Valproic acid as an anticancer therapy-sensitizing agent

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Abstract

Valproic acid is an antiepileptic drug with more than 50 years of clinical use. In the past decade, its anticancer effects were discovered. Analyses in groups of patients who used this drug for years have shown that it decreases the frequency of head and neck cancer. Recent studies show the anticancer effect of combining valproic acid with chemotherapy, biological therapy and antioxidant systems inhibitors, with exceptional results. In this review, we analyze the metabolism of valproic acid and its application against cancer.

KEY WORDS: Valproic acid. Human cancer therapy. Epigenetic therapy. Histone deacetylase inhibitors.

Introduction

Valproic acid (VPA) is used in neurological diseases such as epilepsy, migraine, bipolar disorder and attention deficit.^{1,2} VPA functions as a sensitizer to anticancer treatments by epigenetically modifying gene expression.

Epigenetics and cancer

Malignant neoplasms lose the ability to control their proliferation, invading other tissues (metastasis).³ This alteration is due to mutations in proto-oncogenes and tumor-suppressor genes. The above is generated by genetic and epigenetic changes.^{3,4} Epigenetic changes can be reversible and consist of mechanisms such as methylation of DNA cytokine-rich regions (CpG) or “CpG islands” that when methylated inhibit gene

expression, messenger RNA block by micro-RNA molecules (miRNA) and histone deacetylation.^{3,4}

Histone deacetylases and acetyltransferases in the epigenetic control of genes

DNA is wrapped around histone octamers (H2A, H2B, H3 and H4) that form the nucleosome, which when condensed integrate chromatin.⁵ Each histone has a “tail” in the amino terminus, which is rich in basic amino acids such as lysine, a post-translational modification target, thus making for accessibility to DNA to be partially controlled by changes in this structure.⁶ Histones regulatory mechanisms include modifications by methylation, acetylation, phosphorylation and ubiquitination, among others.⁷ Acetylation and deacetylation of histones and cytoplasmic proteins are

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Date of reception: 15-10-2018
Date of acceptance: 29-10-2018
DOI: 10.24875/GMM.M19000307

Gac Med Mex. 2019;155:384-388
Contents available at PubMed
www.gacetamedicademexico.com

Table 1. Classification of histone deacetylases

HDAC Group	HDAC Enzyme	Proteins it interacts with
Class I (homologous to Rpd3)	HDAC1, HDAC2, HDAC3, HDAC8	p53, RB, MYOD, NF- κ B, DNMT1, DNMT3a, MBD2, Sp1, BRCA1, MeCP2, ATM, Smad7
	HDAC2	RB, NF- κ B, BRCA1, DNMT1
	HDAC3	RB, NF- κ B, Smad7, Stat3, SRY
	HDAC8	ND
Class IIIa (homologous to Hdac)	HDAC4	MEF2
	HDAC5	MEF2
	HDAC7	MEF2
	HDAC9	
Class IIb	HDAC6	Smad7, α -tubulin, Hsp-90
	HDAC10	
Class III (homologous to Sir2)	SIRT1	p53, FOXO1, p300, NF- κ B, α -tubulin
	SIRT2	α -tubulin
	SIRT3	
	SIRT4	
	SIRT5	
	SIRT6	Heterochromatin-related
	SIRT7	Nucleolus-related
Class IV(similar to classes I and II)	HDAC11	

reversible and are controlled by two enzymes: histone acetyltransferases and histone deacetylases (HDAC).⁸ Histone acetyltransferases transfer acetyls to histone tails' lysine, which eliminates lysine positive charge, thus decreasing binding with DNA. As a consequence, transcription factors and RNA polymerase do access.^{7,8} Conversely, HDACs remove acetyl groups, thus increasing DNA attraction towards positive histone charges; this compacted DNA does not allow the entry of transcription factors or RNA polymerase. In cancer, suppressor genes are preferably deacetylated.^{7,8} Acetylation also controls cytoplasmic proteins, regulating gene expression, cell cycle, splicing, transport and actin nucleation.⁹

The HDAC family

In humans, 11 HDACs are known, divided into classes I, II, III and IV, whose classification is based on their homology with yeast HDACs:⁶

- Class I HDAC, proteins that use Zn⁺ as cofactor and express themselves ubiquitously and include HDAC1, HDAC2, HDAC3 and HDAC8.

