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Phase I Clinical Trial of 9,10-Anthracene Dicarboxaldehyde (Bisantrene) Administered in a Five-Day Schedule[1]

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ABSTRACT

Bisantrene is a substituted anthracene derivative which preclinically demonstrated a spectrum of activity similar to that of doxorubicin but without associated cardiotoxicity. A Phase I evaluation of the drug has been performed using daily i.v. administrations for 5 days. Sixty courses of treatment were administered to 23 patients at doses from 2.5 to 90 mg/sq m/day. Courses were repeated at 4-week intervals. Dose-limiting toxicities were leukopenia and local cutaneous reactions. The leukopenia was dose related, noncumulative, and of brief duration. Local reactions occurred in 14 of 37 courses administered at doses >60 mg/sq m and in 3 patients resulted in clinical cellulitis of the infused extremity. Gastrointestinal side effects were mild. No alopecia or cardiotoxicity was observed. Two mixed responses were obtained in patients with hypernephromas. Using a daily schedule for 5 days, approximately 40% more drug can be delivered per course than by single-day i.v. administration. However, with this schedule, local cutaneous reactions may prove additionally dose limiting. Phase II studies of Bisantrene in a daily i.v. schedule for 5 days are planned at a dose of 80 mg/sq m/day to be repeated every 4 weeks.

INTRODUCTION

The anthracycline antibiotics now play an important role in the clinical management of numerous solid tumors and hematologic cancers. Moreover, the mechanism of action and antitumor selectivity of this exciting class of drugs are beginning to be delineated through ongoing investigations of the structure-activity relationships responsible for DNA intercalation and the inhibition of macromolecular synthesis as well as other related areas of research. For these reasons, extensive studies have been undertaken to develop new antibiotics and synthetics in order to produce a "second generation" of intercalating anticancer drugs with antitumor activity equal to or greater than that of doxorubicin but with less toxicity.

It has been proposed that the cardiotoxicity of doxorubicin and daunomycin may be dependent upon the amino sugar moiety of the anthracycline molecule (1). Therefore, one active area of drug development has involved the replacement of the amino group by an alkylamino-substituted side chain (5). A number of anthracene and anthracenedione derivatives have been synthesized, and 9,10-anthracenedicarboxaldehyde bis[(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone] dihydrochloride hydrate or Bisantrene hydrochloride (NSC 337766; American Cyanamid CL216,942) represents the second such compound to enter clinical trial. The related compound 1,4-dihydroxy-5,8-bis{{ {2-[(2-hydroxethyl)amino]ethyl} amino}}-9,10-anthracenedione dihydrochloride (Mitoxantrone) is currently in Phase I-II trials (9).

In preclinical trials, Bisantrene demonstrated a spectrum of antitumor activity similar to that of doxorubicin. Significant activity was found in the following animal tumor systems: P388 and L1210 leukemia; Lieberman plasma cell tumor; B16 melanoma; Colon Tumor 26; and Ridgway osteogenic sarcoma. There was no significant activity against Adriamycin-resistant P388 leukemia or Lewis lung carcinoma. Antitumor activity was not dependent on a specific phase of cell growth cycle in cell culture studies. Although the mechanism of action of Bisantrene has not been defined, it contains an appropriately sized planar electron-rich chromophore to be a DNA intercalating agent, and *in vitro*, it is a potent inhibitor of DNA and RNA synthesis (2).

Animal toxicology studies in mice, rats, dogs, and monkeys demonstrated predictable dose-response effects on the bone marrow and the lymphoid and gastrointestinal systems. The major toxicity was hematopoietic. Dose-related transient marrow hypoplasia accompanied by peripheral blood changes was observed. Other toxic manifestations included anorexia, fever, diarrhea, and limb swelling at the i.v. site. Muscle weakness, tremor, and convulsions were noted in dogs and monkeys receiving lethal doses. Histopathology demonstrated visceral congestion and pulmonary edema, enterocolitis, bone marrow hypocellularity, and muscle degeneration and necrosis at the injection site. However, in animal toxicity studies, no alopecia, testicular degeneration, or cardiotoxicity was noted (2). In special cardiotoxicity studies, beagle dogs were exposed to equivalently leukopenic doses of Bisantrene and doxorubicin and were followed both clinically and with sequential endomyocardial biopsies. Specimens were examined for evidence of cardiomyopathy by electron microscopy and the light microscopy technique of Billingham *et al.* (4). All dogs receiving doxorubicin at these dosages demonstrated progressive myocardial lesions, whereas no animals receiving Bisantrene had clinical or histological findings of cardiotoxicity.

