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Pharmacokinetics of ametantrone acetate (NSC-287513)*

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Summary. Ametantrone is the second anthracene derivative to enter clinical trials. The pharmacokinetic parameters for ametantrone acetate (CI-881) were characterized in six patients concurrently with the phase I clinical trial. Biological samples were assayed by a specific and sensitive high-performance liquid chromatography procedure. Plasma levels of ametantrone declined in a triexponential fashion, with a mean terminal half-life ($t_{½}$ *gamma) of 25 h. The estimated mean total-body plasma clearance was* 25.9± 14.7 l h^{-1} m^{-2}. The steady-state volume of distribution (Vdss) was large, averaging 568 ± 630 l/m^2. Excretion of unchanged ametantrone in the urine over 48 h averaged 5.7% of the total dose, indicating that there is another major route of elimination.

Introduction

Ametantrone, 1,4-bis{[2-[(2-hydroxyethyl)amino]ethylamino}9,10-anthracenedione, diacetate is the second anthracene derivative to enter clinical trials [9]. Ametantrone is one of the original three (NSC-196473, 287513, 279836)[1] aminoanthraquinone derivatives screened in the National Cancer Institute's tumor panel [7]. This intensely blue ink dye has a broad spectrum of antitumor activity against a variety of animal tumor models [3, 7]. Structurally, ametantrone differs from mitoxantrone in the lack of the hydroxyl groups on the ring adjacent to the quinone moiety (Fig. 1). Its antitumor activity is presumed to be by DNA binding through intercalation with the basic aminoalkyl side-chain stabilizing the intercalation [7].

Lu and Loo determined the pharmacokinetic and distribution characteristics of this agent in beagle dogs using an HPLC assay [11]. The plasma clearance of ametantrone followed a biexponential decline, with a distribution half-life of 9.4 min and an elimination half-life of 115.2 min. Twenty-four percent of the dose administered was excreted in the urine, while 39.5% of unchanged drug was excreted in the bile in 5 h. There were no detectable amounts in the cerebrospinal fluid. At necropsy, the liver contained the highest percentage of the dose (7%) 5 h after drug administration, followed by the kidney (3.5%), pancreas (3.1%), small intestine (1.5%), and stomach (1.3%). The liver and intestines retained significant concentrations of ametantrone up to 24 h after dosing.

Using a fluorometric assay, Holsztynska et al. [6] studied the metabolic disposition of ametantrone in rats. A single 5 mg/kg dose was given i.v. to male rats. The rate of elimination from plasma was rapid, with a half-life of approximately 9 min. This was mainly due to tissue sequestration. Peak tissue levels occurred within 5 min, with the highest concentrations appearing in the kidneys, spleen, lung, liver, and heart. The visible presence (blue color) of drug was noted in the urine, bile ducts, and upper small intestine as soon as 5–15 min after dosing. Tissue elimination was prolonged (> 16 h). Only 1.3% of the dose was excreted unchanged in the urine over 16 h.

This study was conducted as part of a phase I clinical trial to characterize the disposition of ametantrone on a single dose schedule.

Materials and methods

Patient selection and characteristics. Six adult male patients with histologically proven metastatic malignancy and having a mean age of 61.7 years (range 59–70) were entered in this study. Prior to entry, written informed consent was obtained from all patients in accordance with federal and institutional policies. Patients with impaired liver function tests (alkaline phosphatase > 150 IU/ml, bilirubin > 1.5 mg/dl) or renal function (creatinine > 2 mg/dl) were not eligible for the study.

Treatment schedule. Ametantrone acetate was provided by the Warner-Lambert/Parke-Davis Company, Ann Arbor, Mich as a sterile dark blue solution for injection in 10-ml ampules containing 100 mg ametantrone as the diacetate salt. Each patient's specified dose was withdrawn from the ampule and further diluted in 250 ml 5% dextrose in water. The resulting dark blue solution was infused over 30–41 min. Blood and urine samples were obtained on day 1 of the first course of therapy.

