Journal of Pediatric Surgery (2012) 47, 1267–1271

ELSEVIER

Journal of Pediatric Surgery

www.elsevier.com/locate/jpedsurg

Efficacy of class I and II vs class III histone deacetylase inhibitors in neuroblastoma ☆

Timothy B. Lautz[a,b], Jessica A. Naiditch[a,b], Sandra Clark[b], Fei Chu[b,*], Mary Beth Madonna[a,b,*]

[a]*Department of Surgery, Children's Memorial Hospital, Northwestern University, Chicago, IL 60614, USA*
[b]*Cancer Biology and Epigenetics Program, Children's Memorial Research Center, Northwestern University, Chicago, IL 60614, USA*

Received 3 March 2012; accepted 6 March 2012

Key words:
Histone deacetylase inhibitor;
Vorinostat;
Neuroblastoma;
Chemotherapy resistance

Abstract

Background: Histone deacetylase (HDAC) inhibitors have shown promise in the treatment of resistant and refractory tumors including neuroblastoma. The goal of the study was to compare the efficacy of a class III HDAC inhibitor (cambinol) to a class I and II inhibitor (vorinostat).

Methods: In vitro efficacy of vorinostat and cambinol, alone or in combination with doxorubicin, was assessed by 2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide calorimetric assay using both wild-type (WT) and doxorubicin-resistant (DoxR) SK-N-SH neuroblastoma cells. In vivo efficacy was determined using the same drug combinations in nude mice bearing xenograft implants of WT and DoxR cells on opposite flanks.

Results: Vorinostat and cambinol were efficacious against WT and DoxR neuroblastoma cells in vitro. In WT cells, the potency of the doxorubicin itself overshadowed any effect of cotherapy with vorinostat or cambinol. The effect of vorinostat and/or cambinol on the DoxR cells was constant across progressively increasing doses of doxorubicin. In the in vivo model, the efficacy of doxorubicin itself (88% reduction in tumor volume) again overshadowed any effect of cotreatment with vorinostat or cambinol on the WT tumors. However, in the DoxR tumors, doxorubicin alone had no efficacy, but cotreatment with either cambinol or vorinostat suppressed tumor growth (70% and 91% reduction in tumor volume, respectively).

Conclusions: Both the class III HDAC inhibitor cambinol and the class I/II HDAC inhibitor vorinostat have efficacy against SK-N-SH neuroblastoma cells, including those resistant to doxorubicin.

Neuroblastoma is the most common extracranial solid tumor of childhood and accounts for 15% of cancer-related deaths in children. The natural history of neuroblastoma and its responsiveness to standard treatment regimens are highly variable. Survival in infants is excellent, and tumors in the youngest children often regress without therapy [1]. In contrast, older children with neuroblastoma, especially those with unfavorable histology and metastatic disease, have a poor prognosis. Survival in this population remains only 20% to 35% despite intensive chemotherapy and autologous

☆ There are no disclaimers or funding sources to report.
* Corresponding authors. 2300 Children's Plaza, Box 63, Chicago, IL 60614, USA. Tel.: +1 773 880 4912; fax: +1 773 880 4588.
E-mail addresses: MMadonna@luriechildrens.org (M.B. Madonna), FChu@luriechildrens.org (F. Chu).

0022-3468/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.jpedsurg.2012.03.039

bone marrow transplant [2]. Acquired resistance to chemotherapeutic agents remains a primary barrier to successful treatment of neuroblastoma and drives the search for alternative therapeutic agents to treat children with relapsed and resistant disease.

Histone deacetylase (HDAC) inhibitors are a novel class of drugs that have demonstrated promise in the treatment of a wide variety of pediatric malignancies. The HDAC family consists of 18 members classified into 4 groups (classes I-IV) based on their sequence homologies [3]. Histone deacetylases are important regulators of gene transcription via their role in deacetylating the amino-terminal tails of histones. In addition, epigenetic modification of a growing list of nonhistone HDAC substrates has been implicated in various aspects of cancer physiology and tumorigenesis [3-5]. Histone deacetylase inhibitors have demonstrated a wide range of effects on cancer cells, including growth inhibition, induction of cell death, differentiation, and antiangiogenesis [4,6,7]. Vorinostat is the first Food and Drug Administration–approved HDAC inhibitor and is currently in phase I trials for a number of hematologic and solid organ malignancies [8]. Histone deacetylase inhibitors have shown therapeutic promise against neuroblastoma in preclinical studies, and vorinostat has been well tolerated in early clinical studies of children with recurrent solid tumors [9-11].