Based on the above information, 7 Phase I studies of Bisantrene have been initiated by the American Cyanamid Company, each utilizing one of 3 schedules of administration (weekly for 3 weeks, daily for 5 days, or single dose). At our institution, a Phase I clinical study was conducted to determine the dose-limiting toxic effects and antitumor activity associated with a daily i.v. administration schedule for 5 days.

PATIENTS AND METHODS

Twenty-three adult patients with histologically confirmed

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Table 1
Patient characteristics

Patients entered				Prior therapy				Diagnoses										
Total	Sex	Median age	Age range	Chemotherapy	Chemotherapy + radiotherapy	Hormonal therapy	None	Hypernephroma	Melanoma	Lung (oat cell)	Lung (adeno)	Sarcoma	Colon	Gastric	Hepatocellular	Thyroid	Ovary
23	15 M, 8 F	61	23–70	14	7	1	1	8	4	2	1	2	2	1	1	1	1

Table 2
Dose escalation and courses

Dose (mg/sq m/day)	Initial courses	Subsequent courses	Total
2.5	1		1
5.0	3		3
10.0	1		1
30.0	4		4
40.0	6	1	7
50.0	7		7
60.0	8	5	13
75.0	7	4	11
80.0	9	1	10
90.0	1	2	3

cancers refractory to conventional modes of treatment were entered into this trial (Table 1). The median age of the patients was 61 years. Fifteen males and 8 females were treated. Patients were eligible who had an estimated life expectancy >8 weeks and no significant electrocardiographic abnormalities. Patients were required to have recovered from the effects of prior therapy and to have adequate peripheral blood counts (granulocytes, >1,500/ml; platelets, >100,000/ml). Acceptable hepatic and renal functions were defined as: bilirubin, <2.0 mg/dl; creatinine, <1.5 mg/dl; normal prothrombin time; serum glutamic-oxaloacetic transaminase, <40 IU/dl; and normal urinalysis. Informed consent was obtained according to federal and institutional policies.

Bisantrene, an orange hygroscopic solid, was supplied by the American Cyanamid Company, Pearl River, N. Y., as a sterile lyophilized powder (10 or 80 mg/vial). The drug was reconstituted with 2.0 ml of water for injection (USP) and was initially diluted in 250 ml of 5% dextrose in water (USP) (final concentration, 0.1 to 10.0 mg/ml). The solution was infused over a 30-min period into a freshly started i.v. line. Due to the frequent occurrence of phlebitis and local cutaneous reactions at daily doses >50 mg/sq m, the drug was subsequently dissolved in 500 ml of 5% dextrose in water (USP) and infused over 45 to 60 min. Bisantrene was administered each day for 5 consecutive days, and if no toxicity was observed, patients received another course on Day 28. The initial dose of 2.5 mg/sq m daily for 5 days was equivalent to one-third of the toxic dose, low, in the most sensitive large animal species, the beagle dog. Dose levels were increased as noted in Table 2 with initial dose doubling and subsequent 20 to 25% increments. Dose escalations in individual patients were permitted. Recruitment of at least 3 patients at each dose level was initially envisioned; however, reports of patient tolerance to single doses 2 logs greater than the 2.5-mg starting dose in other clinical trials being conducted simultaneously led to rapid progression through the first 3 dose escalations (8) (Table 2). At doses >40 mg/sq m daily, a minimum of 6 patients was treated at each dose level. If no toxicity was observed at a particular dose level after 4 weeks of observation, the next dose level was started.

Patients were closely observed during the 5-day period of drug administration and were seen on at least a weekly basis thereafter, as long as they remained on the study. Prior to each course of therapy, a complete blood count with differential and platelet counts, prothrombin and partial thromboplastin times, SMA-12 and chemistry panels, urinalysis, electrocardiogram,

[3] The abbreviation used is: USP, United States Pharmacopeia.

chest X-ray, and studies necessary for tumor evaluation were obtained. During drug administration, patients had vital signs monitored every 15 min. Complete blood counts were obtained daily during the 5 days of drug administration and weekly thereafter. Serum chemistries and clotting studies were obtained weekly.

