Chemotherapy of Human Tumor Xenografts in Genetically Athymic Mice*†

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ABSTRACT

A series of studies were undertaken to evaluate the chemotherapeutic response to various antineoplastic drugs of human breast (MX-1) or colon (CX-2) tumor xenografts growing in genetically athymic (nude) mice. Fragments (2mm³) of either tumor type were implanted subcutaneously into the subaxillary region of NIH Swiss nude mice, and single drug therapy was started when tumors became palpable and were growing progressively. 5-Fluorouracil (5-FU) administered on a Q7DX3 schedule starting on Day 21 post tumor implantation elicited significant retardation in the growth rate of CX-2 tumor. A single treatment with methyl-CCNU induced temporary tumor regression. Against MX-1 tumor, both cyclophosphamide and melphalan induced tumor regressions with no recurrence. 5-FU slowed but did not arrest growth of MX-1 tumor. These tumor systems grown in nude mice appear to be suitable models for in vivo screening of anticancer agents that would prove clinically active.

Introduction

The athymic condition in mice arose as a spontaneous mutation and is inherited as an autosomal recessive.5,20 The gene responsible for this trait is designated nu for nude or no haircoat which is the more obvious phenotypic type. Athymic nu/nu (nude) mice are an excellent animal model for immunological investigations7,11 and also for tissue grafting studies9,24. Human cancer tissues have been successfully grown in nude mice (figure 1). Established xenografts of these tissues implanted subcutaneously into nude mice remain localized and do not usually metastasize.23 Human tumors serially passaged in nude mice retain their original histology, karotype and tumorigenicity through many transplant generations8,22,23. The nude mouse/human tumor xenograft system provides a useful model for experimental cancer therapy studies involving human neoplasms. Moreover the system lends itself to the development of screening protocols for the identification of both normal13,16 and neoplastic cells9,24.
of potential anticancer drugs which would be clinically effective against a given type of cancer. The chemotherapeutic responses are reported for various antineoplastic drugs of transplantable human colon and breast tumor xenografts growing progressively in nude mice. These human tumor xenografts were responsive to certain drugs that are generally active against the corresponding types of clinical cancers.

Materials and Methods

The experimental tumors* employed in this study included (1) a human colon adenocarcinoma (CX-2) which was cultured *in vitro* from cancerous tissues resected from a 50 year-old patient and subsequently transplanted serially in nude mice and (2) a human mammary carcinoma (MX-1) established from a surgical specimen derived from a 29 year-old female and serially grafted into athymic mice. In our laboratory, these tumor lines were maintained *in vivo* by implanting 2 mm³ fragments of aseptically collected fresh tumor tissue subcutaneously into the right subaxillary region of nude mice. The volume doubling time of subcutaneous tumors at the exponential growth phase is approximately four to five days for MX-1 and five to six days for the CX-2 tumor. In the present experiments, the tumors employed were derived from transplant generation numbers 2 to 7.

The nude mice used in these experiments were of NIH Swiss background.† The nude mice were maintained in our laboratory in sterilized cages fitted with molded filter (polyester fiber) covers. Autoclaved food pellets and chlorinated water were provided *ad libitum*. Experimental mice were implanted with tumor fragments as previously described and subsequently randomized into test groups of four to six mice each when the tumors became palpable and growing progressively. Treatment was initiated at this time.‡ Dosages expressed as mg per kg per injection were selected on the basis of LD₁₀ for Swiss mice¹² and previous toxicity studies performed with nude mice in our laboratory (unpublished). The selected dosages for the present ex-

* These two tumor lines were kindly supplied by B. C. Giovanella of the Stehlin Foundation for Cancer Research, Houston, TX.

† The mice were obtained from the Frederick Cancer Research Center, Frederick, MD.

‡ All drugs used in these studies were furnished by the Drug Evaluation Branch, Division of Cancer Treatment, National Cancer Institute.
Experiments encompassed a wide dose range that included a toxic dose.

Test mice were weighed and their tumors measured (length and width) with vernier calipers twice weekly during and after the treatment period. Estimates of tumor weight (W) in mg were calculated from the linear measurements using the formula $W = (a^2 \times b)/2$ where $a$ is the width in mm and $b$ is the length in mm. This simplified formula for calculating volume of an ellipsoid is considered a valid estimation for weight assuming unit density. To standardize the variability in tumor weights among the different test groups at the initiation of treatment, the relative weights (RW) at different times were obtained using the formula $RW = Wi/Wo$ where $Wi$ is the mean tumor weight of a group at any given time and $Wo$ is the initial mean tumor weight of that group. Comparisons between treated and control groups were made on the basis of the RW's. A significant response to treatment is declared when a test group demonstrates an RW $\leq 42$ percent of that of the control.6

**Results**

**Response of Colon Xenograft**

Two clinically active drugs, 5-fluorouracil (5-FU) and 1-[2-chloroethyl]-3-[4-methylcyclohexyl] - 1 - nitrosourea (methyl-CCNU), were evaluated against the CX-2 human colon xenograft in nude mice. In figure 2 is shown the response of the tumor to three weekly treatment with 5-FU. The drug was dissolved in physiologic saline and administered intraperitoneally in a volume of 0.01 ml per gram body weight. 5-FU did not arrest tumor growth; the relative tumor weight was never less than one during the observation period. However, the rate of tumor growth was slowed considerably. The mean tumor weight of the 100 mg per kg group doubled (relative to mean weight at initial treatment) after approximately 22 days compared to six days for that of the untreated control. The relative tumor weight of the treated group on Day 22 was about 38 percent of the control group. The apparent decrease in tumor size for the 67 mg per kg dose level on Day 20 was due to the death of one mouse with a relatively large tumor mass and should not be interpreted as drug-induced tumor regression.

