Cancer Chemother Pharmacol (1981) 6: 141–144

Cancer Chemotherapy and Pharmacology

Activity of 9-10 Anthracenedicarboxaldehyde bis[(4,5-dihydro-1 *H*-imidazol-2-yl)hydrazone]dihydrochloride (CL216,942) in a Human Tumor Cloning System

Leads for Phase II Trials in Man

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Summary. *We have utilized a recently developed human tumor cloning system to screen for antitumor effects in vitro of a new anthracene derivative, CL216,942. The object was to determine whether the system is useful for pinpointing the types of tumors in patients which should be studied in early phase II clinical trials. Tumors from 684 patients were placed in culture (27 different histologic tumor types). Two hundred seventy-three tumors both grew and formed enough colonies for drug sensitivity assays. In vitro antitumor activity was noted for CL216,942 against human breast cancer, ovarian cancer, renal cancer, squamous cell, small cell and large cell lung cancer, lymphoma, acute myelogenous leukemia, melanoma, adenocarcinoma of unknown origin, adrenal cancer, gastric cancer, pancreatic cancer, and head and neck cancer. The drug definitely showed no in vitro activity against colon cancer. These date indicate that CL216,942 has a wide spectrum of in vitro antitumor activity. A comparison of these in vitro results with the results of phase II clinical trials with the drug should allow an evaluation of the utility of the human cloning system for predicting clinical activity of a new compound.*

Introduction

CL216,942 or 9–10 anthracenedicarboxaldehyde bis [(4,5-dihydro-1 *H* imidazol-2-yl)hydrazone] dihydrochloride is a new antitumor agent which has recently been evaluated in a phase I clinical trial in man (Fig. 1). This compound has had excellent antitumor activity in animals [1]. In the phase I study in man the dose-limiting toxicity was leukopenia. Other toxicities included drug-induced phlebitis and hypotension [10]. The maximally tolerated dose on a single dose every 4 weeks schedule of administration is 260 mg/m^2. The compound is now ready for phase II trials in man.

The recently developed human tumor cloning system of Hamburger and Salmon [2, 3] offers a new system for screening a new compound for in vitro activity against human tumors [5, 7]. It is a yet unknown whether this in vitro activity of a new compound will predict for antitumor activity of the drug in man. Initial studies have indicated the cloning system may predict which conventional chemotherapeutic agents will or will not work against an individual patient's tumor [4, 8, 9].

We have utilized the human tumor cloning system to screen for antitumor activity of CL216,942. Results of these in vitro studies can be compared with phase II clinical trial results with the drug as they become available. If the cloning system appears to be predictive for future phase II activity of CL216,942 it will represent a new method for pinpointing the tumors against which a drug should be tried in phase II clinical trials. This would provide maximal utilization of patient resources, and decrease the time requirement for development of a new anticancer agent.

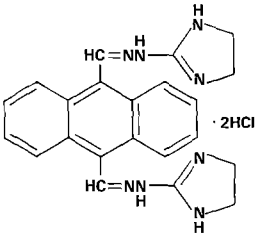

Fig. 1. Structure of CL216,942

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0344-5704/81/0006/0141/$ 01.00

Patients and Methods

A total of 684 patients undergoing surgery, bone marrow examination, thoracentesis or paracentesis had solid tumor, ascites, bone marrow or pleural fluid sent for cloning in soft agar. All procedures were done after informed consent as part of diagnostic work-ups or therapeutic maneuvers. One-half of the patients had received prior chemotherapy, 292 having received prior therapy with 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 and 1% penicillin and streptomycin solutions (all materials, Grand Island Biologic Company, Grand Island, New York). Bone marrow specimens were collected in heparinized syringes and processed in the same manner as the effusions, except that after centrifugation only the buffy coat was removed and processed. Solid tumors removed by biopsy or at operation were immediately placed in McCoy's 5A medium + 10% heat-inactivated fetal calf serum + 1% penicillin and streptomycin in the operating room and transported to the laboratory within 1 h. They were then mechanically dissociated with scissors and subsequent forcing through a wire mesh gauze into Hank's balanced salt solution with 10% fetal calf serum. Finally they were passed through progressively smaller needles and processed in the same manner as the effusions. Viability of cells in all cases was determined by trypan blue dye exclusion. Viability ranged from 40%–90% (median 75%) in fluids and marrow specimens, while cell viability from solid tumor specimens ranged from 10%–80% (median 40%). Only viable cells determined the final concentration of cells plated.