Standard Eastern Cooperative Oncology Group criteria of response were used to evaluate the antitumor effect of the drug. Patients were removed from study if objective tumor progression occurred following 2 or more courses of Bisantrene or if significant drug-related clinical toxicity occurred.

RESULTS

Twenty-three patients with a variety of nonhematologic cancers were entered into the study over a 10-month period. Sixty courses of treatment were administered, with a range of 1 to 8 courses per patient. Twenty-one of 23 patients had been treated previously with multiple chemotherapeutic agents, and 7 of 23 had received radiotherapy. Twenty-two of 23 patients had measurable disease at the time of entry into this study.

Patient characteristics are listed in Table 1. Twenty-two patients received full 5-day courses of treatment, and all but 5 of the 60 courses were evaluable for toxicity. One patient experienced a local allergic reaction on the first day of treatment and was not treated thereafter. Four other courses had inadequate data for analysis. Two patients had clinical responses.

Toxicity. The limiting toxic effects of Bisantrene administered in this daily schedule for 5 days were myelosuppression and local cutaneous reactions. Leukopenia (WBC, <3000/ml) was the dose-limiting toxicity. As noted in Table 3, leukopenia occurred in 6 of 13, 4 of 10, and 4 of 10 of the courses administered at the 60-, 75-, and 80-mg/sq m/day dosages, respectively. However, these figures belie the actual myelosuppression observed, since 2 patients at each of the above dosages had pretreatment WBC counts ranging from 11,500 to 23,600. These patients account for 2, 3, and 4 of the courses administered at the 60-, 75-, and 80-mg/sq m dosages, respectively. Without their inclusion, leukopenia is noted in 6 of 11, 4 of 8, and 4 of 6 courses given to patients with base line counts in the normal range. Similarly, the single patient who tolerated treatment at 90 mg/sq m/day for 3 courses was receiving steroids for treatment of hypercalcemia, and her WBC prior to each treatment was >19,000/ml. In all patients, WBC nadirs occurred from Days 9 to 16 and in all cases recovered by Day 24. Four of 7 and 7 of 9 patients receiving the 75- and 80-mg/sq m dosages, respectively, were new patients, and no cumulative myelosuppression was observed in patients receiving multiple courses. Thrombocytopenia was minimal, and no patients had platelet counts <100,000 following therapy. No anemia was observed after drug administration.

The most severe nonhematologic toxicities observed were local cutaneous reactions (Table 4). These included local erythema, phlebitis, and an unusual delayed erythema and edematous swelling which was seen in 14 of 37 courses administered at doses ≥60 mg/sq m/day. Only one drug administration was complicated by obvious extravasation. This patient was treated immediately with local infiltration of hydrocortisone and ice packs as described for doxorubicin extravasation (3), and no skin ulceration, discoloration, or scarring occurred. Nine patients had local phlebitis or erythema at the site of infusion within 0.5 to 4 hr after infusion, and in most cases, the patency of the vein was not recovered. In 6 patients who

Table 4
Nonhematological toxicity

Dose	Evaluable courses	Local reactions	Nausea + vomiting	Other
2.5	1			
5.0	3			
10.0	1	1		
30.0	4	1		Drowsiness (1)[a]
40.0	6	2	4	
50.0	6	4	1	Allergic cutaneous reactions (1)
60.0	13	4	4	Allergic reaction (1), hypotension (1), visual loss (1)[b]
75.0	10	3	1	Fever, myalgia, and diarrhea (1)[c]
80.0	10	6	3	Anorexia (1), hypotension (1), fever, myalgia, and diarrhea (1)[c]

[a] Numbers in parentheses, number of patients.
[b] Patient sustained visual loss due to progressive disease of retroorbital tumor.
[c] Same patient.

