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Childhood Rhabdomyosarcoma Xenografts: Responses to DNA-interacting Agents and Agents Used in Current Clinical Therapy*

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Abstract—*A laboratory model of childhood rhabdomyosarcoma (RMS) has been used to evaluate cytotoxic agents used in current clinical protocols, and DNA-reacting agents that have had either limited or no evaluation in this histiotype. Seven lines of RMS each derived from a different patient were grown as xenografts in immune-deprived mice, six of these being from specimens derived from previously untreated patients. Of the 'conventional' agents, vincristine was the most effective. Of the other agents evaluated [L-phenylalanine mustard (L-PAM), cis-dichlorodiammineplatinum (cis-DDP), mitomycin C and 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC)], L-PAM caused complete regressions in six of seven lines, including those resistant to cyclophosphamide. DTIC had marked activity in five tumors, and mitomycin C in three lines. Cyclophosphamide was active in five tumors, although efficacy was less marked in two lines in comparison to DTIC and mitomycin C.*

INTRODUCTION

RHABDOMYOSARCOMA (RMS) arises *de novo* in skeletal muscle, and represents between 5 and 15% of all malignant solid tumors in children under 15 yr of age [1–3]; it is the most common soft tissue sarcoma in children [4, 5]. Rhabdomyosarcomas are, in general, moderately sensitive both to radiation therapy [6, 7] and to several chemotherapeutic agents [8]. Using the combined modalities of surgery, chemotherapy and radiotherapy, patients with early stage disease may be cured. However, the prognosis for children with more advanced disease is poor [9]. Relatively few chemotherapeutic agents have been evaluated fully in childhood RMS [10], and of those that have been examined, cyclophosphamide [11, 12], actinomycin D [13, 14], vincristine [15, 16] and doxorubicin [17, 18] have shown activity. Complete responses to chemotherapy alone occur in approximately 20% of patients. Tumor regrowth during therapy suggests that conventional treatment is unable to eradicate disease before the development of a drug-resistant clone, or that a resistant clone was present at the onset of therapy. In the treatment of RMS this presents a major problem, as cross-resistance between vincristine, actinomycin D and doxorubicin, three of the four agents used in primary treatment, has been well documented using model systems [19–23].

For the past several years, work in this laboratory has been directed toward developing a model of childhood RMS by growing specimens of neoplastic tissues as heterografts in immune-deprived mice. These xenografts show many biological similarities to the tumor of origin [24], and respond to chemotherapeutic agents known to be active against the clinical disease [25]. In this article we update our experience with 'conventional' agents used in the treatment of RMS using additional lines of tumor derived from previously untreated patients, and have used the model to evaluate different classes of agents that interact with DNA. Of particular note is the marked activity of the bifunctional alkylating agent L-phenylalanine mustard (L-PAM) against tumors intrinsically resistant or less sensitive to cyclophosphamide, and in one tumor derived from a child refractory to therapy with a regimen utilizing cyclophosphamide.

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MATERIALS AND METHODS

cis-Dichlorodiammineplatinum (*cis*-DDP) and mitomycin C were gifts from Bristol Laboratories (Syracuse, NY). L-Phenylalanine mustard (L-PAM) was purchased from Sigma Chemical Co. (St. Louis, MO), and other agents were supplied through the pharmacy at this hospital.

Immune-deprivation

Female CBA/CaJ mice were thymectomized at 4 weeks of age and received a single administration of cytosine arabinoside 48 hr prior to whole-body irradiation (850 rad, 170 rad/min, ^{137}Cs source) at 7 weeks of age, as described previously [26].

Tumor lines

Tumor lines HxRh12 and HxRh18 have been described previously [24].

Xenograft HxRh28 was derived from a metastatic mass in the axilla. Both human specimen and xenograft have alveolar histology. HxRh30 and HxRh35 were grown from bone marrow samples and are poorly differentiated embryonal rhabdomyosarcomas. HxRh39 was derived from an abdominal tumor and is also a poorly differentiated embryonal rhabdomyosarcoma. With the exception of HxRh10, each line of xenograft was established from tissue specimens taken prior to any chemo- or radiation therapy. Each of these tumor lines is human, as determined by lactate dehydrogenase isoenzyme profiles [24] or karyotype analysis.

