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Mini-review

Anticonvulsant valproic acid and other short-chain fatty acids as novel anticancer therapeutics: Possibilities and challenges

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Results from numerous pre-clinical studies suggest that a well known anticonvulsant drug valproic acid (VPA) and other short-chain fatty acids (SCFAs) cause significant inhibition of cancer cell proliferation by modulating multiple signaling pathways. First of all, they act as histone deacetylase (HDAC) inhibitors (HDIs), being involved in the epigenetic regulation of gene expression. Afterward, VPA is shown to induce apoptosis and cell differentiation, as well as regulate Notch signaling. Moreover, it up-regulates the expression of certain G protein-coupled receptors (GPCRs), which are involved in various signaling pathways associated with cancer. As a consequence, some pre-clinical and clinical trials were carried out to estimate anticancer effectiveness of VPA, in monotherapy and in new drug combinations, while other SCFAs were tested in pre-clinical studies. The present manuscript summarizes the most important information from the literature about their potent anticancer activities to show some future perspectives related to epigenetic therapy.

Keywords: valproic acid, short-chain fatty acids, anticancer activities, histone deacetylase inhibitors, pre-clinical and clinical studies

INTRODUCTION

Valproic acid (VPA) is a branched short-chain fatty acid (SCFA) with two propyl groups linked to an acetic acid moiety, exerting valuable chemical and pharmacodynamic properties (Fig. 1). The most important is its high solubility both in water (sodium or magnesium salts) and lipids (an acidic form) which allows easy distribution of the drug throughout the body (1, 2). Valproic acid, as a potent drug with a broad spectrum of antiseizure effects, has been used for the treatment of epilepsy as well as bipolar and migraine diseases for several decades (3). Moreover, VPA is not expensive and, due to its positive side-effect profile, is well tolerated by patients (1, 4).

In recent years, due to strong evidence of its anticancer activity *in vitro*, VPA was proposed as an attractive candidate for therapy of different types of cancer (4). VPA, by affecting numerous signaling pathways, can modulate different processes in cancer cells and suppress

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tumor growth (5). First of all, VPA acts as a histone deacetylase (HDAC) inhibitor (HDI), being involved in the epigenetic regulation of gene expression (6). Additionally, VPA was shown to induce apoptosis and cell differentiation as well as regulate Notch signaling (7). Moreover, VPA was found to up-regulate the expression of certain G protein-coupled receptors (GPCRs), being involved in various signaling pathways associated with cancer (6, 8).

The presented review compiles the newest and most relevant information in relation to the use of VPA as an anticancer drug, regarding its activity as an HDI or in association with the Notch and GPCR signaling. It summarizes the results of important pre-clinical and clinical studies with VPA in solid tumors and malignant hematological diseases. Some data about anticancer activities of other SCFAs, *i.e.*, propionic acid (PA), butyric acid (BA) and phenylbutyric acid (PBA) (Fig. 1) are also included.

VALPROIC ACID AND ITS ANTICONVULSANT ACTIVITY

Extensive anticonvulsant activity of VPA can be explained by the complex mechanism of action. It is known that VPA has the ability to potentiate γ-aminobutyric acid (GABA) transmission, inhibit *N*-methyl-D-aspartate (NMDA) receptor-mediated excitatory transmission and modulate serotoninergic and dopaminergic transmissions. In addition, the potentiation of calcium-activated potassium currents, the blockade of calcium and sodium channels, and a reduced release of the excitatory β-hydroxybutyric acid can be important (2).

On the other hand, these various mechanisms of action can cause side-effects such as neurological symptoms (tremor, sedation, dizziness, fatigue, mood changes), mild gastrointestinal side-effects and weight gain (1). Therapy with VPA may also result in liver failure, platelet and coagulation disorders, hyperammonaemia and teratogenicity. However, the risk of these effects can be reduced by individual dose regulation in patients (2).

HISTONE DEACETYLASES AND HISTONE DEACETYLASES INHIBITORS

HDACs belong to a group of enzymes responsible for the removal of acetyl groups from histones (deacetylation), leading to modulation of expression of many genes. At the same time, alterations of acetylation level, as well as overexpression of numerous HDACs, were reported in many cancer cells. Consequently, it was shown that HDACs could play a key role in the initiation, promotion and progression of cancer (9).

To date, eighteen human HDACs have been identified and divided into four classes, based on their enzymatic activities, subcellular localization and homology to yeast proteins (10). Classes I, II and IV include zinc ion-dependent metal proteins, whereas class III, known as sirtuins (SIRTs), include nicotinamide adenine dinucleotide-dependent enzymes. Class I includes HDACs 1–3 and 8, which are mostly located in nucleus. Class II, divided into class IIa (HDACs 4, 5, 7, 9) and IIb (HDACs 6, 10), can migrate between the nucleus and the cytoplasm. Class III (SIRTs 1–7) is located in the cytoplasm, nucleus and mitochondrion, while class IV (HDAC11) is expressed mainly in the nucleus (9).

