Eur J Cancer Clin Oncol, Vol. 23, No. 11, pp. 1621–1626, 1987.
Printed in Great Britain

0277-5379/87$3.00+0.00

Elliptinium, a DNA Intercalating Agent with Broad Antitumor Activity in a Human Tumor Cloning System

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Abstract—*We have utilized a human tumor cloning system to determine the* in vitro *antitumor effects of elliptinium, a new DNA intercalating agent. The purpose was to determine which human tumors should be studied in phase II clinical trials with this agent. Eighty-eight out of* 282 *tumors plated in culture were evaluable for drug-sensitivity assays. The overall* in vitro *response rate (defined as a* ≤50% *survival of tumor colony-forming units compared to control) was* 28% *at* 0.4 μg/ml (1/10 *of peak plasma level*). In vitro *activity was noted for elliptinium against breast cancer, renal cell carcinoma, small-cell lung cancer and non small-cell lung cancer. Elliptinium had a higher* in vitro *activity than adriamycin against this same group of tumors. Six of* 25 (24%) *adriamycin-resistant tumors were sensitive to elliptimium. Our* in vitro *response rate in breast cancer correlates with the response rate in phase II clinical trials with this drug. Further phase II clinical trials with elliptinium in patients with renal cell carcinoma, non small-cell lung cancer and small-cell lung cancer are indicated.*

INTRODUCTION

ELLIPTINIUM (9-hydroxy-2-*N*-methylellipticine) is a synthetic quaternary derivative of the indole alkaloid ellipticine that has high affinity for DNA, intercalating between base pairs [1]. Phase I trials in humans revealed very little to no myelosuppression. Dose-limiting toxicities included xerostomia, phlebitis, emesis and reversible azotemia [2, 3]. The xerostomia is probably related to the accumulation of the drug in the salivary glands [4] and the documented affinity of this drug for muscarinic receptors [5]. The renal dysfunction has a tubular etiology [6]. An important toxicity of elliptinium is acute intravascular hemolysis, seen more often with weekly rather than less frequent schedules of administration [7]. The development of an assay for anti-elliptinium antibodies may reliably predict which patients will develop this complication [7]. Although a dose of 100 mg/m^2 i.v. every week had been recommended for phase II clinical trials, because of an appreciation of the risk of hemolysis with this schedule, for future trials a dose of 80 mg/m^2/day for 3 days every 3 weeks is planned (A. Goodman, personal communication). A small number of trials in patients with a limited group of tumor types has been reported [8–15]. These trials have mainly demonstrated a significant antitumor activity in patients with previously treated advanced breast cancer.

The human tumor cloning system first described by Hamburger and Salmon [16, 17] is still widely used for screening new chemotherapeutic agents for *in vitro* activity against human tumors. As suggested by Shoemaker *et al.*, this system can identify active new agents potentially missed by the conventional xenograft moels [18]. Drug screening with this system is particularly feasible with breast, colorectal, kidney, lung, melanoma and ovarian tumors [18]. Another application of this methodology is the identification of specific tumor types that warrant phase II clinical trials with the drug(s) being tested in the system.

We tested the *in vitro* activity of elliptinium in a human tumor cloning system against a variety of primary human malignancies. In this system, elliptinium showed a broad spectrum of *in vitro* antitumor activity especially against breast cancer, renal cell carcinoma, small-cell lung cancer and non small-cell cancer. These results provide a basis for clinical testing of elliptinium in these malignancies.

Accepted 18 May 1987.
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MATERIALS AND METHODS

After informed consent, a total of 282 patients underwent surgery, thoracentesis, paracentesis or bone marrow aspiration with biopsy, as part of diagnostic or therapeutic work-up. Excess material consisting of solid tumor, pleural fluid, ascites or bone marrow was sent for cloning in soft agar and drug-sensitivity studies. Seventy-two per cent of the patients had received prior chemotherapy with 123 having received prior adriamycin.

Collection of cells

Effusions were collected in preservative-free heparinized vacuum bottles, centrifuged at 150 ***g*** for 10 min and washed twice in Hank's balanced salt solution with 10% heat-inactivated fetal calf serum plus 1% penicillin and streptomycin (all materials, GIBCO, Grand Island, NY). Bone marrow specimens were collected in heparinized syringes and processed in a similar way as the fluid specimens, only that after centrifugation the buffy coat was removed and processed. Solid tumors were, immediately after surgery, placed in McCoy's 5A medium (GIBCO, Grand Island, NY) plus 10% fetal calf serum plus 1% penicillin and streptomycin and transported within 1 h to the laboratory, where they were mechanically dissociated by forcing through a wire mesh gauze into Hank's balanced salt solution plus 10% fetal calf serum. They were then passed through progressively smaller needles and processed in the same manner as were the effusions. Viability of cells was determined by trypan blue dye exclusion. Only viable cells were counted for further plating in agar.

