Activity of Mitoxantrone in a Human Tumor Cloning System\textsuperscript{1}

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ABSTRACT

We have utilized a recently developed human tumor cloning system to screen for antitumor effects \textit{in vitro} of a new anthracenedione derivative, Mitoxantrone. The object was to determine if the system is useful for pinpointing the types of tumors in patients which should be studied in early Phase II clinical trials. Tumors from 267 patients were placed in culture (20 different histological tumor types). One hundred seventy tumors both grew and formed enough colonies for drug sensitivity assays. Excellent \textit{in vitro} antitumor activity was noted for Mitoxantrone against human adenocarcinoma of the lung, small cell lung cancer, melanoma, and biliary tree cancer. Good antitumor activity was noted against breast cancer, ovarian cancer, non-Hodgkin’s lymphoma, head and neck cancer, squamous cell lung cancer, soft tissue sarcoma, gastric cancer, and hepatomas. The drug showed \textit{no in vitro} antitumor activity against colon cancer. These data indicate that Mitoxantrone has a wide spectrum of \textit{in vitro} antitumor activity. A comparison of these \textit{in vitro} results with the results of Phase II clinical trials with the drug should allow an evaluation of the utility of the human tumor cloning system for predicting clinical antitumor activity of a new compound.

INTRODUCTION

Mitoxantrone, or 1,4-dihydroxy-5,8-bis(\{2-[(2-hydroxyethyl)amino]ethyl\}amino})-9,10-anthracenedione dihydrochloride (NSC 301739), is a new antitumor agent which has recently been evaluated in a Phase I trial in humans (14). In that study, the dose-limiting toxicities were leukopenia and thrombocytopenia, which were manageable and reversible at a dose of 12 mg/sq m administered as a single dose on an every-3-week schedule. The compound is now ready for Phase II trials in humans.

The recently developed human tumor cloning system of Hamburger and Salmon (3, 4) offers a new system for screening a new compound for \textit{in vitro} activity against human tumors (8, 11, 15). It is as yet unknown whether this \textit{in vitro} activity of a new compound will predict for antitumor activity of the drug in humans. However, initial studies have indicated that the cloning system may predict which conventional chemotherapeutic agents will or will not work against an individual patient’s tumor (9, 10).

We have utilized the human tumor cloning system to screen for antitumor activity of Mitoxantrone. Results of these \textit{in vitro} studies can be compared to 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 Mitoxantrone, it will represent a new method of 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.

MATERIALS AND METHODS

A total of 267 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 workups or therapeutic maneuvers. One-half of the patients had received prior chemotherapy with 117 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 Hanks’ balanced salt solution with 10% heat-inactivated fetal calf serum and 1% penicillin and streptomycin solutions (all materials, Grand Island Biological Company, Grand Island, N. Y.). Bone marrow specimens were collected in heparinized syringes and processed in the same manner as were 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 Medium 5A plus 10% fetal calf serum plus 1% penicillin and streptomycin in the operating room and transported to the laboratory within 1 hr, where they were mechanically dissociated by forcing through a wire mesh gauze into Hanks’ 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 in all cases was determined by trypan blue dye exclusion. Viability ranged from 40 to 90% (median, 75%) in fluids and marrow specimens, while cell viability from solid tumor specimens ranged from 10 to 80% (median, 40%). Only viable cells determined the final concentration of cells plated.

\textit{In Vitro} Exposure to Mitoxantrone. A stock solution of the i.v. formulation of Mitoxantrone was prepared in sterile buffered 0.9% NaCl solution and stored at −70° in aliquots sufficient for individual assays. Subsequent dilutions for incubation with cells were made with 0.9% NaCl solution.

Tumor cell suspensions were transferred to tubes and adjusted to a final concentration of 1.0 × 10^6 cells/ml in the presence of the appropriate drug dilution or control medium. For the first 25 patients, Mitoxantrone was tested at 3 dose levels including 0.05, 0.10, 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 0.5 to 0.8 μg/ml when Mitoxantrone (12 mg/sq m) is given to a patient (14). After the first 25 patients’ tumors were tested with 3 concentrations of the drug, the rest were tested with only one drug concentration (0.05 μg/ml). Cells were incubated with or without Mitoxantrone for 1 hr at 37° in Hanks’ balanced salt solution. The cells were then washed twice with Hanks’ bal-

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anced salt solution and prepared for culture. Cell counts were performed on 16 specimens both before and after the incubation, washing, and resuspension. Overall, the percentage of cells lost during all 3 of these procedures ranged from 5 to 22% (median, 18%). The amount of cell loss was relatively constant throughout both controls and all 3 drug concentrations used.

**Assay for Tumor Colony-forming Units.** The culture system utilized in this study has been described previously (3, 4). In brief, cells to be tested were suspended in 0.3% agar in enriched Connaught Medical Research Laboratories Medium 1066 supplemented with 15% horse serum to yield a final concentration of cells of 0.5 × 10⁶ cells/ml (3, 4). One ml of this mixture was pipetted into each of three 35-ml Petri dishes containing 1 ml of 0.5% agar in enriched McCoy’s Medium 5A (3, 4) but without conditioned medium. Cultures were incubated at 37° in 7% CO₂ in humidified air. All assays were set up in triplicate.

