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Phase I Clinical Trial With Ametantrone (NSC-287513)*

MARTINE PICCART, MARCEL ROZENCWEIG†, RETO ABELE‡, EVELYNE CUMPS, PIERRE DODION, DANIEL DUPONT, DANIEL KISNER and YVON KENIS

Department of Chemotherapy, Service de Médecine et Laboratoire d'Investigation Clinique H. J. Tagnon, Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, rue Héger-Bordet 1,1000 *Brussels, Belgium*

Abstract—*Ametantrone is a new aminoanthraquinone derivative that achieves antitumor activity in a large variety of animal models. It is a dark blue dye. In this phase I study, the drug was given as a* 30-min *i.v. infusion repeated every* 2–3 weeks. *Nineteen patients were entered into the trial and received a total of* 43 *courses. All were adult patients with advanced solid tumors, mainly squamous cell carcinoma of the head and neck and nonsmall cell carcinoma of the lung. Sixteen had undergone prior chemotherapy; only one had not been previously treated. The trial was initiated at a starting dose of* 10 mg/m^2 *and dose levels were escalated up to* 180 mg/m^2. *Leukopenia was dose-related, well predictable, rapidly reversible and dose-limiting. At* 135 mg/m^2, *the median WBC nadir was* 1800/mm^3 (1000–4400) *and the median PMN was* 950/mm^3 (460–2240). *Among all courses, WBC nadir occurred on median* day 12 (8–18) *and recovery was seen on median* day 16 (10–29). *Thrombocytopenia* (<100,000/mm^3) *was encountered in two courses. There was no evidence of cumulative myelosuppression with repeated courses. Non-hematological toxic effects were negligible and included stomatitis in one course, minor alopecia in three patients, and questionably drug-related orthostatic hypotension in three patients. Reversible blue skin discoloration was seen in five patients. All patients treated with* ≧40 mg/m^2 *had dark blue urine for* 2 *or* 3 days. *Antitumor activity with response* >50% *could not be documented. Ametantrone appears to be very well tolerated and easy to handle. Its clinical anticancer potential remains to be determined. A dose-schedule of* 135 mg/m^2 q 2–3 weeks *may be recommended for phase II studies in solid tumors.*

INTRODUCTION

AMETANTRONE or 1,4-bis {2-[(2-hydroxyethyl) amino]ethylamino}-9, 10 anthracenedione diacetate (NSC-287513) is the more soluble salt of the free base (NSC-196473), the experimental antitumour activity of which was discovered by random screening at the National Cancer Institute [1]. Its anticancer potential has stimulated extensive structure–activity relationship studies of bis-(substituted aminoalkylamino)-anthraquinones [1–3]. Ametantrone is a dark blue dye; its chemical structure is shown in Fig. 1.

Antitumor activity of ametantrone has been detected in a variety of experimental murine tumors including P388 and L1210 leukemias, B16 melanoma and colon 26 [1, 4]. There is some suggestion of schedule dependency with

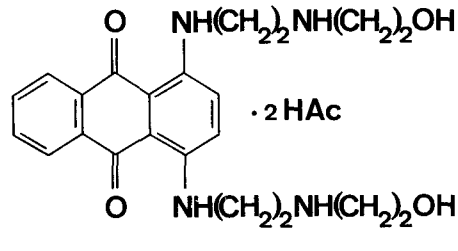

Fig. 1. *Chemical structure of ametantrone.*

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†Reprints requests and correspondence to: Marcel Rozencweig, MD, Head, Investigational Drug Section, Institut Jules Bordet, Rue Héger-Bordet 1, B-1000 Brussels, (Belgium).

‡Fellow of the E.O.R.T.C. Fellowship Programme

daily and every 4-day treatments yielding better results than a single-dose schedule [1].

The mechanism of action of ametantrone has not yet been fully elucidated. It was found to induce a nearly complete block at the G2 phase of the cell cycle [5]. Its planar quinoid ring system suggests intercalating properties. Binding to DNA is supported by its ability to stabilize DNA to thermal denaturation and to inhibit thymidine and uridine incorporation in L1210 cells [1]. Also suggestive of DNA binding is the observed resistance of anthracycline-resistant sublines of P388 leukemia to ametantrone [1, 4].