Chemotherapy

Tumor fragments were implanted subcutaneously into the flanks of mice 1–2 weeks after irradiation. The growth of tumors was assessed from the measurement of two perpendicular diameters at 7-day intervals using Vernier calipers, as described previously [25]. For assessment of chemosensitivity, response of tumors to a single administration of each agent was measured by determining the delay in tumor growth and the incidence of partial ($\geqslant$50% regression) or complete regressions subsequent to treatment. Data are presented for the dose of each agent that was lethal in approximately 10% of tumor-bearing mice (LD_{10}). All agents were administered by intraperitoneal injection.

RESULTS

Data in Table 1 show the responses of seven lines of RMS to vincristine, actinomycin D, doxorubicin and cyclophosphamide, given as a single administration at equitoxic dose levels. In those xenografts derived from untreated specimens, VCR demonstrated the greatest activity in the mouse, with responses in six of seven lines. Complete tumor regressions were measured in HxRh12, HxRh28, HxRh30 and HxRh35 xenografts. Cyclophosphamide and doxorubicin were active ($\geqslant$++) against five and three lines of xenograft respectively. Actinomycin D has shown activity only against HxRh18 and HxRh28 xenografts. None of these agents had significant activity against HxRh10 xenografts derived from a patient refractory to conventional therapy.

We were therefore interested in determining whether classes of agents, other than classical bifunctional alkylating agents, that interacted with DNA were active in the model, and also in determining whether xenografts with intrinsic or acquired resistance to cyclophosphamide were sensitive to 5-(3,3-disubstituted-1-triazeno)imidazole-4-carboximides (e.g. DTIC) or aromatic nitrogen mustards (e.g. L-phenylalanine mustard). The responses of HxRh12 and HxRh18 to a single administration of L-PAM, cyclophosphamide, *cis*-DDP, DTIC and mitomycin C are shown in Fig. 1. Responses of HxRh28 and HxRh39 are presented in Fig. 2. Cyclophosphamide demonstrated some activity in five tumor lines, HxRh12, HxRh18, HxRh28, HxRh30 and HxRh39, but was virtually inactive in HxRh35 and HxRh10. In contrast, L-PAM was very active against each xenograft line irrespective of the sensitivity to cyclophosphamide. In HxRh18 xenografts L-PAM possessed similar activity to cyclophosphamide, whereas in the remaining lines it was considerably more active. With the exception of the relatively uniform response to L-PAM, responses of these xenografts to each of the other agents was individual. Hence *cis*-DDP was active in three lines, and mitomycin C also demonstrated greater activity against three tumors. Five lines responded well to DTIC. The pattern of response in HxRh10 xenografts derived from a patient refractory to treatment with vincristine, cyclophosphamide, actinomycin D and doxorubicin are shown in Fig. 3. This tumor showed a transient response to mitomycin C, DTIC and *cis*-DDP, and although a significant volume reduction occurred after treatment, tumors regrew rapidly. Thus, although these agents demonstrated some activity in HxRh10 tumors, this did not translate into a significant inhibition of tumor growth. Cyclophosphamide was inactive against HxRh10 xenografts, although this tumor was exquisitely sensitive to L-PAM. The responses of the seven lines of xenografts to these agents are summarized in Table 2.

DISCUSSION

It is apparent that for the treatment of disseminated RMS there is need for additional

Table 1. Responsiveness of human rhabdomyosarcoma xenografts to four agents used as primary therapy in the clinical disease*

Agent/tumor‡	LD_{10} dose (mg/kg)	Tumor line HxRh10§	HxRh12	HxRh18	HxRh28	HxRh30	HxRh35	HxRh39
Vincristine	3	+	++++++	+++	++++++	++++++	+++++	++
Cyclophosphamide	150	-	++	+++	++++	++	+	++++
Actinomycin D	0.3	-	-	++	++	-	±	-
Doxorubicin	10	-	++	±	+++	-	-	++

*Tumor response	Representation
No growth inhibition	-
Transient response, inhibition $<Td_2$†	±
Growth inhibition $\geq Td_2$	+
Growth inhibition $\geq 2 \times Td_2$	++
Growth inhibition $\geq 3 \times Td_2$	+++
Growth inhibition $\geq 3\ Td_2$ + volume regression $\geq 50\%$	++++
Complete regression with subsequent regrowth	+++++
Complete regression with no regrowth of any tumors during the period of observation (≥ 84 days)	++++++

†Td_2 = mean time for tumor volume to double.