Vorinostat broadly inhibits class I and II but not class III HDACs. The class III HDACs (sirtuins 1–7), which are evolutionarily distinct and depend on a nicotinamide adenine dinucleotide cofactor, can be inhibited by cambinol [12]. The class III HDAC *sirt1* has been shown to regulate the multidrug resistance molecule P-glycoprotein in neuroblastoma and may, therefore, represent a target for treating drug-resistant tumors [13].

The aim of the present study was to compare the relative efficacy of the class I and II HDAC inhibitor vorinostat with that of the class III HDAC inhibitor cambinol.

1. Methods

1.1. Cell lines and reagents

Human neuroblastoma (SK-N-SH) cells were purchased from American Type Culture Collection (Manassas, VA). Doxorubicin-resistant (DoxR) cells were generated by incubating the wild-type (WT) cells with incremental concentrations of doxorubicin (Sigma, St Louis, MO) ranging from 10^{-9} to 10^{-6} M during a 9-month period. Doxorubicin resistance was confirmed by 2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) calorimetric assay as previously described [14]. Dulbecco modified Eagle medium and fetal bovine serum for tissue culture were obtained from BioWhittaker (Walkersville, MD).

1.2. In vitro drug efficacy

The effect of treatment with vorinostat, cambinol, and/or doxorubicin on cell proliferation was assessed by MTT calorimetric assay. Cells were seeded in 96-well plates and incubated with logarithmic concentrations of doxorubicin (10^{-9}-10^{-5} M), vorinostat (10^{-9}-10^{-5} M), or cambinol (10^{-8}-10^{-4} M) for 96 hours. Ten microliters of 5 mg/mL MTT solution was added to each well of the titration plate and incubated for 4 hours at 37°C. The cells were then solubilized by the addition of 100 μL of 10% sodium dodecyl sulfate/0.01 mmol/L HCl and incubated for 15 hours at 37°C. The absorbance of each well was determined in an enzyme-linked immunosorbent assay–plated reader using an activation wavelength of 570 nm and a reference wavelength of 650 nm. Relative cell proliferation was determined by comparison with untreated control cells.

We then assessed for a synergistic effect of cotreatment of vorinostat and/or cambinol with doxorubicin. The concentration that reduced the number of viable cells by 25% (IC_{25}) of cambinol and vorinostat was determined from the previously described MTT assays. Cell proliferation was then assessed by MTT assay using cells cotreated with the IC_{25} dose of cambinol and/or vorinostat combined with logarithmic concentrations of doxorubicin (10^{-9}-10^{-5} M).

1.3. In vivo drug efficacy

The in vivo efficacy of cambinol and vorinostat treatment was assessed in a nude mouse model. The animal protocol was approved by the Animal Care and Use Committee of the Children's Memorial Research Center (#2010-15). Nude mice (strain CD1; Charles River Laboratories, Wilmington, MA) of 5 to 6 weeks of age and weighing approximately 30 g received subcutaneous tumor injections. Wild-type and DoxR SK-N-SH cells (10^6 cells in 100 μL) were implanted into opposite flanks of 42 nude mice. When tumors were palpable (~50 mm^3), the mice were divided into 7 groups of 6 mice each, as follows: (*a*) control, (*b*) vorinostat (50 mg/kg per dose) alone, (*c*) cambinol (100 mg/kg per dose) alone, (*d*) doxorubicin (2.5 mg/kg per dose) alone, (*e*) vorinostat plus doxorubicin, (*f*) cambinol plus doxorubicin, and (*g*) vorinostat plus cambinol plus doxorubicin. A total of 3 injections were given during a 1-week period. Tumor measurements were obtained biweekly and converted to tumor volume using the equation length × $(\text{width}/2)^2$. Weights were obtained weekly. The mice were humanely killed when the maximal dimension of either tumor reached 15 mm.

1.4. Statistical analysis

Continuous variables are reported as a mean ± standard error, and groups are compared by Student *t* test. All analyses were 2 tailed, and a $P < .05$ was considered significant.