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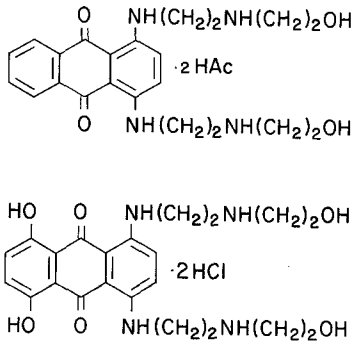

Fig. 1. Structural formulae of ametantrone *(above)* and mitoxantrone *(below)*

Blood sampling and urine collection. Seventeen 10-ml blood samples were collected through a heparin lock at the midpoint and end of the infusion period and at 2, 5, 10, 20, 30, 45, 60, and 90 min and 2, 3, 4, 6, 8, 12, and 24 h after the infusion. Aliquoted urine samples were collected at 8-h intervals for a total of six collections.

HPLC analysis. Ametantrone in both plasma and urine was analyzed by a modified high-performance liquid chromatographic technique being used to assay other anthracene derivatives [8]. A structually related analogue, PD 111,101-1K (9,10-anthracene,1,5-bis((3-((2-hydroxyethyl)amino)propyl)amino)hydrate), supplied by Warner-Lambert/Parke-Davis was added to each plasma sample as an internal standard (I.S.) prior to extraction. Stock solutions and urine/plasma standard curves were prepared on the day of each procedure. A 1 ml volume of plasma or urine was extracted with 2 ml chloroform: methanol: 1 *N* hydrochloric acid mixture (83:16:1 ratio) and agitated in an Eberbach shaker at 180 oscillations/min for 5 min. The phases were separated by centrifugation at 2000 *g* for 5 min. The purpose of this first extraction was to remove lipids. The upper aqueous phase was again extracted by adding 3 ml chloroform, vortexing, and adding 100 µl 28% ammonium hydroxide. The mixture was agitated for 5 min and centrifugated for 5 min. Then 2.5 ml of the organic layer was evaporated to dryness in a 37 °C water bath under a gentle nitrogen system. The extraction yield was 73%±10%. The extracted residue was reconstituted with 200 µl of the mobile phase and centrifuged, and 50–100 µl of clear solution was then injected into a high-pressure liquid chromatography (Tracor model 995) using a Waters µ Bondapak C_{18} reverse phase (3.9 mm i.d. x30 cm). The elution system consisted of 21:5.3:73.7 acetonitrile: 0.44 *M* ammonium formate buffer (pH 6.0): water at a flow rate of 2 ml/min with UV absorbance monitored at 260 nm. Retention times for ametantrone and PD111,101-1K were 3.6 and 5.4 min, respectively. Typical chromatograms are illustrated in Fig. 2. The coefficient of variation was 5% (range 7.43 and 2.57) over the linear range of the assay (25–1000 ng/ml).

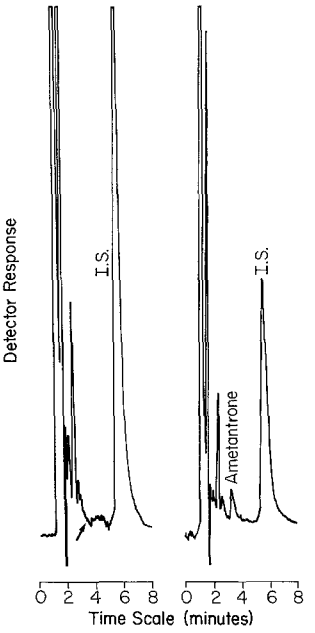

Fig. 2. Chromatograms of a predose and a 4.7 h post-dose plasma sample from patient 3 who, received 285 mg ametantrone. The plasma concentration in the post-dose sample was calculated to be 48.4 ng/ml. Attenuations were 0.01 and 0.02, respectively. The *arrow* in the blank indicates where the drug peak occurs

Pharmacokinetic analysis. Analysis was performed by nonlinear least-squares regression analysis (NONLIN) with a weighting of $1/Y^2$ [12]. The best estimates for the pre-exponential and exponential terms were obtained by fitting the plasma concentration to a two- and three-compartment open pharmacokinetic model, using a zero infusion input and first-order elimination. The F-test and the Akaike information criterion were applied to determine the best presentation for the time-course data [2, 15].

