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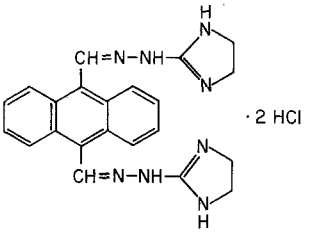
Clinical pharmacokinetics of 9, 10-anthracenedicarboxaldehyde-bis [(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone]dihydrochloride

Katherine Lu, Niramol Savaraj, Boh-Seng Yap*, Lynn G. Feun, Theera Umsawasdi, and Ti Li Loo

Department of Chemotherapy Research, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030, USA

Summary. We studied the clinical pharmacokinetics of the anthracene derivative bisantrene using high-performance liquid chromatographic analysis. We administered the drug to ten patients at 120–250 mg/m^2 IV; one of these patients also received a second dose of 120 mg/m^2 6 weeks later, and another received 150 mg/m^2 weekly for three doses. Bisantrene disappeared from the plasma biphasically, with an initial $t_{1/2}$ of 0.6 ± 0.3 h and a terminal $t_{1/2}$ of 24.7 ± 6.9 h after single doses. The apparent volume of distribution according to the area under the curve was 42.1 ± 5.9 l/kg, and the total clearance was 1045.5 ± 51.0 ml/kg/h. The 96-h cumulative urinary excretion was 3.4% ± 1.1% of the dose; thus, renal excretion was a minor route of elimination for this agent. Bisantrene pharmacokinetics in the patient who received a second dose after 6 weeks showed insignificant changes. However, in the patient who was given this drug weekly for 3 weeks, the plasma $t_{1/2}$ of the drug during the terminal phase became increasingly longer, while the total clearance was significantly reduced. These results suggest that bisantrene may accumulate in the body and that caution is essential in the event of frequent administration.



Bisantrene
NSC-337766

Fig. 1. Structure of bisantrene

Introduction

9,10-Anthracenedicarboxaldehyde-bis-[(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone] dihydrochloride, (C1 216,952, bisantrene, or NSC-337766) (Fig. 1) is a new antitumor agent developed by the American Cyanamid Company. It has demonstrated significant antitumor activity in a number of experimental animal tumor systems, including L1210 leukemia, P388 leukemia, Lieberman plasma cell tumors, B16 melanoma, Ridgeway osteogenic sarcoma, and colon tumors 26 in mice [11]. Also, in the stem cell assay it has shown activity against human breast cancer, ovarian cancer, renal cancer, squamous cell, small cell, and large cell lung cancers, lymphoma, leukemia (AML) melanoma, adenocarcinoma of unknown orgin, adrenal cancer, gastric cancer, pancreatic cancer, and head and neck cancer [2]. Although it bears some structural resemblance to doxorubicin, potentially bisantrene has less myocardial toxicity than doxorubicin at equal doses. Therefore, bisantrene may be a useful antitumor agent in doxorubicin-sensitive tumors [6]. It has been undergoing phase I and II clinical trials in patients with malignancies at our institution and elsewhere. In the phase I study the dose-limiting toxic effects were phlebitis and hypotension [8, 10, 12]. In conjunction with these trials, we have studied its clinical pharmacology.

Materials and methods

Drugs and reagents. Bisantrene was supplied by Lederle Laboratories Division, American Cyanamid Company, Pearl River, NY. For high-performance liquid chromatography (HPLC), glass-distilled-grade methanol was obtained from Burdick and Jackson Laboratories, Muskegon, Mich. All other chemicals and reagents were purchased from regular commercial suppliers.

Patients. Patients with proven malignancies were selected for this study. Prior informed consent was rountinely obtained from all patients. Male and female patients with the characteristics shown in Table 1 received bisantrene in doses ranging from 80 to 250 mg/m^2. Before bisantrene therapy all patients had normal liver function; during the treatment all other medications were suspended if possible. Since it has been reported [7, 8, 10, 12] that local complications of phlebitis and thromboses occur in up to two-thirds of patients given bisantrene by infusion into a peripheral vein, to avoid the likelihood of vasculitis the drug was administered into a central vein. Indwelling silicone elastomer venous catheters were inserted via an arm vein when possible, or subcutaneously via the subclavian vein.

* *Current address:* Cancer Screening & Treatment Center, Mount Elizabeth Medical Centre, Singapore

Offprint requests to: K. Lu, Division of Medicine, The University of Texas M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, 6723 Bertner Avenue, Houston, TX 77030, USA

Table 1. Patient characteristics and bisantrene dose

Patient no.	Age	Sex	Dose (mg/m^2)	Diagnosis
1	59	F	80	Lung carcinoma
2	53	F	120	Breast carcinoma
3	69	F	150	Breast carcinoma
4	66	M	150	Lung carcinoma
5	42	M	180	Melanoma
6	58	F	250	Breast carcinoma
7	35	F	250	Breast carcinoma
8	47	F	250	Lung carcinoma
9	58	M	250	Lung carcinoma
10	62	F	250	Renal cell carcinoma

Once the catheter was in place, the desired dose of bisantrene was mixed in 100 ml normal saline and infused over 1 h. One of the patients (No. 1) who received the lowest dose was given a second dose of 120 mg/m^2 6 weeks later, and another patient (No. 4) received 150 mg/m^2 weekly for 3 weeks.