Table 3
Hematological toxicity

Dose level	Patients	Doses	Toxic patients/evaluable patients[a]	Toxic courses/evaluable courses[a]	WBC median nadir (× 10^3/ml)	WBC nadir range (× 10^3/ml)	Median platelet nadir (× 10^3/ml)	Platelet nadir range (× 10^3/ml)
2.5	1	1	0/1	0/1	11.7	–	300	–
5.0	3	1, 1, 1	0/3	0/3	5.9	4.5–8.4	190	180–300
10.0	1	1	0/1	0/1	5.2	–	175	–
30.0	4	1, 1, 1, 1	1/4	1/4	5.3	2.7–11.9	190	180–200
40.0	6	2, 1, 1, 1, 1, 1	0/5	0/5	5.0	3.4–9.2	331	195–707
50.0	7	1, 1, 1, 1, 1, 1, 1	1/6	1/6	4.3	1.8–10.0	419	200–672
60.0	9	3, 2, 2, 1, 1, 1, 1, 1	4/9 (4/7)	6/13 (6/11)	3.0	0.7–9.9	272	139–600
75.0	7	2, 2, 2, 2, 1, 1, 1	3/6 (3/5)	4/10 (4/7)	3.5 (3.5)	1.0–10.9	306	115–479
80.0	9	2, 1, 1, 1, 1, 1, 1, 1	4/9 (4/6)	4/10 (4/6)	4.1 (1.1)	0.9–16.8	375	106–469
90.0[b]	1	3	0/1 (0/0)	0/2 (0/0)	3.2 (–)	3.2–3.2	317	397–438

[a] As noted in the text, 2 patients at the 60-, 75-, and 80-mg/sq m dosages had a baseline leukocytosis, making their comparability to patients with normal baseline WBCs questionable. The results at each level without their inclusion are shown in parentheses.
[b] A single patient who had a baseline granulocytosis (WBC > 19,000) showed a mixed response to treatment and had no granulocytopenia at lower dosages. She was therefore escalated to a 90-mg/sq m/day dose. It was not felt that other patients with base line WBC in the normal range should be entered at this dosage.

received dosages ≥60 mg/sq m/day, a different type of local reaction was observed. All of these patients had the drug administered through scalp infusion needles into extremities which had no apparent venous or lymphatic obstruction. However, within 2 to 7 days of the first infusion, nonpainful erythema and swelling were noted. In 3 patients, this was mild and persisted for less than 1 week. However, in 3 other patients, the swelling was more severe and edematous in character and persisted for 3 to 4 weeks. In one patient, skin breakdown occurred with a focal 1.5-cm full thickness ulceration forming. In these 3 patients, subsequent venous access in the affected extremities was severely compromised.

Other toxicities were mild and infrequent (Table 4). Notably, no alopecia was observed. Nausea or vomiting occurred in 13 of 54 courses and in all cases was mild. A single patient complained of anorexia during the course of drug administration. Two patients had mild allergic reactions during drug administration. Both had urticaria and pruritus, one localized to the extremity being infused and one generalized to the upper trunk. Both responded to i.v. diphenhydramine, and the latter patient received subsequent drug courses with prophylactic diphenhydramine without incident. One patient developed fever and myalgia, suggestive of a flu-like syndrome, as well as mild diarrhea during 2 treatment courses. A single patient experienced hypotension during 2 drug treatments (at 80 and 60 mg/sq m/day). Blood pressure fell to 80 mm Hg systolic while the patient was in the sitting position and rose immediately when she was placed supine. The first such incident in this patient was also associated with a transient sensation of nonradiating substernal and throat fullness. This resolved immediately when the patient was placed supine, and an electrocardiogram revealed no acute changes. She has received subsequent courses of Bisantrene without incident.

Antitumor Activity. Two patients in this study, both with hypernephroma, had objective clinical responses. A 73-year-old man who had metastatic hypernephroma with pulmonary metastases had a mixed response, with complete disappearance of a 1.8- x 1.2-cm and a 2.3- x 3.0-cm mass. A third 3.3- x 3.0-cm nodule remained unchanged in size. He had undergone a radical nephrectomy 4 years prior to his first disease recurrence. Following resection of a solitary pulmonary nodule, he was treated with depo-provera until recurrent pulmonary nodules appeared. He received 4 courses of Bisantrene at 80, 60, 60, and 60 mg/sq m/day with a clinical response noted after the second dose. He had progressive diminution of the sentinel lesions with subsequent courses of Bisantrene and continued to respond for 6 months at which time new pulmonary lesions reappeared.