Results

The patient characteristics, doses administered, and peak concentrations are listed in Table 1. As noted, peak plasma levels immediately after the infusion ranged from 4.03

Table 1. Patient characteristics

Patient no.	Age (y)	Wt (kg)	BSA (m^2)	Dose[a]		Infusion duration (min)	Infusion rate (mg/min)	Peak conc. (µg/ml)
				(mg/m^2)	(mg)			
1	70	76.8	1.8	140	275	30	9.2	4.36
2	59	45.9	1.5	140	196	36	5.4	4.24
3	59	86.0	2.03	140	285	41	6.9	7.06
4	60	61.9	1.66	160	290	30	9.7	8.62
5	63	83.5	2.0	160	315	35	9.0	10.26
6	59	52.4	1.63	180	290	32	9.1	4.03

[a] Recommended phase II dose 140–160 mg/m^2

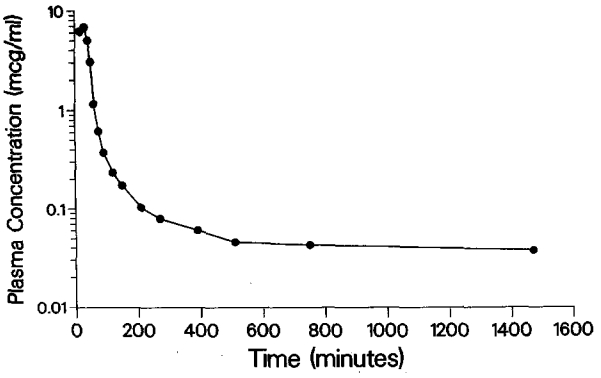

Fig. 3. Semilogarithmic plot of the mean ametantrone plasma concentration versus time for the six subjects

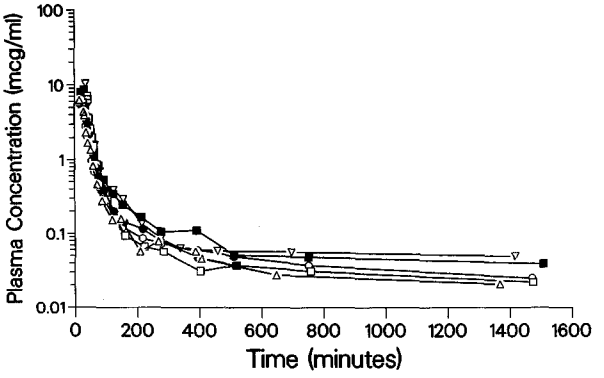

Fig. 4. Plasma concentration-time profiles for the six individual subjects. Subject ID: △1, X2, □3, ■4, ▽5, ○6

Table 2. Ametantrone kinetic parameters

Patient	$t_{1/2}$			Clearance rates			Volumes of distribution		
	Alpha (h)	Beta (h)	Gamma (h)	Plasma ($l\,h^{-1}\,m^{-2}$)	Tissue		V Central (l/m^2)	Tissue	
					P1 ($l\,h^{-1}\,m^{-2}$)	P2 ($l\,h^{-1}\,m^{-2}$)		P1 (l/m^2)	P2 (l/m^2)
1	0.10	0.44	11.8	37.3	13.92	13.62	9.90	6.30	168.9
2[a]	0.02	0.24	2.9	30.9	37.82	8.18	3.04	5.99	26.6
3	0.13	0.60	24.6	22.4	3.66	7.14	6.45	2.74	191.6
4	0.16	1.14	39.8	19.5	7.02	11.58	9.22	9.04	412.6
5	0.14	0.86	192.5	6.8	6.12	20.99	7.10	5.99	1667.6
6	0.13	0.53	24.0	43.7	17.18	25.36	17.55	9.63	315.9
Mean	0.13[b]	0.75[b]	25.20[b]	25.94	9.58	15.74	10.04	6.74	551.32
±SD				14.7	5.7	7.4	4.4	2.8	631.7