‡All agents were given by the i.p. route at equitoxic doses.

§All tumor lines were established from previously untreated patients except for HxRh10, which was derived from a patient refractory to treatment with vincristine, cyclophosphamide, actinomycin D and doxorubicin.

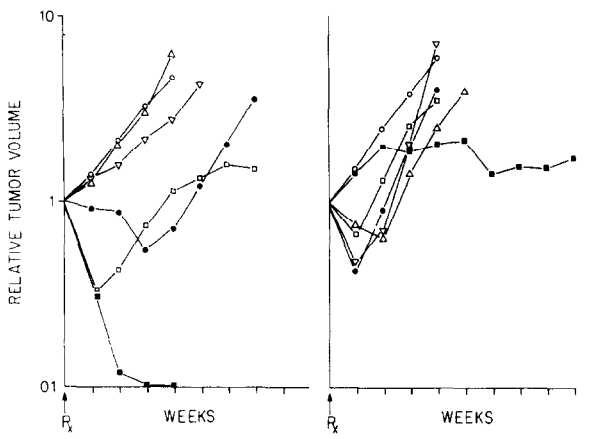

Fig. 1. The responses of HxRh12 (left) and HxRh18 xenografts to a single administration of agent given at the LD_{10} level. ○ No treatment; ● cyclophosphamide; □ DTIC; △ mitomycin C; ◁ cis-DDP; ■ L-PAM. Each curve represents the mean response between 10 and 14 tumors.

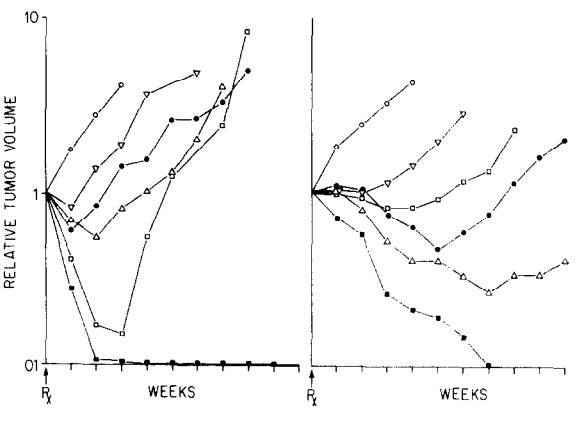

Fig. 2. The responses of HxRh28 (left) and HxRh39 xenografts to a single administration of agent given at the LD_{10} level. ○ No treatment; ● cyclophosphamide; □ DTIC; △ mitomycin C; ▽ cis-DDP; ■ L-PAM. Each curve represents the mean response between 10 and 14 tumors.

and more effective agents that are non-cross-resistant with those used in current therapy. The mechanism by which these agents are identified, however, remains the subject of considerable debate. Human tumors growing in immune-deprived or congenitally immunodeficient mice offer one approach to the development of models of specific human cancers *in vivo*, and there are now substantial data indicating that human tumor xenografts have a similar spectrum of sensitivity to their respective histiotypes in humans [25–29]. Such models may thus be of value in selecting agents that possess activity against a high proportion of tumors of a particular cancer type by virtue of exploiting some as yet unknown metabolic characteristic. Goldin and Venditti [30] have suggested that it may be advisable to employ a small battery of human tumors of the same histologic type to reduce the incidence of false positive and false negative results in current screening programs. In this study we have used seven lines of childhood RMS, six of which were derived from untreated patient specimens. Of the 'standard' agents evaluated, VCR had the greatest activity in the model, causing complete regressions in four lines at the MTD. However, these data must be viewed with the insight that in the mouse dose-limiting toxicity is due to gastrointestinal damage and myelosuppression, whereas in man neurotoxicity precedes myelosuppression. Thus the model may