In vitro Exposure to CL216,942. Stock solutions of the IV formulation of CL216,942 were prepared in sterile buffered saline and stored at −70° C in aliquots sufficient for individual assays. Subsequent dilutions for incubation with cells were made with saline.

Tumor cell suspensions were transferred to tubes and adjusted to a final concentration of 1.0×10^6 cells per milliliter in the presence of the appropriate drug dilution or control medium. For the first 25 patients, CL216,942 was tested at three dose levels: 0.05, 0.50, and 1.0 μg/ml. From early clinical pharmacology studies with the drug it has been shown that peak plasma concentrations of the drug reach 5–8 μg/ml when 260 mg CL216,942/m^3 has been given to a patient [10]. After the first 25 patients' tumors had been tested with three concentrations of the drug the rest were tested with only one drug concentration (0.5 μg/ml). Cells were incubated with or without CL216,942 for 1 h at 37° C in Hank's balanced salt solution. The cells were then washed twice with Hank's balanced salt solution and prepared for culture.

Assay for Tumor Colony-forming Units (T-CFUs). The culture system utilized in this study has been previously described [2, 3]. In brief, cells to be tested were suspended in 0.3% agar in enriched CMRL 1066 medium supplemented with 15% horse serum to yield a final concentration of cells of 0.5×10^6 cells/ml [2, 3]. One milliliter of this mixture was pipetted into each of three 35-ml petri dishes containing 1 ml 0.5% agar in enriched McCoy's 5A [2, 3] but without conditioned medium. Cultures were incubated at 37° C in 7% CO_2 in humidified air. All assays were set up in triplicate.

Colonies (≥ 50 cells) usually appeared in 10–15 days and the number of colonies on control and drug-treated plates was determined by counting the colonies on an inverted stage microscope at 30× magnification. At least 30 tumor colonies per control plate were required for a drug experiment to be considered evaluable for measurement of drug effect.

Data Analysis. Colony counts of the three plates for a particular drug concentration were averaged to obtain each data point. The standard error of the mean for individual data points averaged 14% of the mean. 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 plotted on a linear scale (Fig. 2).

Criteria for in vitro sensitivity and resistance were applied to the colony survival-drug concentration curves. Screening in vitro by means of the cloning system in an attempt to pinpoint the tumor types against which the drug will work clinically is a new methodology. Therefore no criteria have been set for active or inactive agents in this system.

For purposes of this analysis two different definitions of 'active' in vitro have been utilized. These two definitions are:

1) ≥ 70% decrease in T-CFUs in the system is considered active;

2) ≥ 50% decrease in T-CFUs in the system is considered active.

The definition of ≥ 70% decrease in T-CFUs was used because in a prior retrospective study it was satisfactory for predicting which patient would respond to a particular chemotherapeutic agent [8]. The definition of ≥ 50% decrease in T-CFUs was arbitrarily set in an attempt to make the system more sensitive for screening purposes, with a probable decrease in specificity.

Future clinical trial results with CL216,942 will help us to determine whether ≥ 70% of ≥ 50% decrease in T-CFUs is more appropriate for screening for phase II activity of new anticancer agents.

Results

A total of 684 human tumors were placed in culture and had CL216,942 tested against them. This sample represented 27 different histologic types of malignancy (Table 1). A total of 273 of the 684 specimens (40%) placed in culture formed > 30 colonies in control plates and were evaluable for drug sensitivity information.