In vitro *drug exposure to elliptinium*

Stock solutions of elliptinium were prepared in sterile distilled water and stored at −70°C in aliquots sufficient for individual assays. Subsequent dilutions for incubation with cells were made with sterile distilled water. For all tumor types, a single concentration of 0.4 μg/ml of elliptinium was utilized. This represents approx. 1/10th of the peak plasma level after conventional intravenous doses of the drug [19]. Exposure of the malignant cells for 1 h to 1/10th of the peak plasma level can appropriately predict for a clinical response [20]. Breast cancer specimens were exposed to an additional concentration of 4.0 μg/ml of elliptinium. They were also tested with 0.04 μg/ml of adriamycin (1/10th of peak plasma level).

Tumor cell suspensions were transfered to tubes and adjusted to a final concentration of 10^6 cells/ml in the presence of the appropriate drug dilution(s) or sterile distilled water (controls). Cells were incubated with or without elliptinium for 1 h at 37°C in Hank's balanced salt solution. They were then washed twice with Hank's balanced salt solution and prepared for culture.

Assay for tumor colony-forming units (TCFUs)

The human tumor cloning system utilized was a modification of the two-layer system described by Hamburger and Salmon [17]. Cells to be tested were suspended in 0.3% agar in enriched CMRL medium 1066 (Irvine Scientific) containing 15% horse serum to yield a final concentration of 5×10^5 cells/ml. One milliliter of this mixture was pipetted into 35 mm Petri dishes containing 1 ml of 0.5% agar in enriched McCoy's 5A medium but without conditioned medium. All drug concentrations and controls were set up in triplicate. Cultures were incubated at 37°C in 7% CO_2 in humidified air. Colonies (≥ 50 cells) were counted manually on an inverted-stage microscope at 30 × 10–15 days after plating. At least 20 tumor colonies per control plate were required for an experiment to be considered evaluable for measurement of a drug effect. In order to assure that a good single cell suspension had been plated, in some experiments, colony counts were performed immediately after plating with a Bausch and Lomb FAS II automatic counter. Colony count on day 1 needed to be ≤ 30% of the colony count on days 10–15 for the experiment to be considered evaluable.

Data analysis

Colony counts of the three plates for a particular drug concentration were averaged to obtain one data point. For determination of sensitivity to a particular drug, the ratio between the number of colonies surviving at each drug concentration and the number of colonies growing in control plates was calculated. A ≤ 50% survival of tumor colony-forming units (TCFUs) was considered as a definition of *in vitro* antitumor activity. This cutoff of ≤ 50% TCFUs survival has shown a reasonably good predictive value for clinical response [20].

RESULTS

A total of 282 tumors were plated in culture and had elliptinium tested against them. Eighty-eight (31%) formed ≥ 20 colonies on control plates and were evaluable for drug sensitivity. Of these, 71 (80%) had prior exposure to adriamycin. Cloning efficiency for the evaluable specimens varied between 0.004% and 0.104% with a median of 0.01%. In 14 experiments, colony counts on day 1 were available. In all of them, the day 1 count was ≤ 10% of the colony count at the end of the experiment (data not shown). This indicates that at least in this group of experiments an adequate single cell suspension was plated.

Table 1 summarizes the evaluable specimens against which 1 h exposure to 0.4 μg/ml of ellipti-

Table 1. *Summary of* in vitro *activity of elliptinium by tumor type*

Tumor type	No. of evaluable specimens	No. of *in vitro* response (≥50% decrease TCFUs)	Percentage response
Brain	1	0	0
Breast	11	3	27
Colon	10	2	20
Kidney	9	3	33
Lung (non small-cell)	14	6	43
Lung (small-cell)	8	5	63
Melanoma	4	1	25
Mesothelioma	1	0	0
Ovary	25	4	16
Pancreas	1	1	100
Stomach	2	0	0
Thyroid	1	0	0
Unknown primary	1	0	0
Total	88	25	28

Table 2. *Summary of* in vitro *sensitivity of all tumor types to elliptinium according to prior treatment*

	No. of evaluable specimens	*In vitro* responses*	Percentage	
Prior therapy	42	9	21	
				$P = 0.29$†
No prior therapy	46	16	35	
All	88	25	28	

*≥50% decrease in tumor colony-forming units.
†Chi-square analysis.

nium caused ≥ 50% decrease in tumor colony-forming units. *In vitro* antitumor activity was noticed against breast cancer, renal cell carcinoma, non small-cell lung cancer and small-cell lung cancer. A lesser degree of activity was noticed against ovarian and colon cancer. One evaluable pancreatic cancer specimen showed 92% decrease in TCFUs when exposed to elliptinium. There were not enough specimens in the other histological types of tumors to show that the drug is inactive against them *in vitro*.

