasic curve with half-lives of 5.6 min, 1.4 h, and 19.3 h. The C_{max} at 25 mg/m^2 was quite variable, with one patient exhibiting a THP-adriamycin plasma concentration in excess of 1 μg/ml. The increased C_{max} values observed at 25 mg/m^2 resulted in correspondingly greater AUC values at this dose level; there was no evidence of dose-dependent pharmacokinetic parameters. Clearance of THP-adriamycin was relatively constant at all dose levels examined. Finally, its cu-

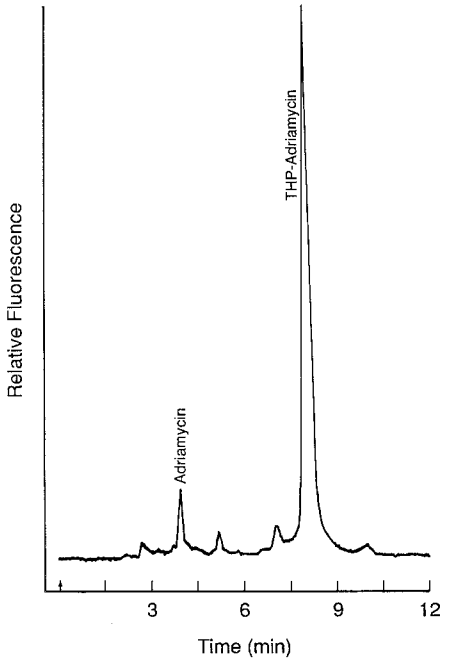

Fig. 2. HPLC chromatogram of a 5-min plasma sample from a patient who received 25 mg/m^2 of drug, demonstrating the early appearance of adriamycin after the injection of THP-adriamycin. The *arrow* indicates the time at which the sample was injected into the HPLC (see *Materials and methods* for analytical details)

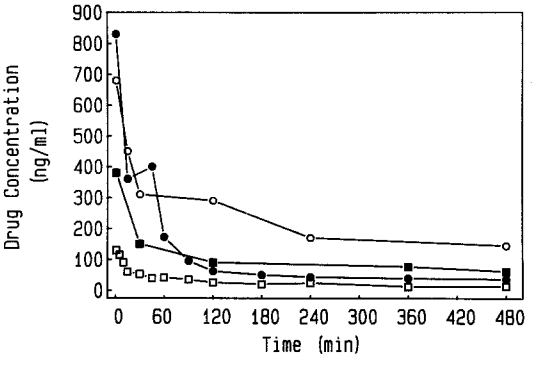

Fig. 3. Plasma and blood cell levels of THP-adriamycin in a patient given 25 mg/m^2 of drug. Data are presented as drug concentration in: ○, polymorphonuclear cells; ●, RBCs; ■, lymphocytes; and □, plasma (see *Materials and methods* for analytical details)

Table 3. THP-adriamycin: pharmacokinetics and urinary excretion

Patient number	Dose (mg/m^2)	$t_{1/2}$			C_{max} (ng/ml)	AUC (ng/ml × h)	Vd_c (l/kg)	Clearance (l/h × kg)	Urinary excretion (% of dose)
		α (min)	β (h)	γ (h)					
1	10	6.0	0.8	10.5	22	109.0	12.5	2.5	ND
2	10	3.4	1.0	12.8	128	98.7	2.1	2.7	ND
3	10	8.1	3.3	28.9	19	256.8	14.3	1.0	ND
4	15	6.3	0.3	23.1	61	203.0	2.0	1.8	–
5	15	4.0	1.4	23.1	106	171.6	3.8	2.4	ND
6	25	7.8	2.7	16.9	113	398.0	6.0	1.7	7.3
7	25	1.2	0.4	8.9	297	446.3	2.3	1.5	6.3
8	25	2.1	0.5	19.3	194	412.5	3.5	1.6	ND
9	25	10.7	2.0	25.0	125	514.2	5.4	1.3	11.5
10	25	6.4	1.7	24.2	1030	731.4	0.7	0.9	10.2
Mean ± SD		5.6 ± 0.9	1.4 ± 0.3	19.3 ± 2.1			5.3 ± 1.4	1.7 ± 1.4	8.8 ± 1.2

$t_{1/2}$, plasma elimination half-life; C_{max}, concentration; Vd_c, volume of distribution; ND, not detectable

mulative urinary excretion was low, with < 10% of the dose being eliminated via this route within the initial 24-h period.