Toxicology studies were performed at the Warner–Lambert/Parke Davis Pharmaceutical Research Division, Ann Arbor, Mi. In mice, the LD_{10}, LD_{50} and LD_{90} were 22, 67 and 206 mg/kg, respectively, with single i.p. injections. In dogs given one or two intravenous injections of ametantrone, the principal target organs of toxicity were the bone marrow, lymphoid tissue, gastrointestinal tract and testes. These experiments revealed the following toxicity parameters: lethal dose = 108.2 mg/m^2, toxic dose high = 54.2 mg/m^2, toxic dose low = 13.6 mg/m^2, highest non-toxic dose = 6.8 mg/m^2. The cardiac effect of ametantrone was evaluated in rats. Preliminary data indicated a lesser potential for cardiotoxicity of ametantrone relative to conventional anthracylines [4].

The pharmacological fate of ametantrone was studied in Beagle dogs, using a high-pressure liquid chromatograph assay [6]. After i.v. administration of the drug at a dose of 300 mg/m^2, the initial plasma $t_{1/2}$ of the agent was 9.4 min and the terminal $t_{1/2}$ was 115.2 min. A maximal plasma concentration of 24.9 mg per liter was attained. In 5 hr, biliary and urinary excretion amounted to 39.5 and 24% of the administered drug, respectively. A trace of a metabolite was detected in the urine.

This paper reports the results of a phase I clinical trial with ametantrone. The trial was designed to define a maximum tolerated dose with single intermittent i.v. treatments.

MATERIALS AND METHODS

All patients had histologic confirmation of advanced solid malignancy. Expected survival was longer than 6 weeks. All patients had leukocyte counts of $\geqq 4000/mm^3$ and platelet counts of $\geqq 100{,}000/mm^3$. Bilirubin and creatinine serum levels did not exceed 2 mg% in any patients.

The starting dose was extrapolated from the LD_{10} of ametantrone in mice [7] and from existing phase I data with mitoxantrone, a closely related derivative [8]. Dose escalation steps followed a modified Fibbonacci series. Escalations within the same patient were carried out if no significant toxicity was encountered in previous courses. Initially, the drug was given as a single administration repeated every 2 weeks. Whenever toxicity occurred, patients were retreated upon complete recovery from toxic effects and the interval between treatments was extended.

During the study period, three complete blood cell counts and one SMA 12 chemistry panel were scheduled per week. In this analysis, the leukocyte nadir for patients receiving two or more courses at the same dose level represents the lowest cell count among all courses. In patients with measurable disease, tumor response was assessed every 3 weeks according to standard criteria of response [9].

Ametantrone was supplied by the Warner-Lambert Co. Morris Plains, NJ, as a lyophilized powder, 30 mg/vial. The drug was diluted in 150 ml of 5% dextrose in water and given as a 30-min i.v. infusion.

Prior to entry, informed consent was obtained from the patients according to institutional policies.

RESULTS

A total of 19 patients entered the trial (Table 1). There were 13 men and 6 women. Their median age was 56 years with a range between 40 and 75 years; the median perfor-

Table 1. *Patient characteristics*

Total number entered	19
Male:female	13:6
Age:	
Median	56
Range	40–75
Performance status:	
Median	70
Range	40–90
Primary tumors:	
Head and neck	9(2)*
Lung	4
Breast	2
Melanoma	2
Osteosarcoma	1
Renal	1(1)*
Previous treatment:	
Chemotherapy only	2
Radiotherapy only	2
Chemo- + radiotherapy	14

*( ) = No. of patients with 2 malignancies.

mance status on the Karnofsky scale was 70, ranging from 40 to 90. Nine patients had tumors of head and neck origin: squamous cell cancer (7), adenocarcinoma (1) or adenoid cystic carcinoma (1). Two of these patients had a second malignancy, i.e. lung cancer and breast cancer. The other patients had nonsmall cell carcinoma of the lung (4), breast cancer (2), melanoma (2), osteosarcoma (1) and renal cell cancer (1). The latter also had melanoma. All but one patient had been previously treated with surgery (16), radiotherapy (16) and/or chemotherapy (16).