- Class IIa HDACs include HDAC4, HDAC5, HDAC7 and HDAC9; they are found both in the nucleus and the cytoplasm,
- Class IIb HDACs are present in the cytoplasm and include HDAC6 and HDAC10.
- Class III HDACs, sirtuins, are located in the cytoplasm and mitochondria; they use nicotinamide and adenine dinucleotide as cofactors.
- Class IV HDACs include HDAC11 and are located in the cytoplasm^{10,11} (Table 1).

HDAC1, HDAC2, HDAC3 and HDAC8 participate in cell proliferation. In apoptosis, HDAC1 and HDAC2 stand out. In chemotherapy resistance, HDAC1 predominates; in differentiation, HDAC3, HDAC4, HDAC5 and HDAC8; in angiogenesis, HDAC4, HDAC6, HDAC7 and HDAC10; and in migration, HDAC6.^{12,13}

Valproic acid metabolism

VPA in children has been associated with liver failure.¹⁴⁻¹⁶ Its elimination depends on its biotransformation into more water-soluble products, a process that is divided in two phases:¹⁴

Figure 1. Valproic acid biotransformation by the cytochrome system; hepatotoxic 4-ene-valproic metabolite formation is shown.

- Phase I, includes oxidation, reduction and hydrolysis reactions.
- Phase II, where conjugation reactions with glucuronate, glutathione, carnitine, coenzyme A or with amino acids such as glycine or glutamic acid do participate.

VPA oxidative metabolism is mitochondrial by means of beta oxidation. In hepatocytes, it is inactivated by phase II, which by conjugation generates renal excretion polar products. VPA is a substrate for the CYP2C6 and CYP2C9 isoforms.^{17,18} Of all the metabolism compounds that are generated, 4-ene-valproic is more hepatotoxic (Fig. 1).¹⁶

HDAC-inhibitors mechanism of action

HDAC inhibitors (HDACis), such as VPA, stop the cell cycle, generate differentiation and apoptosis in human cancer cell lines, inhibit tumor growth in animal models and have shown antitumor activity in controlled clinical trials.¹⁹⁻²¹ In addition, they activate autophagy, generate reactive oxygen species and disrupt the aggressive pathway.¹³ HDAC inhibition causes over-acetylation of these proteins, which reactivates the transcription of tumor suppressor genes and reverts cancer.⁶

In controlled clinical trials, HDACis show acceptable results in the treatment of hematological neoplasms and, therefore, in 2006, the Food and Drug Administration approved the suberanilohydroxamic acid (SAHA, vorinostat) and, in 2009, romidepsin, for the

treatment of T-cell cutaneous lymphoma, bone marrow dysplasia and peripheral T-cell lymphoma.²² In early 2015, panobinostat was approved for the management of multiple myeloma.²³ Notwithstanding the above, the results in solid tumors have been variable.²⁴ HDACis could also be useful in viral diseases and diabetes mellitus.^{2,23-27} Good results have been observed in follicular lymphoma and marginal zone lymphoma.²⁸

Originally, VPA was observed to inhibit murine neuroblastoma and glioma cell proliferation; continued exposure to VPA has also been found to induce cell lines differentiation and apoptosis.²⁹⁻³² VPA has been classified as a selective class I HDAC inhibitor.^{24,33}

VPA *in silico* modeling on HDAC8

The mechanism whereby VPA inhibits HDACs is not known; however, an *in silico* study with HDAC8 using VPA as a ligand suggests that there are two binding sites: the catalytic site and the hydrophobic channel of the active site. VPA's carboxyl group interacts with the catalytic site. On the other hand, acetate is thought to be released into the hydrophobic channel of the active site, thus blocking the enzyme.³⁴