By inhibiting the activity of HDACs and, in consequence, histone deacetylation, HDIs can modulate the chromatin structure. This leads to transcriptional changes of numerous genes involved in the cell cycle, cell differentiation, DNA replication and DNA repair (5, 9). In addition, HDIs have the ability to target non-histone proteins, including transcrip-

tion factors, signaling mediators, nuclear factors and tumor suppressors. Consequently, HDIs can influence various processes such as protein phosphorylation, cell cycle arrest and apoptosis (11). As for apoptosis, it has been found that HDIs increase the expression of pro-apoptotic proteins like BMF, BIM and BID, and decrease the expression of anti-apoptotic proteins such as BCL-X and BCL2 (9).

HDIs are a large group of compounds that are different in terms of their chemical structure, biological activity and specificity in relation to individual HDACs classes, *i.e.*, in relation to class I, II and IV (classic HDIs) and class III (inhibitors of SIRTs). The presented paper is focused on the anticancer activity of VPA and other SCFAs belonging to classic HDIs.

VALPROIC ACID AND ITS HDI ACTIVITY

As far as HDI activity is concerned, VPA acts as an inhibitor of class I and IIa HDACs (5). Efficacy of VPA in this area can be defined by two different pathways, *i.e.*, modulation of chromatin structure through intensification of histone acetylation and acetylation of non-histone proteins (3). Several studies suggest that VPA enhances DNA binding, inhibits cell proliferation and induces apoptosis (12).

What is more, VPA increased the abundance of acetylated histone H3 in bladder cancer cells (12), and the abundance of acetylated H4 histone in hepatocellular carcinoma (HCC) cells (8). Additionally, the influence of VPA on histone acetylation was confirmed in neuroblastoma, glioblastoma, glioma, teratocarcinoma, leukemia, endometrial stromal sarcoma, as well as colon and cervical cancers (6). Due to its additive effect on non-histone proteins, VPA was shown to inhibit the angiogenesis, metastasis and survival of different cancer cells (4).

NOTCH SIGNALING

Activation of Notch signaling, upon the ligand-receptor binding, leads to up-regulation of the transcriptional complex which targets genes involved in cell proliferation and differentiation, *i.e.*, hairy/enhancer of split as well as neoplastic vasculature development (hairy/enhancer of split related with YRPW motif) (13). Due to its involvement in cell fates, Notch signaling can be associated with a wide range of diseases including cancers, both solid tumors and hematological malignancies (14).

In cancer cells, four Notch receptors can be involved, *i.e.*, NOTCH1 which is important for cell proliferation, invasion and chemoresistance, NOTCH2 associated with hepatic tumors, NOTCH3 stimulating cell migration and proliferation (linked to chemotherapy response) and NOTCH4 involved in epithelial-mesenchymal transition and endocrine therapy resistance in breast cancer. Also, five ligands for Notch receptors are known, *i.e.*, Jagged 1-2, Delta-like 1, 3 and 4 (13).

Due to their broad spectrum of activity in cancer, the ligands for Notch receptors are currently estimated in several preclinical and clinical trials (13). However, depending on the type of cancer and cross-talks between other signaling pathways, Notch signaling can act either as an inhibitor or promoter of cancer (14). Activation of Notch signaling leads to cancer progression in leukemia, lymphoma, ovarian, colorectal and breast cancer. On the other hand, in small cell lung cancer, medullary thyroid cancer, skin cancer and neuroblastoma, Notch signaling can play a tumor-suppressive role (15).

G PROTEIN-COUPLED RECEPTORS AND THE CELL CYCLE

G protein-coupled receptors (GPCRs) are the largest protein family of cell surface receptors involved in signal transduction pathways, with a broad spectrum of physiological functions and related disease processes (16). In vertebrates, there are several groups of GPCRs, classified by sequence homology and pharmacological properties (17). First and the biggest group is class A (rhodopsin-like), which is further divided into four subgroups: α, β, γ and δ. Class B is known as secretin receptor family, class C includes metabotropic glutamate/pheromone receptors, and class F consists of frizzled (FZD) and smoothened (SMO) proteins (16, 17).

GPCRs may control many cancer-associated signaling pathways. They can regulate tumorigenesis, cell proliferation and invasion (16). Several members of class A GPCRs, such as protease-activated receptors, have been detected in cancer cells and can be used for the receptor-targeted cancer treatment. As a consequence, the compounds with activity towards GPCRs appear as crucial agents in tumor growth and metastasis (6). GPCRs were shown to exist in more than one conformation, which allows binding of more than one ligand and affects various signaling pathways. This "biased agonism" provides functional selectivity of signaling and offers potential benefits in clinical development (16).

