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Evaluation of the Retinoids with Cisplatin and Vincristine in Xenograft Models of Neuroblastoma

Robin E. Norris, M.D.^{1,*}, Vu T. Nguyen, B.S.², and Peter C. Adamson, M.D.²

¹Division of Pediatric Hematology/Oncology, Rainbow Babies and Children's Hospital

²Division of Clinical Pharmacology & Therapeutics, Division of Oncology, The Children's Hospital of Philadelphia

Abstract

Retinoids have been studied for the treatment of children with neuroblastoma for more than 25 years. Post-transplant administration of isotretinoin is standard of care for children with high-risk neuroblastoma, while fenretinide remains investigational. Previous preclinical studies have evaluated the interaction of retinoids and cytotoxic agents with conflicting results. We evaluated the schedule dependent interaction of the cytotoxics, vincristine and cisplatin, with the retinoids, isotretinoin and fenretinide, in xenograft models of neuroblastoma. Concomitant administration of isotretinoin or fenretinide with the cytotoxics did not result in any clear potentiation of cytotoxicity.

Keywords

neuroblastoma; fenretinide; isotretinoin; *in vivo*; preclinical

INTRODUCTION

Neuroblastoma is the most common extra-cranial solid tumor in children and accounts for 15% of deaths from cancer in children in the United States. The retinoids, both naturally occurring and synthetic derivatives of vitamin A, have been evaluated as therapeutic agents for the treatment of children with high-risk neuroblastoma. Isotretinoin (13cRA) causes neuroblastoma cells to differentiate leading to decreased proliferation¹⁻³. Treatment with 13cRA following myeloablative therapy with autologous stem cell transplantation resulted in an improved 3-year event-free survival in children with high-risk neuroblastoma⁴, although the long-term impact of 13cRA on cure is less clear⁵. Fenretinide (4HPR) is a potent trigger of apoptosis in preclinical models of neuroblastoma^{6,7}. Pediatric phase 2 clinical evaluation of 4HPR in pediatric patients with relapsed or refractory neuroblastoma resulted in 1 partial response and 13 subjects with stable disease amongst 42 subjects enrolled⁸.

*Correspondence to: Robin Norris, MD, Division of Pediatric Hematology & Oncology, Rainbow Babies and Children's Hospital, 11100 Euclid Avenue, Mailstop RBC 6054, Cleveland, OH 44106. Tel: (216) 844-3345, Fax (216) 844-5431, Robin.Norris@UHhospitals.org.

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While use of 13cRA is considered standard of care for children with disseminated high-risk neuroblastoma, the current practice of only administering retinoids following cytotoxic chemotherapy is largely empiric. This investigation evaluating the potential role for integration of retinoid therapy with upfront cytotoxic chemotherapy was conducted, in part, based on the observation in adult tumor models that retinoid therapy enhances the activity of cytotoxic chemotherapy^{9–13}. Moreover, the use of retinoids administered concomitantly with cytotoxic chemotherapy has been found to be the most effective use of retinoid therapy in the treatment of patients with acute promyelocytic leukemia,^{13–15} and explored clinically for other cancers including advanced cervical carcinoma^{16–22}. We previously evaluated the schedule dependency of the most commonly used cytotoxic chemotherapeutic drugs (etoposide, doxorubicin, topotecan, irinotecan, mafosfamide, cisplatin, and vincristine) in the treatment of neuroblastoma in combination with retinoids *in vitro*²³. Vincristine and cisplatin were the two cytotoxic agents that most consistently resulted in additive to synergistic effects with concomitant exposure to retinoids. We therefore evaluated the interaction of 13cRA and 4HPR with vincristine and cisplatin in xenograft models of neuroblastoma.

MATERIALS AND METHODS

Xenograft Models

CB17 SCID female mice (Taconic Farms, Germantown, NY) were used to propagate subcutaneously implanted neuroblastoma (SY5Y or NGP) tumors as previously described²⁴. SY5Y and NGP cell lines were generously provided by Dr. John Maris, The Children's Hospital of Philadelphia (CHOP). The SY5Y cell line (MYCN non-amplified, no 1p deletion) was established at the time of relapse following therapy with vincristine, daunorubicin, doxorubicin, cyclophosphamide and radiation^{25,26}. The NGP cell line (MYCN amplified, no 1p deletion, 3p LOH, 11q deletion, no unbalanced gain of 17q) was also established at the time of relapse following therapy with vincristine and cyclophosphamide^{25–27}. Both the NGP and SY5Y cell lines have demonstrated sensitivity to 13cRA and 4HPR²⁸. The methods used in this study were based on the published procedures used in xenograft testing by the Pediatric Preclinical Trials Program (PPTP)²⁴. All experiments were conducted using protocols and conditions approved by the institutional animal care and use committee.

Drugs

Isotretinoin (Sigma, St. Louis, MO) was dissolved in peanut oil to a concentration of 1mg/1mL and administered via oral gavage. Fenretinide (generously provided by the National Cancer Institute, Bethesda, MD) was dissolved in ethanol and diluted in sterile 0.9% saline containing 1.65 mg/mL bovine serum albumin (Sigma, St. Louis, MO) w/v to a final ethanol concentration of 5% (v/v) and administered via intraperitoneal injection. Cisplatin (Sigma, St. Louis, MO) was diluted in 0.9% saline and administered via intraperitoneal injection immediately after preparation. Vincristine (VCR) (kindly provided through the hospital pharmacy) was diluted in sterile water and administered via intravenous tail injection. Fenretinide, cisplatin, and VCR were all diluted such that the final volume administered was 10 μ L/g of body weight.