As illustrated in Table 2, prior exposure to chemotherapy did not seem to influence the possibility of *in vitro* sensitivity to elliptinium.

Ten breast cancer specimens were exposed for 1 h to an additional concentration of 4.0 μg/ml of elliptinium. Figure 1 illustrates the dose–survival curves for these breast cancer specimens. The dose–response curve for these tumors was flat past an elliptinium concentration of 0.4 μg/ml.

Twenty-six evaluable specimens had elliptinium (0.4 μg/ml) and adriamycin (0.04 μg/ml) tested against the same specimen. Both drug concentrations represent 1/10 of peak plasma levels.

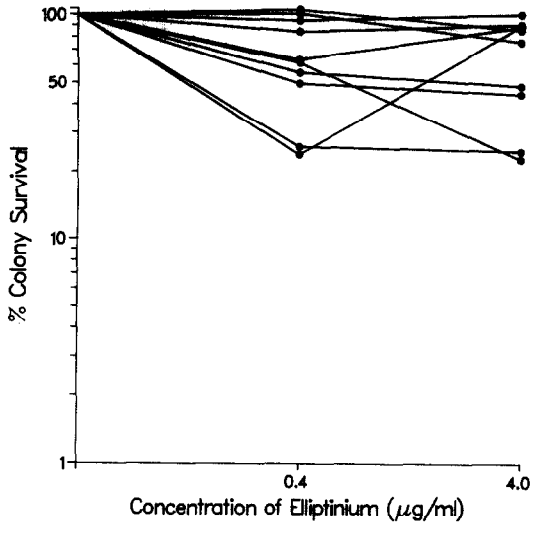

Fig. 1. *Dose survival curve for elliptinium against human breast cancer studied in the human tumor-cloning system. Percentage survival of TCFUs is represented along the vertical axis and* in vitro *concentration of the drug* (1 h *exposure*) *along the horizontal axis.*

Twenty of these specimens were from patients who had failed prior adriamycin. Results are summarized in Fig. 2. Twenty-five of 26 (96%) specimens were resistant to adriamycin. Seven of 26 tumor specimens (27%) were sensitive to elliptinium and six of these were selectively sensitive to this drug, when compared to adriamycin. Of these six tumors, four were small-cell lung cancer and two were large-cell lung cancer specimens. All of these tumors had had prior exposure to adriamycin. In no instance was adriamycin more active than elliptinium when tested simultaneously against a variety of human tumor types in our cloning system.

Table 3 summarizes all the phase II clinical trials to date with elliptinium in different human malignancies. Despite significant prior chemo-

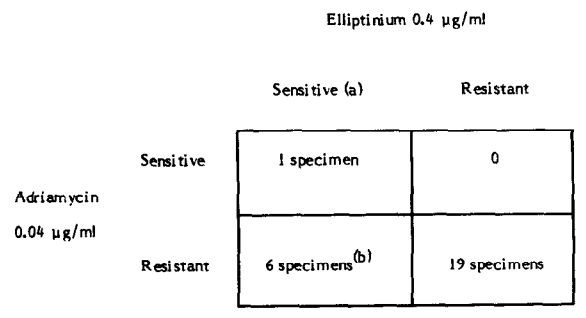

Fig. 2. Activity of adriamycin and elliptinium in the same patient's tumors in the human tumor cloning sytem (n = 26).

therapy, there is significant (for a single agent) antitumor activity in breast cancer patients, for a mean response rate of 25%. Responses in the limited number of phase II clinical trials in few other tumor types have not been as impressive. The only trial with elliptinium in lung cancer patients included only those with squamous histology [10]. There have been no trials in patients with small cell lung cancer.

DISCUSSION

We have investigated the *in vitro* antitumor activity of the DNA intercalating agent, elliptinium (9-hydroxy-2-*N*-methylellipticine), in a human tumor-cloning system. Our objective was to identify which tumor types were sensitive to the drug. Secondly, we wanted to investigate the *in vitro* cross-resistance between elliptinium and another DNA intercalating agent, adriamycin.

In this study, elliptinium demonstrated a broad spectrum of *in vitro* antitumor activity in a human tumor-cloning system. This activity was highest against breast cancer, renal cell carcinoma, non small-cell and small-cell lung cancer. In these same tumors, the *in vitro* cytotoxic activity of elliptinium exceeded the cytotoxic activity of other conventional marketed antineoplastics like adriamycin and cisplatin (data not shown with the latter). The concentration of elliptinium used for *in vitro* 1 h exposure of tumor cells roughly corresponds to one tenth of peak plasma level in humans or one tenth of the area under the curve for 1 h when using doses of 100 mg/m^2 [19]. Since these concentrations for established agents can appropriately predict for a clinical response [20], it is likely that these results reflect the therapeutic efficacy of elliptinium rather than its pharmacological potency.

