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In Vitro Activity of the Novel Antitumor Antibiotic Fostriecin (CI-920) in a Human Tumor Cloning Assay

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Abstract—*A human tumor cloning assay was utilized to evaluate the antineoplastic activity of the novel antitumor antibiotic fostriecin (CI-920). Initial screening with* 10.0 mcg/ml *continuous exposure against a variety of histologic tumor types resulted in* 14/51 (27%) in vitro *responses (defined as* > 50% *decrease in TCFUs). Further investigation of the compound was performed in* 1-hr *preincubation experiments. The* in vitro *response rate at a concentration of* 1.0 mcg/ml *(which was considered to correspond to a clinically achievable concentration) was* 15/43 (35%). *Response rates for specific tumor types included:* 5/15 *in ovarian cancer,* 5/12 *in breast, and* 4/11 *in human lung cancer. The impression of significant antitumor activity of the compound at this dose was further substantiated by comparing its* in vitro *activity with a variety of simultaneously tested standard anticancer agents. In addition, these data indicated the possibility of non-cross resistance of CI-920 to several established cytostatics. CI-920 is a compound with good* in vitro *activity which should be further developed for clinical trials.*

INTRODUCTION

FOSTRIECIN (CI-920) is a structurally novel phosphate ester antitumor antibiotic (Fig. 1) that has recently been isolated from a subspecies of *Streptomyces pulveraceus* [1,2]. While strong antineoplastic activity was documented against murine leukemia and a broad spectrum of other cell lines *in vitro* [3,4], limited activity was found against solid mouse tumors or human tumor xenografts [4]. Elucidation of the structure of the compound [5], and subsequent studies on the biochemical mechanism of CI-920 [6,7] strongly suggested that the lack of solid tumor activity in mice may be caused by a reduced folate transport deficiency. However, despite this transport similarity to methotrexate and its classical analogues, the antitumor mechanism of CI-920 appears to be directly related to an inhibition of macromolecular synthesis [6], rather than by antifolate effects.

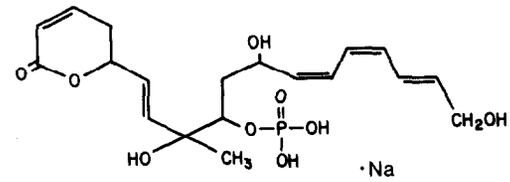

Fig. 1. Chemical structure of the new antitumor antibiotic fostriecin (CI-920) (2-H-pyran-2-one,5,6-dihydro-6-{3,6,13-trihydroxy-3-methyl-4-(phosphonoxy)-1,7,9,11-tridecatetraenyl}-monosodium salt).

The main objective of the present investigation was to utilize a human tumor cloning assay to screen for *in vitro* activity of this new agent. Should this compound proceed into clinical trials, the cloning assay might predict areas to be explored in initial phase II studies.

MATERIALS AND METHODS

Collection of cells

Histologically confirmed malignant tumors were obtained from 338 patients during the course of routine diagnostic and therapeutic surgical procedures. All patients had given their informed consent for removal of the tumor specimen. Fifty-six per cent of the patients had been previously treated with chemotherapy, 32% had no prior therapy,

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and no information was available from 10%. The mode of collecting, processing and culturing the tumor samples has been previously described in detail [8.9]. Briefly, malignant effusions and bone marrow aspirates were collected in preservative-free heparin solution, and were washed twice in tissue culture medium enriched with 10% heat-inactivated fetal calf serum (all reagents were obtained from Grand Island Biological Co., Grand Island, NY). Solid tumor specimens were processed by mechanical dissection, passage through a #400 stainless steel screen (E-C. Apparatus Corp., St. Petersburg, FL), and serial passage through progressively smaller needles until a single cell suspension was obtained.

