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Evaluation of an amsacrine analog in a human tumor cloning system*

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Summary. A human tumor-cloning system was used to compare the antitumor activity of CI-921, a new amsacrine analog with that of its parent compound (amsacrine gluconate). A total of 48 specimens of 9 histologically different types of human malignancy were evaluable for a direct comparison of the cytotoxic activity. Both compounds were tested simultaneously at 10 μg/ml final concentration under continuous exposure. The overall activity was similar for both drugs, but the degree of cross-resistance was low. We concluded that in patients, CI-921 might have a different spectrum of antitumor activity from that of its parent compound.

Introduction

4'-(9-Acridinylamino)methanesulfon-m-ansidine (amsacrine) is an intercalating antitumor compound that was synthesized by Cain et al. in 1974 [3]. Since then it has undergone extensive clinical evaluation in phase II studies. Amsacrine shows significant clinical activity in patients with acute leukemias as well as promising activity in patients with lymphomas [8, 12, 20]; the compound is virtually inactive against solid malignancies [11, 13, 15]. In an attempt to develop drugs with a broader spectrum of antitumor activity, a series of amsacrine analogs has been synthesized [1]. Amsacrine 4-methyl-5(*N*-methylcarboxamide) (CI-921) was selected for further investigation due to its equally good in vitro activity compared with that of amsacrine against leukemias P388, L1210, and Adriamycin-resistant P388 leukemia, and its higher in vitro cytotoxicity against the human colon cancer lines HCT-8, HT-29, and LoVo as well as the human breast cancer lines MCF-7, MDA-231, and T-47D [2].

For preclinical evaluation of the new analog it would be of interest to compare its activity with that of the parent compound amsacrine. We therefore used a human tumor-cloning system [5, 6, 16] to test CI-921 and amsacrine simultaneously for their cytotoxic activity against 48 evaluable specimens of 9 histologically different types of tumors.

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Materials and methods

After giving their informed consent, a total of 132 patients underwent thoracentesis, paracentesis, or surgery as part of routine diagnostic workups or therapeutic maneuvers. Specimens consisting of solid tumor, ascites, bone marrow, and pleural fluid were obtained for cloning in soft agar.

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 a 1% penicillin-streptomycin solution (all materials were obtained from Grand Island Biological Co, NY). Solid tumors were mechanically dissociated and processed in the same manner as the effusions [5, 6].

In vitro drug exposure. The gluconate formulation of amsacrine was used due to its better water solubility. Stock solutions of amsacrine gluconate (hereafter called amsacrine) and CI-921 were prepared in sterile distilled water. Since CI-921 had not entered phase I clinical trials, no pharmacokinetic data were available in man. We therefore arbitrarily chose a final drug concentration of 10 μg/ml and a continuous incubation for comparing the cytotoxic activity of both drugs.

Assay for tumor colony-forming units (TCFUs). The culture system used in the present study has previously been described [5–7, 17]. 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 cell concentration of 5×10^5/ml. The appropriate amount of drug was added to reach a final concentration of 10 μg/ml; 1 ml mixture was pipetted into each of three 35-mm Petri dishes containing 1 ml 0.5% agar in enriched McCoy's 5A medium. Cultures were then incubated at 37° C in 7% CO_2 in humidified air. All assays were set up in triplicate. With a Bausch and Lomb FAS II automatic scanner, the plates were then screened for the presence of cell clusters to assure the plating of only single cells. No more than five features were allowed on day 0. Colonies (≥ 60 μm in diameter) usually appeared within 10–15 days; the number of colonies on control and drug-treated plates were determined with an automatic counter and were also checked in part by visual counting. At least

20 tumor colonies/control plate (above background) were required for a drug experiment to be considered evaluable for the measurement of drug effect.