Most of the patients entered in this *in vitro* trial had been previously exposed to cytotoxic chemotherapy (72%). Since most patients in phase II clinical trials will also have had prior chemotherapy, our tumor population represents a realistic situation for predicting clinical response of new anticancer agents in early clinical testing. Prior treatment did not

Table 3. Phase II trials of elliptinium

Tumor type	Evaluable patients	Dose	Prior chemotherapy	Responses CR	Responses PR	Percentage response	Reference
Breast	63	100 mg/m^2 i.v.qw	51	2	10	19	[8]
Breast	135	100 mg/m^2 i.v.qw	69	0	44	32	[9]
Breast	36	100 mg/m^2 i.v.qw	35	1	6	19	[10]
Breast	7	100 mg/m^2 i.v.qw	7	0	2	29	[11]
Renal	38	100 mg/m^2 i.v.qw	10	1	4	13	[12]
Renal	8	100 mg/m^2 i.v.qw	4	0	0	0	[13]
Sarcoma	19	100 mg/m^2 i.v.qw	19	0	0	0	[14]
Lymphoma	18*	100 mg/m^2/day × 3 q 3 ws	18	0	1†	6	[15]
Lung‡	21	100 mg/m^2 i.v.qw	4	0	1	5	[10]

*Fourteen non-Hodgkin's and four Hodgkin's lymphoma.
†Non-Hodgkin's lymphoma.
‡All were squamous cell histology.

affect the *in vitro* response rate to elliptinium as shown in Table 2.

At similar concentrations (1/10 of peak plasma levels), elliptinium showed antitumor activity in six of 25 tumors that were resistant *in vitro* to adriamycin. Remarkably, four of these were small-cell lung cancer specimens, and two were non small-cell lung cancer. All of these tumors had had a prior exposure to adriamycin. These results suggest that in some previously treated tumors the efficacy of elliptinium may be different from that of its clinical analog, adriamycin.

Finally, our *in vitro* results with elliptinium against breast cancer tumors correlate with the results of phase II clinical trials with this drug in patients with advanced metastatic breast cancer (27% vs. 25%, respectively). In renal cell carcinoma only two trials have been reported (see Table 3). The response rate in the trial by Caille *et al.* [12] is certainly no worse than the one reported for other single conventional agent trials [21]. The clinical experience with elliptinium in lung cancer is rather limited with only one phase II trial in squamous cell lung cancer reported, and none in small-cell lung cancer. There was one partial response in a patient with anaplastic small-cell lung cancer in a phase I trial [3].

The results of this *in vitro* study, the lack of total cross-resistance with adriamycin, and the lack of myelosuppression make elliptinium a potentially good drug for incorporation into drug combinations against small-cell lung cancer. The lack of myelosuppresssion and of cardiotoxicity should encourage a head-to-head comparison with adriamycin and/or incorporation into drug combinations in patients with metastatic breast cancer.

The frequency of immune hemolytic anemia secondary to elliptinium is higher than that of melphalan, cisplatin and teniposide [22]. Approximately 20% of patients receiving weekly courses of elliptinium will develop anti-elliptinium antibodies. In one prospective study of 146 patients undergoing treatment with this drug, 10 patients (6.8%) developed a hemolytic episode [23]. Screening tests for anti-elliptinium antibodies are available and are recommended before each drug dose [6, 24]. In one study, the presence of an antibody titer of $<1:32$ accurately predicted for the absence of risk for hemolysis [7]. In this same study, patients receiving 80 mg/m^2/day for 3 days every 3 weeks did not develop anti-elliptinium antibodies or hemolysis [7]. Routine measurement of the anti-elliptinium antibody titer, the use of a dose of 80 mg/m^2/day for 3 days every 3 weeks in future trials, and discontinuation of treatment if the antibody titer is $\geq 1:32$ should decrease significantly the risk of elliptinium-induced hemolysis. Reversible azotemia has also been reported with elliptinium. Preliminary data suggests that aggressive hydration similar to that used for cisplatin may prevent or decrease the nephrotoxicity of elliptinium [25].

In conclusion, elliptinium demonstrated *in vitro* antitumor activity against breast cancer, renal cell carcinoma, non small-cell and small-cell lung cancer as well as lack of total cross-resistance with adriamycin. Further *in vitro* testing of elliptinium against these and other tumor types should continue. The data presented here and the limited phase II clinical information on elliptinium may lead to further phase II clinical trials with this drug in patients with the above mentioned tumors.

Acknowledgements—This work is supported in part by a grant from Wyeth Laboratories. Karla Clark prepared this manuscript.

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