Sample preparation. Blood samples (10 ml) were drawn from an indwelling heparin lock before drug administration and from the corresponding contralateral sites at predetermined intervals after completion of the infusion. They were put in heparin-coated tubes and placed in ice until centrifugation for 10 min at 12 000 *g* in a Sorvall RC2 centrifuge (Dupont Instruments, Wilimington, Del). The plasma was separated from the red blood cells (RBC) and kept frozen until analysis. Urine samples were collected as voided at 6 h intervals for 24 h and at 24 h intervals for a further 72 h.

All biological fluids were filtered through C_{18} Sep-Pak (Waters Associates, Milford, Mass). Minichromatographic columns before HPLC analysis, as reported earlier [5]. Preparation of the samples by this procedure resulted in 75% recovery of the drug. The integrated area under the curve is linear with drug concentrations ranging from 2 to 2000 ng/ml, and the lowest limit of detection was 2 ng/ml.

Total fluorescence. The total fluorescence of the biological samples was determined by an Aminco fluorometer SPF model 125, with the excitation wavelength set at 418 nm and emission at 503 nm.

Chromatography. The details of HPLC analysis have been published elsewhere [5]. Briefly, all analyses were performed on a Waters Associates model 204 liquid chromatograph (Milford, Mass) equipped with a Schoeffel model SF-970 fluorescent detector (Kratos Analytical Company, Westwood, NJ), a Shimadzu Chromatopac-E1A electronic integrator (Kyoto, Japan), and a Brinkmann Instrument model 2544 recorder. Separation was achieved with an analytical reverse-phase μBondapak C_{18} column (4 mm × 30 cm ID, Waters Associates). The solvent system consisted of 0.02 *M* ammonium acetate in 40% methanol, pH 4.0, and the flow rate was 1.5 ml/min. The fluorescence detector was set at 260 nm for excitation and 550 nm for emission. Bisantrene has a retention time of 9 min under these conditions. The lowest limit of detection was 2 ng/ml.

Computation of the results. Nonlinear regression analysis of the results was performed with the aid of the Prophet Program. Best fit was based on an open two-compartment model [3].

Results and discussion

The disappearance of bisantrene from the plasma of the patients given this agent IV at a dose of 80–250 mg/m^2 was biphasic, with a mean initial half-life of 0.6 h and a terminal half-life of 24.7 h. A typical plasma clearance curve of bisantrene is shown in Fig. 2, and the pharmacokinetic parameters are summarized in Table 2.

The total clearance ranged from 620 ml/kg/h in patients receiving the higher dose to 1903 ml/kg/h in patients with the lower doses. This significant difference was

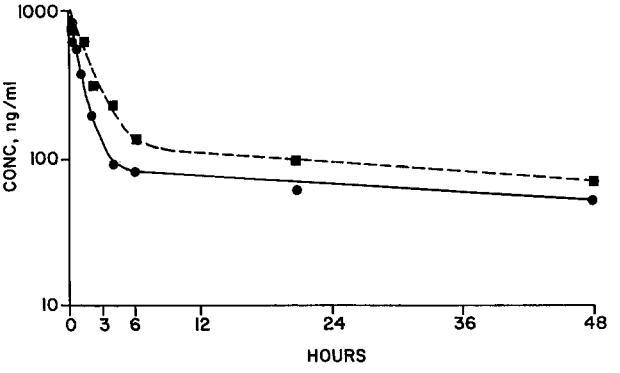


Fig. 2. Plasma concentration of total fluorescence (■) and unchanged bisantrene (●) in a patient after 250 mg/m^2 IV

Table 2. Clinical pharmacokinetic parameters of bisantrene

Pt	t ½ (h)		V_d (l/kg)	Cl (ml/kg/h)	Urinary excretion (% in 96 h)
	α	β			
1	0.2	32.7	41.6	882.5	1.2
2	1.2	12.5	26.4	1467.4	2.3
3	0.9	29.0	79.5	1903.3	N.D.
4	3.4	17.9	39.5	1532.0	0.4
5	0.6	23.1	43.0	1293.9	11.1
6	0.5	13.9	19.3	962.5	2.2
7	0.6	27.6	25.3	637.4	3.6
8	0.6	65.0	44.2	472.5	5.3
9	1.0	43.1	38.5	619.9	3.1
10	0.7	67.9	66.9	683.8	1.6
Mean ±SE	0.6[a] ±0.3	24.7[a] ±6.9	42.4 ±5.9	1045.5 ±151.0	3.4 ±1.1
1	2.3	52.0	83.7	1114.5	1.1
4	0.1	79.0*	17.2	151.0*	2.0
4	0.3	117.0*	34.8	206.8*	2.9