The activity of methyl-CCNU against the CX-2 xenograft is presented in figure 3. A single intraperitoneal treatment with methyl-CCNU dissolved in 10 percent ethanol, 10 percent emulphor and saline induced a brief period of tumor regres-
sion. This short lived period can not be considered unimportant, for although tumor growth subsequently resumed, the RW of the 40 mg per kg groups was still 33 percent of that of the control as late as 32 days after treatment. In a previous experiment, methyl-CCNU administered as a suspension in hydroxypropycellulose (0.3 percent) showed minimal activity against CX-2 tumor and did not induce any tumor regression. Clearly, this would imply that the physical state of drug preparation affects the drug's efficacy.

**RESPONSE OF BREAST XENOGRAFT**

Breast carcinoma is one of the most responsive human solid tumors to many cancer chemotherapeutic agents. In the present experiment, a breast tumor xenograft (MX-1) was assessed for its responsiveness to cyclophosphamide, L-phenylalanine mustard (melphalan) and 5-FU.

The effects of cyclophosphamide on the growth MX-1 xenograft in nude mice are shown in figure 4. Cyclophosphamide was administered intraperitoneally on a Q4DX4 schedule. Total eradication of the tumors was observed at all dose levels tested. The top dose of 150 mg per kg per injection was toxic as all mice in this group were dead 21 days after the initiation of treatment. Five of six mice that received 100 mg per kg per injection and six of six mice in the 67 mg per kg per injection group survived tumor-free beyond 50 days after the treatment. It should be noted that nude mice tolerated doses of cyclophosphamide which would be toxic for other strains of mice especially the two top doses used in this study. Although it has been recently reported that nude mice tolerate higher doses of some drugs than conventional mice, drug toxicity in nude mice has not yet been extensively studied.

Melphalan, which like cyclophosphamide is a mustard gas derivative, displayed a striking activity against MX-1 (figure 5). Both doses given on a Q4DX3 schedule dramatically arrested tumor growth. The recurrence of tumor de-
picted in the figure for the 67 mg per kg dose level was that of only one mouse whose tumor regrew after being almost eradicated. Administration of melphalan has been shown to produce significant numbers of disease-free survivors when used as an adjuvant to radical mastectomy in pre-menopausal patients.3 This may relate to the MX-1 tumor’s remarkable response to melphalan, as the xenograft was established from a 29 year-old woman.

In figure 6 is illustrated the activity of 5-FU against the MX-1 tumor. 5-FU did not induce tumor regressions but delayed the growth of tumor to some degree during and immediately after the treatment period. The tumor increased in size at the same rate as the untreated control following this period. RW was never less than 41 percent of the control throughout the duration of the experiment.

Discussion

The chemotherapy of colorectal cancer has not met with a great deal of success. 5-FU is considered the most effective...
single chemotherapeutic agent in the treatment of colon carcinoma,15 although objective response to the drug is rather low and temporary14 and therapy with the drug does not have a significant effect on the survival of treated patients.17 Among the nitrosoureas, methyl-CCNU appears to be the most active against colorectal cancer eliciting response data comparable to that of 5-FU.2 In combination, these two drugs are synergistic and demonstrate consistent superiority over single drug therapy.17

In this report, methyl-CCNU and 5-FU as single agents both showed significant anti-tumor effect against xenografts of human colon growing in nude mice. However, their activity is limited to temporary regression with subsequent regrowth or mere slowing of tumor growth, which is consistent with the clinical observation that colorectal cancers are relatively refractory to chemotherapy. Our results also agree with the findings of Osieka et al.18

Breast carcinoma generally exhibits a greater response rate to chemotherapy1 than that observed in patients with colorectal carcinoma. Alkylating agents as a class of drugs consistently exhibit activity against breast cancer, and among them cyclophosphamide and melphalan are the most active and commonly used. The antimitabolite 5-FU is also a well-recognized drug for the treatment of breast cancer yielding objective response data comparable to those of the alkylating agents. However, results of an extensive investigation suggest that 5-FU might not be beneficial in the postoperative adjuvant period.4 Combinations involving cyclophosphamide and 5-FU have produced relatively high response rates ranging from 50 to 60 percent.1

The breast tumor xenograft employed in this study exhibited significant sensitivity to cyclophosphamide and melphalan. Treatment with either drug produced long-term, tumor-free survivors. 5-FU did not arrest but simply delayed tumor growth. It is worth noting that the patient from whom this tumor line was obtained received postsurgical therapy involving radiation and 5-FU; she did not respond to such treatment regimen and died six months later. It is reasonable to speculate that either cyclophosphamide or melphalan might have been a better drug choice.

Although the data are limited and thus inconclusive, there appears to be a general agreement between a particular tumor xenograft and its corresponding clinical cancer type with respect to their response to a given drug or set of drugs. Povlsen and Jacobson21 reported similar findings with malignant melanoma xenografts which responded favorably to drugs effective in clinical therapy. For adequate evaluation, it is also necessary to assess the chemotherapeutic response of the xenograft system to known clinically inactive drug(s). Although these studies are not yet complete, the data to date indicate the use-
fulness of the nude mouse/human tumor xenograft system as a tool for detection of potentially active anticancer drugs.

References