The second patient, a 67-year-old woman with hypernephroma and multiple pulmonary metastases, had a mixed response with some nodules increasing in size and some decreasing following therapy. However, drug administration was also accompanied by marked improvement in her constitutional symptoms and subjective sense of well-being. Following treatment, the patient had increased appetite, weight gain, and alleviation of her prior lethargy and easy fatigability. She had been treated previously with radical nephrectomy only and no prior chemotherapy. She received 8 courses of Bisantrene at 40, 50, 60, 75, 75, 90, 90, and 90 mg/sq m/day.

No other patients demonstrated clinical responses during this trial.

DISCUSSION

This Phase I clinical study has identified dose-limiting toxic effects and certain *in vivo* antitumor effects of Bisantrene. The results indicate that this drug can be given safely in a 5-day schedule every 4 weeks and has tolerable toxicity except for local reactions, to be elaborated below. Leukopenia was dose limiting, but given in a 5-day schedule, this was dose dependent, predictable, and rapidly reversible. No cumulative myelotoxicity occurred, and neither thrombocytopenia nor anemia was observed. The present trial suggests that, in Phase II trials using a 5-day schedule, Bisantrene should be tolerable when administered as an 80-mg/sq m/day dose in patients without prior myelosuppressive therapy and as a 60-mg/sq m/day dose in patients with compromised bone marrow reserve. Of note, a Phase I trial of Bisantrene administered as a single dose reported the maximum tolerable dose to be 280 mg/sq m, with leukopenia being dose limiting (8). This suggests that the 5-day schedule may allow for administration of a 40% greater dose per course.

The major nonhematologic toxicities observed were local cutaneous reactions. These are problematic and may prove to be additionally dose limiting unless prophylactic measures or formulary changes are introduced to modify them. Similar reactions have been observed in other centers administering Bisantrene. Phlebitis occurs frequently even following single-dose administration, but both phlebitis and other cutaneous reactions appear to occur with increased frequency in the 5-day schedule[4] (8). Pretreatment with i.v. diphenhydramine and Hydrocortisone has failed to prevent reactions. Dilution of the drug into a 500-ml vehicle administered over 1 hr produced some decrease in the incidence of local reactions but was not entirely successful in preventing reactions in all patients. We have now begun drug administration in 1000 ml (final drug concentration, approximately 1 mg/ml) over 1 hr but cannot yet evaluate the effect of this dilution. It is possible that, in the concentrations used, Bisantrene exerts a direct effect on vessel endothelium, resulting in a loss of venous integrity. The drug then may be entering the perivascular space and producing an inflammatory response and cellulitis. Administration into high flow venous systems may be required, and we have now utilized femoral vein catheters for drug administration on 16 occasions without incident.

Other clinical toxicities were minimal in this trial. Mild hypotensive episodes occurred in one patient on 2 occasions (at 80- and 60-mg/sq m/day doses), but both were more mild than those reported in patients receiving single bolus administration of the drug in 240, 260, and 280 mg/sq m (8). No other patient experienced chest pain, hypotension, or tachycardia, each of which has been described in patients receiving the drug in a high-dose bolus administration. No patient demonstrated clinical cardiac abnormalities during this trial, and no electrocardiographic changes were observed acutely, during the week of drug administration, or during the weeks of follow-up observation. However, as few patients in this Phase I trial received more than 2 courses of treatment, no conclusion regarding the presence or absence of cumulative cardiac toxicity can be made. More definitive observations will come only from trials in which the drug is administered repeatedly to

[4] R. Gams, personal communication.

patients with reasonably long life expectancies.

The antitumor activity seen was encouraging and suggests that further Phase II studies are warranted. Since preclinical drug uptake studies showed the renal concentration of Bisantrene to be 120 to 150 times the concentration found in plasma, and since our 2 responses were seen in patients with hypernephroma, this may be a particularly interesting tumor type in which to further test this compound. Results of *in vitro* testing in human tumor stem cell assays also suggest that Bisantrene might have activity against renal cell carcinoma as well as melanoma (6, 7).

In conclusion, Bisantrene given daily in 5 divided doses is an attractive compound for further clinical study. Reversible leukopenia and local cutaneous reactions are the major predictable toxicities. Given daily over 5 days, a greater dose per course can be delivered than in schedules which utilize single bolus administration, and fewer acute cardiovascular side-effects are seen.

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