Typical drug sensitivity curves for the in vitro activity of CL216,942 against carcinoma of the kidney at doses of 0.05, 0.5, and 1.0 μg/ml are shown in Fig. 2. There is a distinct heterogeneity of tumor sensitivity to CL216,942. For some patients' tumors there is a more profound decrease in T-CFUs with increasing concentrations of the drug. For other patients tumors there is a plateau in the survival curve even with increasing concentrations of CL216,942.

Table 1 details the number of specimens against which CL216,942 caused a ≥ 70% decrease in T-CFUs. The tumor types are arranged alphabetically. CL216,942 demonstrated ≥ 70% decrease in T-CFUs against at least two different patients' tumors for each of breast cancer, small cell and squamous cell lung cancer, lymphoma, melanoma, ovarian cancer, pancreatic cancer, and renal cell cancer. The drug

Table 1. Types of tumor used in the study and number of specimens of each in which there was a decrease in T-CFUs in response to CL216,942

Tumor type	No. which grew > 30 colonies/ total no. plated	No. of specimens with ≥ 50% decrease in T-CFUs	No. of specimens with ≥ 70% decrease in T-CFUs
Adenocarcinoma of unknown origin	12/21	2	1
Adrenal	5/5	1	1
Bladder	4/14	0	0
Brain	9/15	3	0
Breast	45/105	14	6
Cervix	2/2	0	0
Colorectal	20/42	2	0
Head and neck	1/4	1	1
Islet cell	1/1	1	0
Lung			
Adenocarcinoma	9/24	1	0
Bronchoalveolar	1/3	0	0
Large cell	6/12	2	1
Small cell	15/50	4	3
Squamous	10/37	4	2
Leukemia	3/17	2	1
Lymphoma	5/59	2	2
Melanoma	16/26	3	3
Mesothelioma	2/3	1	0
Multiple myeloma	2/13	1	0
Neuroblastoma	2/23	0	0
Ovarian	59/86	14	9
Pancreas	8/28	3	2
Prostate	3/10	1	0
Renal	18/37	9	4
Sarcoma	2/11	1	0
Stomach	7/15	2	1
Testicular	6/21	1	0

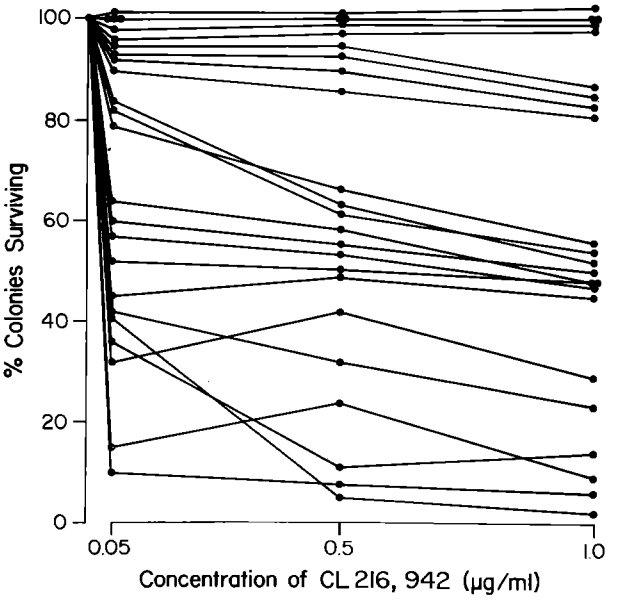

Fig. 2. In vitro activity of CL216,942 against 23 patients' tumor (renal cell carcinoma) growing in a human tumor cloning system. Note heterogeneity of effect of CL216,942 on the various tumors

gave a ≥ 70% decrease in T-CFUs against a least one patient's tumor for each of adenocarcinoma of unknown origin, adrenal cancer, head and neck cancer, large cell lung cancer, leukemia (AML), and gastric cancer. If a ≥ 70% decrease in tumor kill is used as a definition for activity, then CL216,942 is definitely inactive in vitro against colorectal carcinoma. There are not enough evaluable tumor specimens (at least 14 are needed) in the other histologic types of tumors to call the drug definitely inactive against those tumor types (Table 1).

