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Histone deacetylases inhibition and tumor cells cytotoxicity by CNS-active VPA constitutional isomers and derivatives

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Abstract

The tumor cells toxicity of the antiepileptic drug valproic acid (VPA) has been associated with the inhibition of histone deacetylases (HDACs). We have assessed, in comparison to VPA, the HDACs inhibition and tumor cells cytotoxicities of CNS-active VPA's constitutional isomers, valnoctic acid (VCA), propylisopropylacetic acid (PIA), diisopropylacetic acid (DIA), VPA's cyclopropyl analogue 2,2,3,3-tetramethylcyclopropanecarboxylic acid (TMCA) and VPA's metabolites, 2-ene-VPA and 4-ene-VPA, all possessing, as does VPA, eight carbon atoms in their structures. The aim was to define structural components of the VPA molecule that are involved in HDACs inhibition and tumor cells cytotoxicity.

HDACs inhibition by the above-mentioned compounds was estimated using an acetylated lysine substrate and HeLa nuclear extract as a HDACs source. SW620 cells were used for assessing HDACs inhibition in vivo. The cytotoxicity of these compounds was assessed in SW620 and 1106mel cells.

HDAC inhibition potency was the highest for VPA and 4-ene-VPA (IC_{50} = 1.5 mM each). 2-Ene-VPA inhibited HDACs with IC_{50} = 2.8 mM. IC_{50} values of the other tested compounds for HDACs inhibition were higher than 5 mM, 4-ene-VPA and VPA induced histone hyperacetylation in SW620 cells. 4-Ene-VPA and VPA at 2 mM each were also most potent in reducing cell viability, to 59 ± 2.0% and 67.3 ± 5.4%, respectively, compared to control. VCA, PIA, DIA, TMCA, 2-ene-VPA and valpromide (VPD) did not reduce viability to less than 80%. All tested compounds did not significantly affect the cell cycle of SW620 cells.

In conclusion, in comparison to the VPA derivatives and constitutional isomers tested in this study, VPA had the optimal chemical structure in terms of HDACs inhibition and tumor cells cytotoxicity.

JEL classification: Molecular pharmacology

Keywords: Valproic acid; Valproic acid constitutional isomers; Anticancer drugs; Histone deacetylase; Antiepileptic drugs

1. Introduction

Valproic acid (VPA), Fig. 1 is an eight-carbon, branched side chain carboxylic acid with a broad spectrum of antiepileptic activities, which is used also for the treatment of bipolar disorder, neuropathic pain and migraine prophylaxis [1,2]. The anti-proliferative properties of VPA were first demonstrated in 1985 [3]. VPA inhibited at anticonvulsant therapeutic concentrations the mitotic index of murine neuroblastoma and glioma cells. Prolongation of the cell cycle has been attributed to its arrest in the G1 phase [3–5]. Continued exposure to VPA-induced differentiation in various cell lines [6,7] as well as in transformed hematopoietic progenitor cells [8] and leukemic blasts [5]. VPA induced apoptosis in breast carcinoma cells [8], in leukemia cells from patients with acute myeloid leukemia [5] and in vivo, in neuroblastoma xenografts in athymic mice, and this effect may contribute to VPA's effect on proliferation rate [9–11]. Furthermore, administration of

Abbreviations: AED, antiepileptic drug; BuA, butyric acid; HDAC, histone deacetylase; DIA, diisopropylacetic acid; PI, propidium iodide; PIA, propylisopropylacetic acid; TMCA, 2,2,3,3-tetramethylcyclopropanecarboxylic acid; TSA, trichostatin A; VCA, valnoctic acid; VPA, valproic acid; VPD, valpromide

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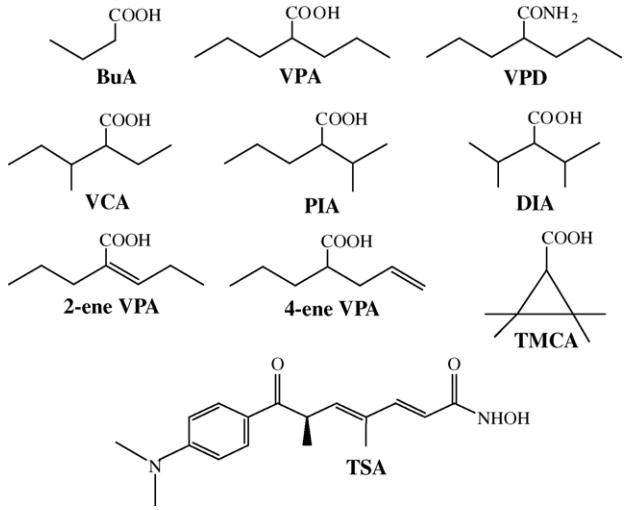

Fig. 1. Chemical structures of the tested compounds. Abbreviations: VPA, valproic acid; VPD, valpromide; VCA, valnoctic acid; PIA, propylisopropylacetic acid; DIA, diisopropylacetic acid; TMCA, 2,2,3,3-tetramethylcyclopropanecarboxylic acid; BuA, butyric acid; TSA, trichostatin A.