Drugs

Fostriecin (CI-920) was supplied by Dr. Edward Elslager, Warner–Lambert/Parke–Davis Pharmaceutical Research, Ann Arbor, MI. The solid form of the compound was stored in a dessicator at −20°C. Aqueous solutions were prepared immediately before use to avoid loss of activity. In order to evaluate the overall antitumor activity of the compound, the agent was initially screened at 10 mcg/ml as a continuous exposure. After recognition of a significant *in vitro* response rate, further investigation of the drug was performed at 1.0 and 10.0 mcg/ml as 1-hr drug exposure. Since the drug has not entered phase I clinical trials, no pharmacokinetic data are available in man; however, we based the *in vitro* cut-off concentration of CI-920 (1 mcg/ml for 1 hr) on an LD_{50} of approx. 100 mg/kg in (normal) mice. It is believed that this toxicity data might yield a clinically representative initial starting dose for *in vitro* screening of new antitumor drugs*. A number of other chemotherapeutic agents that were simultaneously tested in order to obtain some information about the relative antitumor potency of CI-920 were standard and investigational agents. The final concentrations corresponded to approx. 1/10 of the peak plasma level in man [10] (see Table 2). Chemosensitivity testing was performed by exposing 3×10^6 cells/ml to the appropriate drug dilution or control medium. After incubation for 1 hr at 37°C, the cells were centrifuged, washed twice in Hank's balanced salt solution plus 10% FCS, and prepared for culture. For continuous exposure studies the drug was incorporated into the plating medium for the entire duration of culture. All colony inhibition assays were done in triplicate, and for each tumor tested, at least three control plates were included. To make sure a good single cell suspension was present, a positive control consisting of chromomycin A3 (100 mcg/ml) was utilized [11].

*S. E. Salmon, personal communication.

Culture technique

The basic double layer soft agar technique used in this study was that of Hamburger and Salmon [12,13]. Briefly, 5×10^5 cells/ml, suspended in 0.3% agar in enriched CMRL 1066 medium were plated into 35 mm Petri dishes, containing 1 ml feeder layer. The latter consisted of 0.5% agar, 15% FCS and McCoys 5A medium supplemented with various nutrients [14]. The cultures were incubated at 37°C in a 7% humidified atmosphere of CO_2.

Data analysis

Control and drug treated plates were scored 14–18 days after initiation of the culture using an electronic FAS II Image Analysis Scanner (Bausch and Lomb, Inc., Rochester, NY). The antineoplastic effects of a particular compound were calculated by comparing the mean number of colonies in drug treated and control plates. *In vitro* response was defined as per cent inhibition of tumor colony growth (TCFU) by more than 50% of the corresponding control count. At least 20 colonies per control plate as well as a < 30% survival in the chromomycin plates (positive control) were required for an experiment to be considered evaluable for measuring drug effects.

RESULTS

Among 338 samples submitted to the laboratory and screened against CI-920, 98 experiments (29%) were considered adequate for drug sensitivity information (> 20 colonies on control plates with a good positive control). Table 1 summarizes the *in vitro* activity of CI-920 in the continuous (10 mcg/ml, 51 patients), and the subsequent 1-hr (1.0 and 10.0 mcg/ml, 47 patients) screening mode. These two series represented different patient populations.

At 10.0 mcg/ml as a continuous exposure, 14 of the 51 evaluable tumors (27%) had an *in vitro* response (defined as < 50% survival of TCFUs) (see Table 1). Responses were noted against 2/14 ovarian cancers, 1/10 breast cancer, 1/4 adenocarcinoma of unknown primary site, and against one case each of acute myelogenous leukemia and Hodgkin's lymphoma. Most notable was the *in vitro* sensitivity of 6/10 non-small cell and 2/3 small cell lung cancers.

The impression of fairly good activity against lung cancer of all histologies was confirmed in the subsequent series of 1-hr exposure experiments (Table 1). In addition, at a concentration of 1.0 mcg/ml a 42% and 33% response rate were noted against human breast and ovarian cancer, respectively. The precise reason for the lesser degree of activity at the higher dose level at a 1-hr exposure in both tumor types is not clear. The

Table 1. Antitumor activity of CI-920 in the human tumor cloning assay

Tumour type	*In vitro* drug sensitivity (> 50% Decrease of TCFUs)					
	Continuous		1 hr			
	(10mcg/ml)		(10mcg/ml)		(1.0mcg/ml)	
Ovary	2/14	(14%)	2/17	(12%)	5/15	(33%)
Breast	1/10	(10%)	4/12	(33%)	5/12	(42%)
Lung/non-small cell	6/10	(60%)	4/10	(40%)	3/8	(38%)
small cell	2/3	(67%)	1/3	(33%)	1/3	(33%)
Colon	0/3	(0%)	0/2	(0%)	0/2	(0%)
Unknown primary	1/4	(25%)	0/1	(0%)	1/1	(100%)
Hodgkin's lymphoma	1/1	(100%)	–		–	
Acute myel. leukemia	1/1	(100%)	–		–	
Miscellaneous*	0/3	(0%)	0/2	(0%)	0/2	(0%)
Total	14/51	(27%)	11/47	(23%)	15/43	(35%)

* Including cervix, kidney, mesothelioma, myeloma, pancreas (continuous); brain and thyroid (1-hr drug exposure).