[a] Harmonic mean

* Significantly different from the mean ($P < 0.05$)

N.D., not done

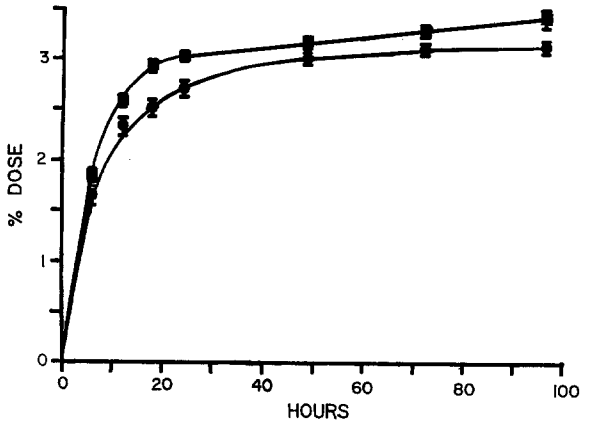


Fig. 3. Average cumulation urinary excretion of total fluorescence (■) and unchanged bisantrene (●) in patients

possibly due to the saturation of the clearance mechanism by the higher doses. Together with the total clearance (average 1045.5 ml/kg/h) the apparent volume of distribution calculated from the area under the curve, 42.4 l/kg, was also large. However, the cumulative urinary excretion of bisantrene was only 3.4% of the administered dose, even 4 days after the initial dose (Fig. 3). These parameters did not change consistently in patient 1, who took part in the study again after 6 weeks. But in patient 4, who received three consecutive weekly doses of bisantrene, the terminal half-lives of the drug were significantly lengthened and the total clearance rates were diminished after both the second and the third doses. Furthermore, at the end of drug administration, the plasma bisantrene concentration in this patient increased from 0.76 μg/ml after the first dose to 1.57 and 2.47 μg/ml after the second and third doses, respectively.

By and large, bisantrene appears not to be metabolized in vivo. However, occasionally an unknown metabolite was detected in the plasma (Fig. 2) and urine (Fig. 3) of a few patients, as demonstrated by the discrepancy between total fluorescence and unchanged bisantrene levels.

The large apparent volume of distribution suggests that in man bisantrene is most probably localized in some body compartment from which it exits slowly. Also, 60% of bisantrene was bound to plasma protein, as reported by Alberts et al. [1], suggesting that binding to tissues was also high in man. This is further supported by the study of Wu and Nicolou [13] on the tissue distribution of radiolabelled bisantrene in dogs and monkeys. The high total clearance is more than offset by the large apparent volume of distribution, so that the half-life of the drug during the terminal phase is relatively long. Since urinary excretion is insignificant with bisantrene and the in vivo metabolism is probably negligible, the clearance of the drug must be principally by tissue sequestration and hepatobiliary-fecal excretion. We have no evidence that bisantrene was indeed so excreted in man. However, Wu and Nicolau [13] reported that in both dog and monkey fecal excretion was the major route of elimination of bisantrene. Their results therefore lend support to the possibility of a similiar fate of this agent in man. Since the total clearance of bisantrene was drastically reduced, while the plasma half-life of the drug was markedly increased, in the patient who received three weekly doses, it definitely seems that to avoid cumulative toxicity it would be prudent to exercise caution in frequent bisantrene administration.

Structurally, bisantrene bears some superficial resemblance to another new anticancer agent, mitoxantrone, insofar as both possess an anthracene moiety with different but similarly hydrophilic side-chains. Even in this respect, the anthracene ring in bisantrene is devoid of other substituents, whereas in mitoxantrone it is actually a 9,10-quinone with two additional 1,4-phenolic groups in close proximity. In man, the two exhibit some common pharmacokinetic characteristics, namely, long plasma half-lives and large apparent volumes of distribution, as well as total clearance rates [9]. Furthermore, only a small fraction of the administered dose of either agent is excreted in the urine [4]. In this connection it may be recalled that pharmacokinetically, in the dog at least, mitoxantrone is strikingly different from its cogener, ametantrone, with which it is entirely identical except for the presence of the 1,4-phenolic groups in the anthracene ring. We previously showed that in three dogs the average values of the relevant pharmacokinetic parameters of mitoxantrone were: terminal plasma half-life, 28.1 h; apparent volume of distribution, 28.1 l/kg; total clearance, 606 ml/kg/h. In contrast, the corresponding parameters for ametantrone are: 1.9 h; 0.7 l/kg; and 1410 ml/kg h. In 48 h, only 2.4% of the administered dose of mitoxantrone is excreted in the urine and 3% in the bile. But with ametantrone, 24% of the administered dose can already be recovered from the urine within 5 h, in addition to the 40% in the bile. Moreover, by 5 h after administration 50% of the mitoxantrone is localized in the liver alone, whereas only 7% of the ametantrone is similarly sequestered [4]. Taking all three agents together, it is thus apparent that their pharmacokinetic behavior is largely determined by the anthracene moiety, with little contribution from the hydrophilic side-chains; polycyclic compounds tend to be excreted largely in the bile. However, the presence of a quinoid structure in an anthracene compound shortens its plasma half-life, reduces its volume of distribution, diminishes its organ localization, and increases its total clearance and elimination. All these effects are nullified by the 1,4-phenolic groups in close proximity to the quinoid moiety, however, presumably due to hydrogen bonding, so that ametantrone is distinctly different pharmacokinetically from mitoxantrone, while the latter resembles bisantrene to a large extent.