Doses were escalated from 10 to 180 mg/m^2. New patients were entered at each dose level. The median number of courses per patient was two, and no patient received more than four courses. A total of 43 courses were given.

Myelosuppression could be adequately evaluated in 41 courses. Ametantrone induced well predictable, dose-related and dose-limiting leukopenia (Table 2). This effect was first noted at the dose of 40 mg/m^2 in one of three patients. At 60 mg/m^2, white blood cells (WBC) below 2000/mm^3 were seen in two patients. One of these had rapidly progressive disease, bone marrow involvement and splenomegaly. The other patient had pleural effusion and had received mitomycin 4 weeks before entering the trial. Retreatment at the same dose level did not produce leukopenia in this patient. A more favorable selection of patients were entered subsequently and a slow decrease in the WBC nadir was found with doses increasing from 75 to 180 mg/m^2. Six patients received eight evaluable courses at 135 mg/m^2. At this dose level, the median nadirs of the WBC and the polymorphonuclear cells were 1800/mm^3 (1000–4400/mm^3) and 950/mm^3 (460–2240/mm^3) respectively. The corresponding figures for the three patients who received four evaluable courses at 180 mg/m^2 were 1400/mm^3 (600–3200/mm^3) and 800/mm^3 (70–1630/mm^3). The median time to nadir was day 12 after drug administration at 135 and 180 mg/m^2. With the former dose, all but one patient had a return to normal WBC ($\geqq$4000/mm^3) by day 16. There was no evidence of cumulative myelosuppression with repeated treatments.

The drug does not seem to affect the platelet count to any significant extent (Table 3). Only two patients experienced thrombocytopenia with platelet counts below 100,000/mm^3.

Non-hematological toxic effects were evaluated in 42 courses and appeared to be minimal (Table 4). All patients had dark blue urine for 24–72 hr at doses of 40 mg/m^2 or more. At doses of 135–180 mg/m^2, almost all patients developed generalized grayish or blue skin discoloration that lasted for several days. Skin discoloration was marked in five patients. This effect did not appear to be cumulative with retreatments.

Mild alopecia was noted in three patients. Other toxic effects were questionably drug-related and consisted of orthostatic hypoten-

Table 2. Drug-induced leukopenia

Dose (mg/m^2)	No. of patients/ No. of courses	WBC ($\times 10^3$/mm^3)	PMN ($\times 10^3$/mm^3)	Median time to: Nadir	Recovery*
10	3/4	8.2 (6.8–12.5)	6.0 (5.2–11.4)	—	—
20	2/3	6.6 (5.2–8.0)	4.4 (3.9–5.0)	—	—
40	3/6	5.1 (2.2–6.0)	3.4 (2.2–4.7)	11 (8–12)	20 (18–26)
60	5/7	3.3 (1.2–4.4)	2.7 (0.9–3.2)	12 (10–18)	21 (17–25)
75	4/5	4.1 (2.0–5.4)	2.5 (1.5–3.4)	11 (10–14)	13 (12–14)
100	3/4	3.0 (2.5–3.2)	1.9 (1.8–2.5)	9 (8–12)	12 (10–14)
135	6/8	1.8 (1.0–4.4)	1.0 (0.5–2.2)	12 (10–15)	15 (13–29)
180	3/4	1.4 (0.6–3.2)	0.8 (0.1–1.6)	12 (10–15)	17 (15–25)

*WBC$\geqq$4000/mm^3.

Table 3. Drug-induced thrombocytopenia

Dose (mg/m^2)	No. of patients/ No. of courses	Nadir platelets $\times 10^3/mm^3$ Median	Range
10	3/4	271	219–430
20	2/3	285	240–330
40	3/6	224	175–355
60	5/7	219	80–275
75	4/5	250	215–280
100	3/4	251	250–280
135	6/8	176	60–400
180	3/4	150	150–189

sion in three patients and one episode of minor stomatitis. Transient EKG abnormalities were found in two patients, but identical changes had already been noted in these patients prior to ametantrone treatment.