Treatment

Four experimental combinations were tested: cisplatin + 13cRA, cisplatin + 4HPR, VCR + 13cRA, and VCR + 4HPR. Mice received drug when tumors reached between 200 mm³ and 250 mm³, approximately 11 days after xenotransplantation. At that time, 35 mice were randomized into 4 groups: retinoid (n=10), cytotoxic (n=10), retinoid + cytotoxic (n=10), and control (n=5). 13cRA and 4HPR were given at a dose of 10mg/kg daily for five days for

a total of six weeks or until mice were sacrificed due to tumor growth or unacceptable toxicity. Vincristine (0.7 mg/kg) and cisplatin (7mg/kg) were given on days 1 and 14. Control mice received 0.9% saline via intraperitoneal injection daily. In mice receiving both retinoid and cytotoxic, the retinoids were given 30 minutes prior to the administration of the cytotoxic. Dexamethasone (1mg/kg) was given subcutaneously 30 min prior to administration of cisplatin to reduce cisplatin-associated emesis. Tumor size was determined at least twice weekly.

RESULTS

At the tolerable doses evaluated, 13cRA and 4HPR demonstrated limited activity in the two xenograft models studied (Figures 1 and 2). The doses selected for evaluation of cisplatin and vincristine were based on results of prior publications^{29,30} and on limited dose tolerability experiments conducted in our laboratory exploring higher doses, which resulted in unacceptable toxicity (data not shown). The described evaluated doses of both retinoids and cytotoxic chemotherapy were at, or in a few cases still exceeded, maximum tolerated doses (weight loss). Vincristine demonstrated modest, reproducible activity in both cell lines (Figure 2) while cisplatin had more variable effects (Figure 1).

Concomitant administration of either 13cRA or 4HPR with vincristine or cisplatin did not result in any clear potentiation of antitumor activity (Figures 1 and 2), although no evidence of antagonism was noted.

DISCUSSION

In vitro models used to evaluate the interaction of 13cRA with cytotoxic chemotherapy have yielded conflicting results^{23,31–33}, with concern that pre-treatment with 13cRA induces differentiation and subsequent resistance to cytotoxic agents, including cisplatin^{32,33}. In contrast, a number of *in vitro* studies have found a synergistic interaction when all-*trans* retinoic acid, the active isomerized form of 13cRA, is administered either as pretreatment or concomitantly with cytotoxic agents, including cisplatin^{9,34–37}. Similarly, most studies with 4HPR have found that the addition of 4HPR enhances the activity of cisplatin in *in vitro* models of cancer^{10,31}. *In vivo* models using ovarian carcinoma have also demonstrated that 4HPR potentiates the effects of cisplatin^{38,39}, but similar data is lacking for neuroblastoma. We therefore evaluated concomitant administration of the two best-studied retinoids in neuroblastoma with cytotoxic chemotherapy in two xenograft models of neuroblastoma. The cell lines evaluated were chosen based on our prior *in vitro* studies and on the differing MYCN status of the lines. Doses of the agents that proved tolerable and would allow for assessment of drug interactions were employed. There was no evidence of a negative interaction, nor was there evidence to suggest marked enhancement of cytotoxicity by 13cRA or 4HPR. Although evaluation using higher doses of either retinoid or cytotoxic chemotherapy could result in a more favorable interaction profile, due to unacceptable toxicity, such doses could not be studied in our model system.

There are a number of potential pitfalls when extrapolating from *in vitro* data to the clinic. Although xenograft model results are not necessarily predictive of clinical efficacy, such models do overcome a number of limitations of *in vitro* models, including to varying extent the impact of drug distribution and drug clearance. The data presented unfortunately did not support our preliminary *in vitro* findings suggesting favorable interaction between retinoids with cytotoxic chemotherapy, and thus we did not expand our evaluation to other xenograft models. Although limited in scope, our data do not lend support for evaluation of concomitant administration of retinoids with cytotoxic chemotherapy in neuroblastoma.

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A Cisplatin and 13cis Retinoic Acid

B Cisplatin and Fenretinide

Figure 1.

Responses of neuroblastoma xenografts to cisplatin in combination with the retinoids. Tumor bearing mice were treated with cisplatin (7mg/kg on days 1 and 14) or retinoid (10mg/kg daily $\times$ 5 repeated weekly for a total of six weeks) as single agents or in combination. (A) Interaction between cisplatin and 13cRA. (B) Interaction between cisplatin and 4HPR. Control (—); Cisplatin (---); Retinoid (.....); Cisplatin + Retinoid (- · -).

A Vincristine and 13cis Retinoic Acid

B Vincristine and Fenretinide

Figure 2.

Responses of neuroblastoma xenografts to vincristine in combination with the retinoids. Tumor bearing mice were treated with vincristine (0.7mg/kg on days 1 and 14) or retinoid (10mg/kg daily $\times 5$ repeated weekly for a total of six weeks) as single agents or in combination. (A) Interaction between vincristine and 13cRA. (B) Interaction between vincristine and 4HPR. Control (—); Vincristine (---); Retinoid (.....); Vincristine + Retinoid (- · -).