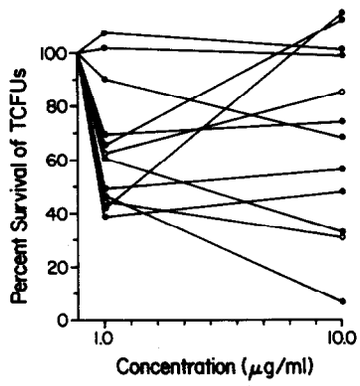

Fig. 2. In vitro activity of CI-920 against 12 breast cancer specimens in a human tumor cloning assay using a 1-hr drug exposure. Each point represents the mean number of colonies surviving for three plates. Only two patients in this series (marked with closed circles) had not received prior chemotherapy.

observed heterogeneity in dose response effect against breast cancer specimens with the compound is shown in Fig. 2.

In addition to tests with CI-920 for the 1-hr incubation, aliquots from the same tumors were also exposed to a variety of standard anticancer drugs at 1/10 of their peak plasma concentration. The median number of compounds per specimen included in this comparative analysis of drug activity was 5 (range: 2–15). Overall, with 15/43 *in vitro* responses (35%), CI-920 ranked among the most active cytostatics tested (Table 2). If an *in vitro* cut-off concentration of 1.0 mcg/ml can be verified as a clinically representative *in vitro* dosage level for CI-920, our results would also suggest the possibility of non-cross resistance to several established anticancer drugs. As shown in Table 2, in 12% of a total of 236 drug intercomparisons sensitivity to both CI-920 and the other agent was found. Twenty-seven per cent of specimens were sensitive to CI-920 but resistant to other drugs. Ten per cent of specimens were sensitive to the other agent but resistant to CI-920, and in 51% cross-resistance was found. A more detailed analysis of the relationship between CI-920 and other cytostatics is shown in Table 3 and 4 for human breast and lung cancer, respectively. The potential clinical relevance of these findings, namely the relative efficacy of CI-920 in both tumor types, is supported by the observation that the *in vitro* response activity of known cytostatics tested in these specimens corresponded closely to the known clinical activity for these agents.

DISCUSSION

Despite increasing evidence for certain limitations to the human tumor cloning assay for routine clinical use in assessing chemosensitivity for individual patients' tumors [15–17], this system undoubtedly represents a potential means of screening new anticancer compounds [11,18]. Its usefulness as a tool in preclinical drug development has been shown for several analogues and novel antitumor agents [19–24]. In this study we have investigated the antineoplastic activity of CI-920, a recently discovered structurally new antitumor antibiotic [1,2]. Our objective was to define tumor types that should be considered as targets for early phase II clinical trials.

It should be emphasized that the computation of the frequency of sensitivity to CI-920 throughout the study, as well as the comparison of its antitumor potential against a variety of standard anticancer drugs must be considered as preliminary. The *in vitro* test concentration of 1.0 mcg/ml for

Table 2. Comparison of activity of CI-920 with that of standard drugs in the human tumor cloning assay as 1-hr *exposure*

Standard drug	Conc. mcg/ml	Overall sensitivity to standard drug (<50% survival)		S/S*	S/R	R/S	R/R
BCNU	0.10	1/3	(33%)	1	0	0	2
Bisantrene	0.50	8/21	(38%)	3	5	5	8
Bleomycin	0.20	2/15	(13%)	1	5	1	8
Cis-platinum	0.20	2/22	(9%)	2	5	0	15
Cyclophosphamide	3.00	5/14	(36%)	3	5	2	4
Doxorubicin	0.04	7/38	(18%)	3	11	4	20
Echinomycin	0.01	0/2	(0%)	0	1	0	1
Etoposide	3.00	1/8	(13%)	0	2	1	5
5-Fluorouracil	6.00	3/21	(14%)	3	6	0	12
Hexamethylmelamine	1.00	3/5	(60%)	2	1	1	1
Melphalan	0.10	2/9	(22%)	1	2	1	5
Methotrexate	0.30	4/9	(44%)	2	3	2	2
MGBG	10.00	2/12	(17%)	1	3	1	7
Mitomycin C	0.10	3/14	(21%)	1	3	2	8
Mitoxantrone	0.05	0/4	(0%)	0	1	0	3
Vinblastine	0.05	7/34	(21%)	4	9	3	18
Vincristine	0.01	1/4	(25%)	0	2	1	1
Total		51/235	(22%)	27 (11%)	64 (27%)	24 (10%)	120 (51%)

* S/S indicates a tumor sensitive to CI-920 (1.0 mcg/ml) and sensitive to the drug in first column, tested for 1 hr at 1/10 of its maximal achievable plasma concentration (sensitive to CI-920/sensitive to second drug).