VPA sensitizes cancer and helps chemotherapy

In cancer therapy, VPA has been applied as monotherapy or in combination with demethylating epigenetic agents, chemotherapy and immune system modulators.³⁵

In a phase II investigation, VPA monotherapy was observed to be able to induce the expression of Notch I (a tumor suppressor) in neuroendocrine carcinoma; the study included eight patients, out of which one did partially respond and five evolved to stable disease.³⁶ In vitro, in myeloid neoplasms, VPA has shown apoptosis and differentiation induction in non-differentiated leukemic cells, which has stimulated the use of VPA as monotherapy or in combination with all-trans retinoic acid in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). In a phase II study with 75 patients treated with VPA associated with all-trans retinoic acid, 18 patients (24 %) were reported to have achieved adequate responses. In another study with VPA and all-trans retinoic acid, 20 patients with MDS were treated: clinical benefit was observed in 30 % of patients with AML and MDS.^{37,38} In another approach, 5-azacitidine plus VPA was used, with better response than with conventional therapy being reported in phase I, II and III trials in older adults with AML and MDS.^{37,39,40} In a phase II trial of VPA with all-trans retinoic acid and 5-azacitidine applied to patients with AML and with low-risk MDS, responses were observed in 23 % of patients and a 12.4-month survival.⁴¹

Combinations of VPA with chemotherapy include agents that damage the DNA. In a study where it was combined with epirubicin (topoisomerase II inhibitor), responses were observed in 22 % of the 44 patients included, among them patients with tumors regarded as anthracycline-resistant, such as melanomas.⁴² In a phase I-II trial, VPA was combined with 5-fluorouracil, epirubicin and cyclophosphamide in a cohort of 15 breast cancer patients; acceptable toxicity was observed in 64 % of patients.³⁵ In a phase II clinical trial of 16 patients with cisplatin-resistant inoperable malignant mesothelioma, synergistic results were detected with the combination of VPA and doxorubicin; seven patients out of 45 showed partial responses.⁴³ In another phase I/II clinical trial of patients with metastatic melanoma, VPA was used in combination with karenitecin, a topoisomerase I inhibitor; the result was disease stabilization in 47 % (seven out of 15 patients in the dose escalation cohort).⁴⁴ In a phase III randomized analysis that included 36 patients with advanced cervical cancer, the hydralazine, VPA, cisplatin and topotecan combination resulted in a significant improvement in progression-free survival.⁴⁵ Other in vitro work showed that VPA increases histone H3 acetylation. With these histone changes, this drug prevents mTOR inhibition-mediated resistance by the RAD001 compound (everolimus) in renal carcinoma CaKi-1 cells.⁴⁶

VPA to prevent head and neck cancer

In a retrospective study of a cohort of 439,628 older adults treated with VPA for different diagnoses (bipolar disorder, migraine, epilepsy), a lower frequency of smoking-related head and neck carcinoma was observed in 26,911 individuals who chronically used VPA.⁴⁷

Interestingly, in vitro trials of cultures of cancer cell lines, in vivo trials with animal models and clinical studies show that VPA decreases the resistance to conventional cancer therapy.^{45,47}

Conclusions

In studies with large patient populations, VPA has been shown to prevent head and neck cancer; in addition, some clinical trials reveal its usefulness as combination therapy. Recently, the combination of VPA, chemotherapy and agents that block antioxidant systems (glutathione) has been used with interesting results in cancer cell lines.^{34,48}

The works using the VPA as a base-molecule for designing new compounds focus on molecules with HDAC inhibition related to cancer with low hepatotoxicity. In this regard, our group has worked with the design and testing of VPA-derived drugs, with N-(2-hydroxyphenyl)-2-propylpentanamide (o-OH-VPA) standing out, which has shown its antiproliferative effect in Hela, sarcoma and MCF7 cell cultures.⁴⁹

Funding

Gabriela Luna Palencia is Conacyt (grant 91136) and Secretaría de Investigación y Posgrado del Instituto Politécnico Nacional (project 20151399).

Conflict of interests

The authors declare that there were no conflicts of interests that might have affected the execution of this work.

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