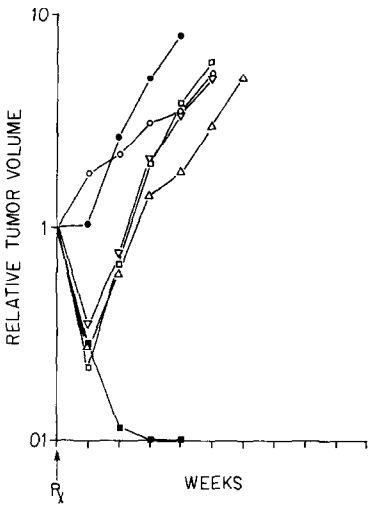

Fig. 3. The responses of HxRh10 xenografts to single agents. ○ No treatment; ● cyclophosphamide; □ DTIC; △ mitomycin C; ▽ cis-DDP; ■ L-PAM. Each curve represents the mean response between 10 and 14 tumors.

be more sensitive to VCR in the mouse than in man.

This, of course, raises the question of how results derived using xenografts may be extrapolated to a clinical situation. For example, the *N*-demethylation of DTIC, required for activation, may proceed more rapidly in the mouse than in man. An analogous situation, the *N*-demethylation of pentamethylmelamine to form the cytotoxic *N*-methylolmelamine, proceeds more rapidly in the mouse and may contribute to the greater activity of this agent against human tumor xenografts than has been observed in the clinic [31]. Thus, with these limitations taken into consideration, we have evaluated the activity of several agents known to be active against RMS. The model ranks these agents as VCR > cyclophosphamide > doxorubicin > actinomycin D, which is a similar order to that reported in clinical evaluation against RMS.

Cyclophosphamide was used in these studies as the standard agent against which other DNA-reacting agents were compared. Against xenografts derived from untreated specimens cyclophosphamide was active in five of six lines and *cis*-DDP in three tumors. Mitomycin C was very active in HxRh28, HxRh35 and HxRh39 tumors, whereas DTIC caused complete regressions in HxRh28 and HxRh35 and significant growth inhibition in three other lines (Table 2). None of these agents, or those used for conventional therapy (Table 1), had significant activity against the HxRh10 xenograft line. In contrast, L-PAM had marked activity in all seven tumor lines, causing complete regressions in six lines, with no regrowths in four lines over the duration of the experiment (≥84 days). Of note is the activity of L-PAM in tumor lines that are considerably less sensitive to cyclophosphamide (HxRh10, HxRh12, HxRh28, HxRh30, HxRh35 and HxRh39). The HxRh10 xenograft developed from a surgical specimen derived from a heavily pretreated patient refractory to VCR, actinomycin D, cyclophosphamide and doxorubicin would appear to have the pleiotropic drug-resistant phenotype. However, in contrast to a cloned line of Chinese hamster ovary cells selected for resistance to colchicine, in which cross-resistance between natural products and L-PAM was observed [32], HxRh10 remains very sensitive to this alkylating agent.

Data derived using these xenografts suggest that L-PAM in particular, but in addition DTIC and mitomycin C, may be of value in the clinical management of RMS. DTIC has demonstrated activity against RMS when used in combination [33, E. Etcubanas, personal communication]. We are aware of only one report in which L-PAM has been used in childhood RMS [34]. This patient had a complete response subsequent to L-PAM (≥120 mg/m²), with autologous bone marrow rescue. The data derived using the xenograft model suggest that RMS may be very sensitive to this agent at conventional dose levels. Whether this heterograft model identifies agents useful in the treatment of pediatric rhabdomyosarcoma remains to be tested prospectively.

*Table 2. Responsiveness of xenografts of childhood rhabdomyosarcoma to DNA reacting agents**

Agent/tumor	LD_{10} dose (mg/kg)	HxRh10†	HxRh12	HxRh18	HxRh28	HxRh30	HxRh35	HxRh39
L-PAM	13	++++++‡	+++++	+++	++++++	+++++	++++++	++++++
Cyclophosphamide	150	-	++	+++	++++	++	+	++++
cis-DDP	7	±	+	++	++	+	+	++
Mitomycin C	3.25	+	-	+	++++	+	++++	+++++
DTIC	200	±	+	+++	+++++	++++	+++++	+++

*Agents administered as a single i.p. injection at equitoxic doses.
†HxRh10 derived from a patient refractory to vincristine, actinomycin D, doxorubicin and cyclophosphamide.
‡Responses were determined as for Table 1.

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