Discussion

Preclinical evaluation of THP-adriamycin suggests that it has greater inherent antitumor activity than and only slight cross-resistance to adriamycin [15]. In animal models it appears to be less cardiotoxic. Japanese and European investigators have reported a number of phase II clinical trials demonstrating that its spectrum of activity is similar to that of adriamycin, with greater activity in head and neck tumors, less apparent cardiotoxicity, and less alopecia [11, 12].

The present phase I trial confirms that on a weekly schedule THP-adriamycin has minimal acute toxicity. The maximum tolerated dose is 25 mg/m^2 per week and the recommended phase II dose is 20 mg/m^2 weekly. At the maximum tolerated dose alopecia is rare and nausea and vomiting are mild. Although cardiac function was closely monitored in all patients receiving multiple courses of therapy, given the small number of patients treated, we are unable to comment on the incidence of cardiac toxicity.

The pharmacology of this drug has been well characterized in dogs [7] and mice [6], although it has received relatively little attention in man. Preclinical studies in the dog, for example, have demonstrated that, as in man, the plasma decay of THP-adriamycin levels can be simulated with a three-compartment open model with α, β, and γ half-lives of < 1 min, 9 min, and 7 h, respectively [7]. These values are similar to those reported in the present study as well as those recently reported by Miller and Schmidt [11] in their report of a European phase I trial. The extremely short α and β phases of plasma elimination half-life most probably reflect the rapid uptake of this lipophilic drug into tissues and blood cell components, as previously suggested by Majima [9].

As shown in the present study, THP-adriamycin was rapidly taken up by polymorphonuclear cells, lymphocytes, and red blood cells, where it achieved much higher concentrations than those determined in plasma at corresponding times. Miller and Schmidt [11] also observed its accumulation in packed RBCs and found that levels in cells exceeded plasma levels by 10-fold. Other investigators have reported a rapid cellular uptake of THP-adriamycin under in vitro conditions [8, 13]. Its relatively large volume of distribution also reflects extensive tissue uptake with minimal excretion. It is likely that this high degree of tissue uptake explains the rapid α phase of the drug-disappearance curve and might represent a "reservoir" from which the drug is slowly released. In the present study, < 8% percent of the dose was eliminated in the urine within the initial 24 h following drug administration. Although there was considerable variation within the small patient population examined in this study, the AUC values appear to correspond with the dose and are dependent upon the dose-related increases in C_{max}; the low clearance of the drug remained constant at all dose levels.

THP-adriamycin appears to be catabolized in a fashion similar to that of doxorubicin, with formation of a 13-OH derivative (THP-OH) and subsequent formation of aglycones. An alternative and probably minor metabolic pathway, involving cleavage of the tetrahydropyranyl moiety to yield adriamycin, has been suggested by Fujita et al. [4], based their studies using liver homogenates from various species. These investigators demonstrated that, in contrast to mouse and rabbit liver homogenates, detectable but relatively little adriamycin is formed from THP-adriamycin by human liver homogenates under anaerobic conditions of incubation.

In the present study, a detectable peak of adriamycin was apparent as early as 5 min following the administration of THP-adriamycin (Fig. 2); at this time the adriamycin peak area was < 6% of the THP-adriamycin peak area. Drug determinations made at later times (e.g., 30 min postinfusion) demonstrated that adriamycin reached levels as high as 33% of those of the parent THP-adriamycin. The latter observation is no doubt a reflection of liver/tissue metabolism, which has been well documented in in vitro liver homogenate studies [4] and clinical pharmacology reports [9, 11].

The appearance of adriamycin as early as 5 min after drug infusion, however, remains an anomaly. Although degradation of THP-adriamycin at low pH levels has been shown to be extensive [11], the low metabolic rate of this drug from human liver homogenates [4] and temperature-dependent plasma degradation studies [11] suggests that

levels of adriamycin are unlikely to be detectable as early as 5 min after drug infusion. An alternative explanation for the early appearance of adriamycin is that this drug coexists in the clinical preparation of THP-adriamycin. Miller and Schmidt [11] observed that their clinical supply of THP-adriamycin was contaminated by 2%–3% with adriamycin; in the present study, such contamination amounted to as much as 5%.

From preclinical studies it would appear that THP-adriamycin is an anthracycline with similar cytotoxicity but less cardiotoxicity and alopecia than adriamycin. Based on this phase I study and phase II studies in Europe and Japan, the maximum tolerated doses of THP-adriamycin and adriamycin appear to be similar. If further phase II and III studies confirm these results, THP-adriamycin may be an attractive alternative to adriamycin.

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