P1, peripheral compartment one; P2, peripheral compartment two
[a] Patient omitted from calculation of mean and SD
[b] Harmonic means

Table 3. Excretion of unchanged ametantrone in the urine

Time period (h)	Amount excreted by patients per time interval (mg)						Mean (± SD)
	1	2	3	4	5	6	
0– 8	11.127	3.272	18.588	15.505	13.262	19.408	
8–16	0.302	0.403	0.971	2.185	0.976	0.566	
16–24	0.467	0.433	0.417	1.288	0.688	0.511	
24–32	0.257	0.125	0.301	1.029	0.478	0.434	
32–40	0.406	0.386	0.710	0.673	0.378	0.318	
40–48	0.180	0.135	0.240	0.282	0.578	0.407	
Total	12.739	4.754	21.227	20.962	16.360	21.644	5.73
% of dose	4.63	2.43	7.45	7.23	5.19	7.46	(2.03)

to 10.26 μg/ml, depending on the total dose administered and the duration of infusion.

The time-course for the ametantrone plasma concentration for all six subjects was best described by a triexponential equation with zero-order input. The mean plasma concentration-time data for all six subjects is illustrated in Fig. 3. The subjects' individual plasma concentration-time profiles are shown in Fig. 4. The minimum sensitivity for the analytical procedure occurred at the beginning or during the final disposition phase. This precluded quantitation of the plasma samples for a full 24 h for subject 2. The mean pharmacokinetic parameters for the subjects having detectable plasma concentrations for approximately 24 h after an infusion were a harmonic mean terminal half-life of 25.2 h, an estimated plasma clearance of $25.9 \pm 14.7\ l\ h^{-1}\ m^{-2}$ and an apparent volume of distribu-

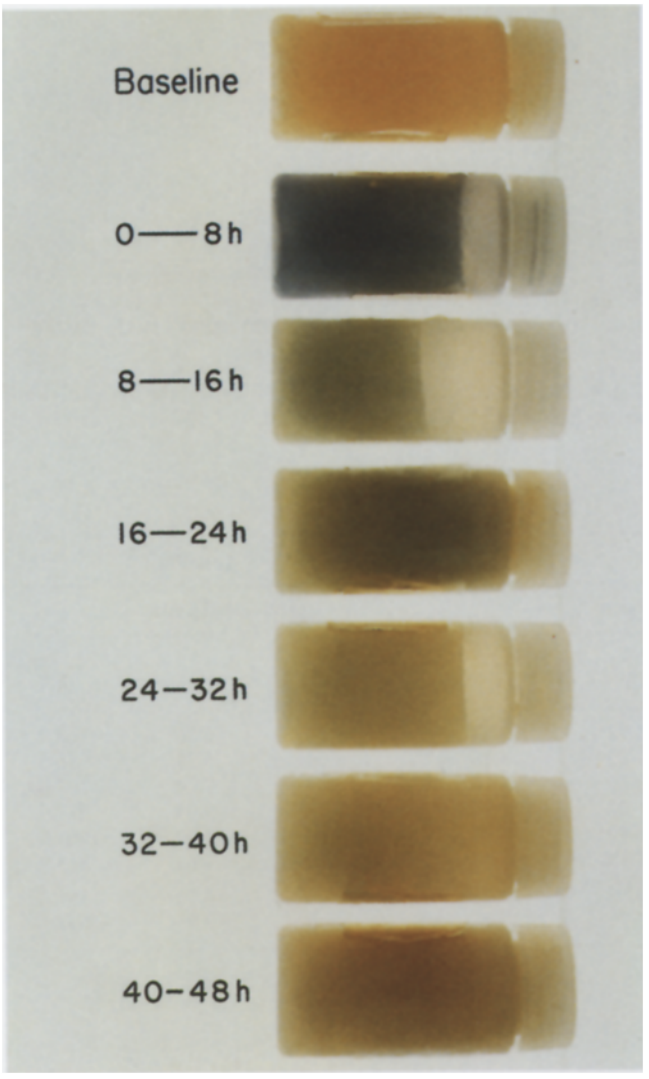

Fig. 5. Color intensity of urine following ametantrone administration (pt. 4)