Discussion

We have utilized a human tumor cloning system to test for in vitro antitumor activity of the new antitumor agent CL216,942. The first object of the study was to identify tumor types that were sensitive to the drug. These in vitro results could then be compared with results of clinical pase II trials as they became available. If ≥ 70% decrease in T-CFUs is utilized as a definition of in vitro sensitivity then

CL216,942 has in vitro antitumor activity against human breast cancer, ovarian cancer, renal cancer, squamous cell, small cell, and large cell lung cancer, lymphoma, leukemia (AML), melanoma, adenocarcinoma of unknown origin, adrenal cancer, gastric cancer, pancreatic cancer, and head and neck cancer. The drug appears to be inactive against colon cancer in this in vitro system. From this information it is clear that CL216,942 has a broad spectrum of in vitro antitumor activity even when a $\geq 70\%$ decrease in T-CFUs is used to define in vitro activity.

As clinical phase II trials with CL216,942 are completed it will be of great interest to see how the in vitro results presented above compare with the results obtained in the phase II trials in man. Even though the patients tested in these in vitro trials are not the same as those entered in the phase II clinical trials, it will be of interest to see whether phase II trials carried out in vitro are predictive for in vivo phase II results. If this in vitro phase II methodology looks promising it has the potential for pinpointing the tumor types against which the drug should be used in phase II clinical trials. This methodology would provide maximal utilization of patient resources and decrease the time required for development of a new antitumor agent. It might also pinpoint more unusual tumor types where a particular drug might work, such as renal cell cancer, as was noted in this study. Clinical trial efforts could also be directed against those unusual tumor types.

Acknowledgements. This work was supported by a grant from the Medical Research Division, American Cyanamid Company.

References

1. Citarella RV, Wallace RE, Murdock KC, Angier RB, Durr FE (1980) Anti-tumor activity of CL216,942: 9,10 Anthracenedicarboxaldehyde bis ((4,5-dihydro-1 *H*-imidazol-2-yl)hydrazone)dihydrochloride. Abstracts of the 20th Interscience Conference on Antimicrobial agents and Chemotherapy. (Abstract no. 23)
2. Hamburger AW, Salmon SE (1977a) Primary bioassay of human tumor stem cells. Science 197: 461–463
3. Hamburger AW, Salmon SE (1977b) Primary bioassay of human myeloma stem cells. J Clin Invest 60: 846–854
4. Salmon SE, Hamburger AW, Soehnlen B, Durie BG, Alberts DS, Moon TE (1978) Quantitation of differential sensitivity of human tumor stem cells to anticancer drugs. N Engl J Med 298: 1321–1327
5. Salmon SE, Alberts D, Meyskens F, Durie B, Jones S, Soehnlen B, Young L (1980) A new concept: In vitro phase II trial with the human tumor stem cell assay (HTSCA). Proc Am Assoc Cancer Res/ASCO 21: 329
6. Von Hoff DD, Rozencweig M, Muggia FM (1979) The evaluation of cytotoxic drugs. Cancer Treat Rev 6: 1–8
7. Von Hoff DD, Coltman CA Jr, Forseth B (1981a) Activity of mitoxantrone in a human tumor cloning system. Cancer Res 41: 1853–1855
8. Von Hoff DD, Casper J, Bradley F, Sandbach J, Jones D, Makuch R (1981b) Association between human tumor colony forming assay results and response of an individual patient's tumor to chemotherapy. Am J Med 70: 1027–1032
9. Von Hoff DD, Page C, Harris G, Clark G, Cowan J, Coltman CA Jr (1981c) Prospective clinical trial of a human tumor cloning system. Proc Am Assoc Cancer Res 22: 154
10. Von Hoff DD, Myers JW, Kuhn J, Sandbach JR, Pocelinko R, Clark G, Coltman CA Jr (1981d) 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

Received December 5, 1980/Accepted May 26, 1981