Data analysis. The results of the in vitro cloning assay were expressed as the percentage of survival of TCFUs for amsacrine or CI-921 relative to that in the nontreated control. This quantity was calculated as the ratio between the mean number of colonies surviving on triplicate plates and those growing on triplicate control plates. A survival of $\leq 50\%$ of TCFUs was considered to be a definition of activity in vitro. The statistical methods used to compare the in vitro activity of CI-921 and amsacrine were the McNemars chi-square test and the Kappa statistical assay [4].

Results

A total of 128 human tumors were placed in culture and tested against CI-921. In all, 48 specimens (38%) formed at least 20 colonies on control plates and were evaluable for drug sensitivity measurements involving 9 histologically different tumor types. The plating efficiency on control plates for evaluable specimens varied between 0.004% and 0.088%, with a median plating efficiency of 0.011%.

Table 1 details the cytotoxic activity of CI-921 and amsacrine for each type of tumor. At the investigated concentration, both compounds showed cytotoxic activity against breast, colon, and lung cancer as well as melanoma. With an overall activity of 29% for CI-921 and 37% for amsacrine, there was no significant difference in the cytotoxic activity of the drugs (McNemars chi-square test; $P = 0.39$).

Table 2 shows the in vitro activity of CI-921 and amsacrine when the results were analyzed for sensitivity or resistance in the same patient's tumor specimen ($\leq 50\%$ survival = sensitive; $>50\%$ survival = resistant). There was concordance of the results in only 58% of the specimens (12% sensitive/sensitive and 48% resistant/resistant); Kappa = 0.07 ($P = 0.75$). Thus, the cytotoxic activity of the drugs for individual specimens was not correlated; this means that in individual human tumors the compounds show different activities.

Table 1. Summary of sensitivity results for CI-921 and amsacrine in the human tumor-cloning system

Tumor type	Number of evaluable specimens	Number of specimens with $\leq 50\%$ survival of TCFUs	
		CI-921	Amsacrine
Breast	14	4	6
Colon	12	4	2
Lung	11	4	5
Melanoma	5	2	4
Miscellaneous[a]	6	0	1
Totals	48	14 (29%)	18 (37%)

[a] Bladder, corpus uteri, kidney, ovary, stomach, unknown primary

Table 2. Cross-resistance of CI-921 and amsacrine in the same patient's specimen in the human tumor-cloning system

		Amsacrine	
		Sensitive[a]	Resistant
CI-921	Sensitive	6 (12%)	8 (17%)
	Resistant	12 (25%)	22 (46%)

[a] $\leq 50\%$ survival of tumor colony-forming units

Discussion

In retrospective [14, 18] and prospective clinical trials [19], the human tumor-cloning system has shown some promise for prediction of the response or resistance of an individual patient's tumor. Based on this experience, the human tumor-cloning system has also been used for predicting the cytotoxic activity of investigational drugs prior to their entrance into phase II clinical trials [9]. In addition, the system has been explored as a method for the preclinical comparison of analogs of clinically useful drugs [10]. The object of the present study was to determine whether the amsacrine analog CI-921 has a higher degree or different pattern of cytotoxic activity against solid human malignancies than its parent compound amsacrine. For this purpose, both drugs were simultaneously tested against a variety of solid human tumors.

Both CI-921 and amsacrine showed in vitro activity against breast, colon, and lung cancer as well as melanoma. Although the overall activity of amsacrine was higher than that of CI-921, the difference was not statistically significant. When the in vitro results were examined for the sensitivity or resistance of an individual patient's tumor, there was a rather high percentage of discordance (Table 2). In comparison with previously published results [10], this high incidence of discordance indicates a relatively low level of cross-resistance between the analog and the parent compound. From the information obtained in the present study, one would predict that, overall, CI-921 would not be superior to amsacrine against human tumors in vivo.

The purpose of the study was to compare CI-921 with its parent compound. Both drugs were tested at an arbitrary concentration; therefore, the results should not be extrapolated to expectations of the antitumor activity of CI-921 in clinical trials. However, if plasma levels of CI-921 approximate those obtained with amsacrine, CI-921 could possibly be active against some tumors that are resistant to amsacrine in vivo.

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