Patients were closely monitored for possible disease regression. None of these experienced partial remission according to standard criteria. One patient with renal cell cancer and melanoma had a very brief shrinkage in one of her lung metastases. One patient with adenoid cystic carcinoma had an apparent stabilization of her disease.

DISCUSSION

Ametantrone is a new anticancer agent which is clinically well tolerated and easy to handle. Toxic manifestations include essentially neutropenia and skin discoloration. Leukopenia is well predictable and increases moderately with increasing doses. A dose–response relationship may, however, not be accurately estimated from Table 2 because of re-entries according to previous toxicity. Neutropenia was dose-limiting but was rapidly reversible in a majority of patients. In our experience, a dose of $135\,mg/m^2$ seemed suitable for outpatient treatments despite a median PMN nadir of $950/mm^3$.

Skin discoloration was difficult to quantify, but was generally mild. No risk factor could be clearly identified in the five patients who developed marked blue discoloration. Other toxic effects encountered with ametantrone were negligible. Orthostatic hypotension was striking in three patients, but was not clearly drug-related.

Mitoxantrone is a dihydroxylated ametantrone derivative which has been recently introduced in phase II trials. The maximum tolerated dose of the former was reported to be $14\,mg/m^2$ [8] and its higher potency seems favorable in that a lower load of dye is required to achieve similar biologic activity. In contrast, the dose–response relationship observed in the phase I trial of mitoxantrone was much steeper than with ametantrone, which may be a disadvantage for a wide clinical use of this drug.

Anthracenedione derivatives are structurally related to anthracycline antibiotics. Animal studies suggest cross-resistance to these compounds and have indicated the cardiotoxic potential of these new derivatives. There was no firm evidence of a cardiac effect of ametantrone in this study. More prolonged treatments would be required to rule out possible chronic damages to the heart.

No clear antitumor activity could be documented with this drug in our trial. However, patients included in the study had usually chemoresistant tumor types or were extensively pretreated. In addition, a number of patients received suboptimal doses of ametantrone. Prior therapy with adriamycin could also account at least for part of these findings.

We have recently undertaken a pharmaco-

Table 4. Non-hematological toxic effects

Dose (mg/m^2)	No. of patients/No. of courses	No. of patients with: Blue skin discoloration	Hair loss	Orthostatic hypotension	EKG changes	Stomatitis
10	3/4	—	—	—	—	—
20	2/3	—	—	—	—	—
40	3/6	—	—	1	—	—
60	5/7	—	—	—	1	—
75	4/5	1	—	—	1	—
100	3/4	—	1	—	—	—
135	7/9	2	1	2	—	1
180	3/4	2	1	—	—	—

kinetic study with ametantrone using a high-pressure liquid chromatograph assay described earlier [6]. Blood and urine samples have been analyzed in four patients with normal renal and hepatic functions receiving 135–180 mg/m^2 of ametantrone over a 15- to 30-min period. Preliminary results indicate that peak plasma levels vary between 4.1 and 15 μg/ml. Peaks occur at the end of the infusion and are followed by a rapid decline in plasma levels down to the limit of sensitivity of the assay (0.5 μg/ml) with an initial $t_{1/2}$ ranging from 8 to 16 min. Urinary recovery appears to be nearly complete within 48 hr and accounts for 10–33% of the administered dose. No metabolite has been identified. Thus currently available data suggest a similar drug disposition in man and in dogs. More complete elucidation of the clinical pharmacology of ametantrone will probably require a more sensitive assay.

Ametantrone leads a series of new compounds with interesting potential for cancer chemotherapy. Delineation of the therapeutic role of this compound and its derivatives must still await phase II investigations. A dose schedule of 135 mg/m^2 repeated every 2–3 weeks may be recommended to assess the antitumor activity of ametantrone in solid tumors

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