VPA to rodents reduced tumor growth and metastasis [8,12]. Based on these results, VPA is currently being used in clinical trials for the treatment of gliomas in children [10,11,13].

More recently it has became evident that similar cellular targets, such as activation of peroxisome proliferator-activated receptor δ (PPARδ) and modulation of the Wnt signaling pathway, may be involved in both VPA's antitumor activity and in its teratogenicity [6,8,14,15]. Both the antitumor and the teratogenic effects of VPA as well as its interaction with some of the aforementioned molecular targets have also been associated with its activity as histone deacetylases (HDACs) inhibitor [8,15,16]. HDACs inhibitors with diverse chemical structures, such as butyric acid (BuA) and trichostatin A (TSA) (Fig. 1), induce histone hyperacetylation and directly alter the transcription of a subset of genes, with resulting antiproliferative, apoptotic and differentiating effects [8,13,17–20]. However, the use of some of these inhibitors is limited by their toxicities or poor pharmacokinetics [17–20]. Compared to other HDACs inhibitors, VPA is generally a well-tolerated drug that is administered orally and has a relatively long half-life [8,17].

Characterization of HDACs inhibition profile and related induction of differentiation of transformed cells by a series of VPA analogues have provided further evidence for the involvement of HDACs inhibition in the antitumor action of VPA [8,15,21]. However, the contribution of specific elements in the structure of VPA molecule for HDACs inhibition has only been partially investigated.

Based on different structural requirements for antiepileptic activity, hepatotoxicity and teratogenicity for VPA and its analogues, second-generation VPA derivatives and analogues have been developed with improved antiepileptic activity and/or reduced teratogenicity and hepatotoxicity [22]. These second-generation drugs include constitutional isomers of VPA, such as propylisopropylacetic acid (PIA) and valnoctic acid (VCA), which exert antiepileptic potencies similar to those of VPA in animal models, but are non-teratogenic [23,24]. In addition, numerous cyclpropyl analogues of VPA have been synthesized which possessed similar or better antiepileptic activities in rodents, compared to VPA [25,26].

The aim of the current work was to assess HDACs inhibition and tumor cell toxicity by CNS-active analogues and constitutional isomers of VPA and two VPA metabolites, 2-ene-VPA and 4-ene-VPA. All tested compounds, including VPA, possess eight carbon atoms in their chemical structures, which enable us to define specific structural components in the aliphatic moiety of the VPA molecule responsible for HDACs inhibition and tumor cell toxicities. We demonstrate here that structural isomers of VPA are distinct from VPA in terms of their potencies as HDACs inhibitors.

2. Materials and methods

2.1. Drugs

VPA was a gift from Teva Pharmaceutical Industries, Petach Tikva, Israel. 2-ene-VPA and 4-ene-VPA were gifts from the Department of Pharmaceutics, University of Washington, Seattle. 2,2,3,3-Tetramethylcyclopropanecarboxylic acid (TMCA), sodium butyrate and TSA were purchased from Sigma–Aldrich, Rehovot, Israel.

VPA's constitutional isomers were synthesized according to the synthetic procedures previously described in the following references: PIA, [24]; DIA, [27]; VCA, [28]. Solvents and drugs were purchased from Sigma–Aldrich.

Sodium butyrate, which served as a positive control, was dissolved in either the incubation buffer or cell culture media. VPA, its analogues and derivatives and TSA were dissolved in DMSO, (Sigma–Aldrich) and diluted in buffer or medium up to a final concentration of 1% DMSO, except for HDACs inhibition assay with TMCA, where the highest final DMSO concentration was 10%.

2.2. Cells

SW620 and 1106mel cells were cultured in RPMI 1640 (Biological Industries Ltd., Beit Haemek, Israel) and supplemented with 10% fetal calf serum (Biological Industries Ltd.) and 1% penicillin/streptomycin (Biological Industries Ltd.). Cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2/95% O_2.

Cells were incubated with 300 nM TSA or the indicated concentrations of other tested compounds in culture medium for the indicated periods. Controls were prepared by incubating cells for the same periods with the medium containing DMSO at the corresponding dilutions. pH