References

1. Alberts DS, Peng YM, Davis TP (1981) Comparative pharmacology of mitoxantrone and bisantrene. Third NCI-EORTC symposium on New Drugs in Cancer Therapy, Abstract 35. Institut Jules Bordet, Brussels, Belgium Det 15–17, 1981
2. Citarella RV, Wallace RE, Murdoch KC, Angier RB, Durr FE, Forbes RM (1982) Activity of a novel α-anthracenyl bishydrazone, 9,10-anthracenedicarboxaldehyde bis[(4,5-dihydro-1*H*-imidazol-2-yl)hydrozone] dihydrochloride, against experimental tumors in mice. Cancer Res 42: 440–444
3. Gibaldi M, Perrier D (1975) Drugs and the pharmaceutical sciences. Dekker New York, p 45 (Pharmacokinetics, vol 1)
4. Lu K, Loo TL (1980) Pharmacological disposition of 1,4-bis2-[(2-hydroxyethyl)amino] ethylamino-9,10-anthracenedione diacetate in the dog. Cancer Res 40: 1427–1430
5. Lu K, Savaraj N, Huang MT, Moore D, Loo TL (1982) High-performance liquid chromotography (HPLC) of the new an-

tineoplastic 9,10-anthracenedicarboxaldehyde bis[4,5-dihydro-1*H*-imidazol-2-yl)hydrazone] dihydrochloride (CL 216, 942; bisantrene). J Liq Chromatogr 5: 1323–1328
6. National Cancer Institute, Clinical Brochure (1981) Orange Crush (CL 216,942) 9,10-anthracenedicarboxaldehyde bis [(4,5-dihydro-1*H*-imidazol-2-yl) hydrazone dihydrochloride. NSC 337766, Pearl River, New York (For Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI, Bethesda, MD 20205, USA)
7. Powis G, Kovach JS (1983) Disposition of bisantrene in humans and rabbits: Evidence for intravascular deposition of drug as a cause of phlebitis. Cancer Res 43: 925–929
8. Salmon S, Alberts DS, Mackel C, Pocelinio R (1982) Phase I clinical investigation of 9,10-anthracenedicarboxaldehyde bis[(4,5-dihydro-1*H*-imidazol-2-yl) hydrazone]dihydrochloride with correlation in vitro human tumor clonogenic assay. Cancer Res 42: 1170–1175
9. Savaraj N, Lu K, Valdivieso M, Loo TL (1982) Pharmacology of mitoxantrone in cancer patients Cancer Chemother Pharmacol 8: 113–117
10. Spiegel RJ, Blum RH, Levin M, Pinta CA, Wernz JC, Speger JL, Hoffman KS, Maggia FM (1982) Phase I clinical trial of 9,10-anthracenedicarboxaldehyde (bisantrene) administered in a five-day schedule. Cancer Res 42: 354–358
11. Van Hoff DD, Coltman CA, Forseth B (1981) Activity of 9,10-anthracenedicarboxaldehyde bis[(4,5-dihydro-1*H*-imidazol-2-yl) hydrazone]dihydrochloride (Cl 216, 942) in a human tumor cloning system. Cancer Chemother Pharmacol 6: 141–144
12. Van Hoff DD, Myers JW, Kuhu J, Sandbach JF, Pocelinki R, Clark G, Coltman CAJ (1981) Phase I clinical investigation of 9,10-anthracenedicarboxaldehyde bis[4,5-dihydro-1*H*-imidazol-2-yl)hydrazone] dihydrochloride (Cl 216,942). Cancer Res 41: 3118–3121
13. Wu WH, Nicolau G (1982) Disposition and metabolic profile of a new antitumor agent CL 216,942 (bisantrene) in laboratory animals. Cancer Treat Rep 66: 1173–1184

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