Table 3. In vitro *activity of CI-920 and standard drugs against breast cancer*

Drug*	Conc. (mcg/ml)	*In vitro* response rate (%)	
CI-920	1.00	5/12	(42)
Doxorubicin	0.04	3/12	(25)
Mitomycin C	0.10	3/12	(25)
5-Fluorouracil	6.00	2/11	(18)
Vinblastine	0.05	3/11	(27)
Cyclophosphamide	3.00	3/9	(33)
Bisantrene	0.50	2/8	(25)

* Drugs with < five tests are not listed.

Table 4. *Comparative analysis of* in vitro *cytotoxic drug activity in human lung cancer*

Drug*	Conc. (mcg/ml)	*In vitro* response rate (%)	
CI-920	1.00	4/10	(40)
Doxorubicin	0.04	1/9	(11)
Cis-platinum	0.20	1/7	(14)
Vinblastine	0.05	1/7	(14)
MGBG	10.00	1/7	(14)
Etoposide	3.00	1/5	(20)

* Drugs with < five tests are not listed.

1 hr was based on the concept that for several anticancer drugs the toxicity data in mice had some bearing on the appropriate dose for *in vitro* testing in the human tumor cloning assay. The absolute percentage of responders should therefore be considered as an attempt to evaluate prospectively the validity of the LD_{50} in mice as a reliable starting dose for *in vitro* drug screening of new anticancer compounds. The potential advantage of such an approach would be that new drugs could be selected in terms of their antitumor activity, rather than by their potency, as with the current use of a fixed starting dose of 10 mcg/ml in the current NCI screen.

Our results suggest that CI-920 is an active antineoplastic with potential utility for treatment of human breast, ovarian and lung cancer. Assuming the use of a reliable *in vitro* concentration, the activity in breast cancer is impressive, especially considering that more than two-thirds of the patients had received prior chemotherapy. In addition, the effective growth inhibition in several specimens resistant to other cytostatics that had been tested simultaneously, indicates the possibility of non-cross resistance. The data we have obtained in lung cancer might have to be interpreted more carefully. As previously shown by Lathan *et al.* [23], the definition of a < 50% survival of tumor colony forming units as a crite-

rion for *in vitro* drug sensitivity did not reveal an optimal correlation to the clinical situation when the experimental cytotoxicity of the two anthracene derivates bisantrene and mitoxantrone was analyzed prospectively. This overprediction of drug efficacy in the HTCA, (which was also noted for melanoma and kidney cancer), however, could be significantly reduced by selective use of the more stringent operational definition of a < 30% survival in these three tumor types. Even with that more stringent definition, CI-920 would have resulted in a 50% response rate in continuous drug experiments. In the 1-hr mode it would have caused 2/10 responses.

The heterogeneity in dose–response effect, especially the lesser degree of activity at a higher concentration in several patients' tumors, remains difficult in its interpretation. Since both concentrations had been tested in the same specimens under similar experimental conditions, and because of the quality control of each individual assay (including a positive chromomycin A3 control), a technical failure in the performance of the assay is not likely to account for these findings. Furthermore, the drug is readily water-soluble, and effective growth inhibition at the 10 mcg/ml level occurred in several patients' tumors. A definitive biochemical rationale for the lack of dose–response effect in some tumors is not available at present. However, it had been postulated that the reduced folate carrier system might influence the chemotherapeutic behaviour of CI-920 [7]. Since the compound appears to irreversibly interact with this transport mechanism, it may eventually affect its own cellular uptake, thus resulting in a possible self limitation of cytotoxic efficacy. A hypothetical dose dependency or an individual susceptibility of target cells to this phenomenon might serve as a possible explanation for the variable chemotherapeutic effects of different CI-920 concentrations.

The continuous and 1-hr drug exposure experiments had been performed in different patient populations, and speculations on schedule dependency of the drug can therefore not be performed [25]. Our data, revealing a comparable overall response rate for both *in vitro* schedules, would indirectly suggest that prolonged drug exposure might not necessarily increase efficacy in human tumors. This interpretation of our results would be in conformity with biochemical studies of Fry *et al.* []6], who described a maximal decrease of DNA, RNA, and protein synthesis in L1210 leukemia cells within 60 min of CI-920 exposure. The relevance of these findings in regard to the most therapeutically efficient mode of drug administration in man, however, remains to be established in upcoming clinical trials. The outcome of these trials will also further elucidate the potential role of the cloning assay for new drug screening and for performance of *in vitro* phase II trials.

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