tion at steady state of 568.1 ± 630 l/m^2 (Table 2). The kinetic parameters for subject 2 were not included in the calculations of the means, since the parameters were only derived over an 8.6 h interval. Because of patient 5's contribution to the overall arithmetic mean volume of distribution at steady state, a closer reflection of the "true" value may result if the harmonic mean, which was 308.4 l/m^2, is considered. The cumulative urinary excretion of ametantrone was 5.3% ± 1.96% at 24 h and 5.73 ± 2.03% at 48 h (Table 3). Urine discoloration (dark blue to green) occurred in all patients up to 48–72 h after infusion (Fig. 5). The prolonged time-course of colored urine versus parent drugs excreted suggests the possibility of (a) colored metabolite(s). A trace metabolite of ametantrone has been detected in the urine of dogs [11]. Lack of detection of urinary metabolites in our study could have resulted from the extraction and loss of the more polar compounds.

Discussion

The plasma clearance for ametantrone was rapid and followed a triexponential time-course which is similar to that observed for the dihydroxy analogue mitoxantrone [14]. Recently, Alberts et al. described a triexponential plasma disappearance pattern for mitoxantrone with a rapid plasma clearance (34.2 l h^{-1} m^{-2}) and a terminal $t_{½}$ gamma of 42.6 h [1]. Visual inspection of the plasma concentration-time curves suggests that the terminal half-life of 25 h for ametantrone may be an underestimation of the true terminal half-life. However, assay sensitivity limits further exploration of this observation and its clinical implication.

The apparent steady-state volume of distribution was large (568.1 l/m^2), indicating sequestration outside the plasma in one or more tissue compartments from which the drug is slowly released. This would account for the rapid disappearance of ametantrone from the plasma and the relatively long terminal half-life. The extensive tissue distribution and retention of ametantrone in dogs [11] and rats [6] lends support to this contention. In humans, one of the tissue compartments that might contribute to the large apparent volume of distribution is the skin. A blue-gray skin discoloration has been described in all three of the phase I clinical trials [5, 10, 13]. Unfortunately, no drug could be demonstrated in biopsy specimens submitted to various pathological procedures, which included observing a quick-frozen section, without staining, with a regular or fluorescent (390–400 nm bandwidth) light microscope [10].

Total urinary excretion of ametantrone was low, which suggests another major route of elimination. Hepatobiliary excretion constituted the primary route of drug elimination in both the dog [11] and the rat [6]. Reportedly, one patient in the phase I trial had blue stools [10]. The exact contribution of hepatobiliary excretion to the elimination of ametantrone in humans is yet to be determined.

Ametantrone's disposition patterns in humans have also been described by Piccart et al. [13] and Gams et al. [5]. Piccart analyzed the blood and urine samples from four patients receiving 135–180 mg/m^2 of drug over 15–30 min. Peak plasma levels ranged from 4.1 to 15 μg/ml. Plasma levels rapidly declined to the limit of assay sensitivity (0.5 μg/ml), with an initial half-life between 8 and 16 min. Within 48 h after administration, 10%–33% of the administered dose was eliminated in the urine. Gams et al. evaluated ametantrone on the daily times five schedule, escalating doses from 15 to 35 mg/m^2. In nine patients, the harmonic mean terminal half-life was 0.38 h, with mean apparent volume of distribution and plasma clearance of 26.3 l/m^2 and 39.42 l h^{-1} m^{-2} respectively. The differences between these kinetic parameters and the present study can be related to the lower doses evaluated and the relative assay sensitivities.

In conclusion, our results suggest that ametantrone is avidly taken up by tissue from which it is released slowly, and hepatobiliary excretion is probably the major route of excretion. It is likely that patients with hepatic impairment or third space compartments will have a decreased clearance of ametantrone similar to that observed with mitoxantrone [4, 14].

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