Colonies (≥50 cells) usually appeared in 10 to 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. 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 3 plates for a particular drug concentration were averaged to obtain one data point. The standard error of the mean for individual data points averaged 10% 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 (see Chart 1).

Criteria for in vitro sensitivity and resistance were applied to the colony survival-drug concentration curves. Screening in vitro using 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, 2 different definitions of “active” in vitro have been utilized. These 2 definitions included: (a) ≥70% decrease in T-CFU³ in the system is considered active; and (b) ≥50% decrease in T-CFU in the system is considered active. The definition of ≥70% decrease in T-CFU was used because, in a prior retrospective study, it was satisfactory for predicting which patient would respond to a particular chemotherapeutic agent (10). The definition of ≥50% decrease in T-CFU 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 Mitoxantrone will help determine whether ≥70% or ≥50% decrease in T-CFU is more appropriate for screening for Phase II activity of new anticancer agents.

**RESULTS**

A total of 267 human tumors were placed in culture and had Mitoxantrone tested against them. This represented 20 different histological types of cancer (see Table 1). A total of 170 of the 267 specimens (64%) placed in culture formed colonies and were evaluable for drug sensitivity information (i.e., grew ≥30 colonies on control plates). Evidence for the malignant nature of cells growing in these colonies has been presented previously (1–7, 12, 13).

Typical drug sensitivity curves for the in vitro activity of Mitoxantrone against adenocarcinoma of the lung are noted in Chart 1. There is a distinct heterogeneity of tumor sensitivity to Mitoxantrone. For some patients’ tumors, there is a more profound decrease in T-CFU with increasing concentrations of the drug. However, for the majority of patients’ tumors, the dose-response curve is flat past a Mitoxantrone concentration of 0.05 μg/ml.

Table 1 details the number of specimens against which Mitoxantrone caused a ≥70% decrease in T-CFU. Mitoxantrone demonstrated ≥70% decrease in T-CFU against at least 2 different patients’ tumors for adenocarcinoma of the lung, small cell lung cancer, melanoma, biliary tree cancer, and adenocarcinoma of unknown origin. The drug gave a ≥70% decrease in T-CFU against at least one patients’ tumor for breast cancer, ovarian cancer, squamous cell lung cancer.
head and neck cancer, non-Hodgkin’s lymphoma, soft tissue sarcoma, gastric cancer, and hepatoma. If <70% decrease in tumor kill is used as a definition of inactive, then Mitoxantrone is inactive in vitro against neuroblastoma, hypernephroma, and colorectal carcinoma. There are not enough tumor specimens in the other histological types of tumors to show that the drug is definitely inactive against these tumor types (see Table 1).

If ≥50% decrease in T-CFU is used as a definition of active, then Mitoxantrone would be considered active in vitro against more tumors, as outlined in Table 1.

DISCUSSION

We have utilized a human tumor cloning system to test for in vitro antitumor activity of the new antitumor agent Mitoxantron. The first object of the study was to identify tumor types which were sensitive to the drug. These in vitro results could then be compared with results of clinical Phase II trials as they became available.

The shape of the dose-response curves noted in this study of Mitoxantrone are of interest. The majority of the curves demonstrate a plateau after a 0.05-µg/ml dose of the drug. Mitoxantrone is not thought to be a cell cycle-specific agent (14). It is more likely that this plateau represents tumor cells which are resistant to the higher doses of Mitoxantrone.

If ≥70% decrease in T-CFU is utilized as a definition of in vitro sensitivity, then Mitoxantrone has good in vitro antitumor activity against adenocarcinoma of the lung, small cell lung cancer, melanoma, and biliary tree cancer. Less dramatic in vitro activity has been demonstrated against breast cancer, ovarian cancer, squamous cell lung cancer, head and neck cancer, non-Hodgkin’s lymphoma, soft tissue sarcoma, gastric cancer, and hepatoma. The drug is definitely inactive against colon cancer in this in vitro system.

From this information, it is clear that Mitoxantrone has a broad spectrum of in vitro antitumor activity even when a ≥70% decrease in T-CFU is used to define in vitro activity. When a less stringent definition of in vitro activity is used (≥50% decrease in T-CFU), Mitoxantrone has in vitro antitumor activity against even more tumor types. Most notably, these include breast cancer, neuroblastoma, hypernephroma, and mesothelioma. When interpreting this information, it is important to remember that one-half of all the breast cancers, small cell lung cancers, neuroblastomas, sarcomas, lymphomas, and gastric cancers placed in culture had had prior in vivo exposure to Adriamycin (i.e., the patients had failed treatment with Adriamycin as a single agent or in combination). With the data in this study, we are not able to make a statement on the possibility of cross-resistance between Adriamycin and Mitoxantrone.

As clinical phase II trials with Mitoxantrone are completed, it will be of great interest to see how the in vitro results presented above compare to the results obtained in the Phase II trials in humans. 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 if 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 would provide maximal utilization of patient resources and decrease the time required for development of a new antitumor agent. The system might also pinpoint rare tumor types where a particular drug might work, such as biliary tree cancer as was noted in this study. Clinical trial efforts could also be directed against those unusual tumor types.

REFERENCES

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