PRECLINICAL STUDIES OF ANTICANCER ACTIVITY OF VALPROIC ACID

Ovarian cancer

It was found that VPA acting as an HDI, down-regulated the expression of HDAC7 and HDAC2 in ovarian cancer cells, which resulted in lowering of chemoresistance and better prognosis (18, 19). The influence of VPA in the combination with paclitaxel or doxorubicin was demonstrated to ameliorate expression of *BCL-2*, *BCL-XL* and *CDK4* genes associated with cell cycle regulation and apoptosis. Moreover, it was shown that VPA, in cooperation with paclitaxel or doxorubicin, decreased PARP protein expression, which led to inhibition of DNA repair in ovarian cancer cells. Inhibition of PARP enzymes which act as enzymatic co-activators in number of physiological and pathological processes, such as DNA repair, maintaining genomic stability, cell survival and cell death, could be a potent therapeutic strategy in cancers with specific DNA repair defects (20).

Breast cancer

To date, the routine treatment of patients with this type of cancer is capecitabine, a prodrug of 5-fluorouracil. The newest studies showed that the combination of capecitabine with VPA could be an innovative antitumor strategy. It was found that VPA, through transcriptional mechanisms, induced up-regulation of thymidine phosphorylase and down-regulation of thymidylate synthase mRNA and protein expression, both *in vitro* and *in vivo*. As thymidine phosphorylase is a key enzyme in the metabolic transformation of capecitabine to 5-fluorouracil in tumor cells, the increased expression of thymidine phosphorylase might result in higher sensitivity of tumor cells to this drug. Furthermore, the down-regulation of thymidylate synthase overexpression, which occurred in aggressive breast cancer phenotypes, could slow down the disease progression. Consequently, the

combined therapy with capecitabine and VPA may result in synergistic action leading to pro-apoptotic and antiproliferative effects in breast cancer cells (21). In addition, pretreatment of breast cancer cells with VPA resulted in their notably increased sensitivity to radiotherapy (3).

Another study showed that VPA strongly decreased the viability of MCF-7 breast cancer cells. It was also showed that VPA up-regulated expression of melatonin receptors 1 (MT1) in these cells, providing the higher inhibition of tumor cells proliferation (22).

Hepatocellular carcinoma (HCC)

VPA by acting as an HDI leads to HDAC4 suppression and H4 acetylation in HCC HTB-52 (SK-HEP-1) cells, resulting in the induction of cell cycle arrest and cell apoptosis. It is also supposed that HCC progression is closely related to Notch signaling activity. According to literature data, HCC cells showed highly expressed NOTCH1 signatures, confirming the progressive role of Notch signaling in this cancer the development of this cancer type. It was found that VPA could down-regulate Notch signaling and its target gene *HES1* which led to significant inhibition of HCC cells (8).

It was also observed that VPA significantly increased the expression of GPCRs such as gastrin-releasing peptide receptor and somatostatin receptor type 2 in HCC cells (6). Moreover, several cell surface receptors have their specific ligands and antibodies, which can be conjugated with different anticancer agents for better drug delivery. These findings provide novel opportunities for the receptor-targeted anticancer therapy with VPA as a tumor suppressor and GPCRs regulator (6, 8). To date, there are several reports confirming the efficiency of VPA combined with gastrin-releasing peptide receptor and somatostatin receptor type 2 targeted conjugates (6).

Prostate cancer

It was shown that VPA, by acting as an HDI, increased histone acetylation of the *CCND2* gene, resulting in re-expression of cyclin D2 and inhibition of cancer cell proliferation. It was demonstrated that treating prostate cancer cells with VPA, through the presented molecular mechanisms, reduced cell proliferation, migration and invasion. Furthermore, it was found that VPA affected separately the re-expression of cyclin D2 from a whole D-type cyclin family group. Cyclin D2 is an important member of highly conserved cell cycle regulators playing a key role in the cell cycle progression. However, its role in cancer development depends on the tumor type. VPA can be useful for the treatment of cancers in which cyclin D2 shows a tumor suppressor function (23).

VALPROIC ACID IN CLINICAL STUDIES: PHASES I-III

Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)

Some *in vitro* studies suggested that VPA affected AML cells which showed lower proliferation and higher apoptosis. Moreover, *in vitro* studies with MOLT4 and HL60 leukemic cells demonstrated that VPA had synergized with decitabine, resulting in growth inhibition and apoptosis. These results provoked clinical studies to evaluate the combined

therapy with VPA and other cytostatics (24). However, the phase II randomized study demonstrated that the treatment of patients with MDS or older patients with AML with VPA combined with decitabine did not improve outcomes compared to decitabine alone (25). On the other hand, the next study proved significant activity of VPA combined with 5-aza-2′-deoxycytidine (5-AZA) in patients with MDS and advanced, relapsed-refractory AML (26). Furthermore, several clinical studies were conducted to investigate the combination of VPA with *all-trans* retinoic acid. It was shown that this combination gave clinically notable antileukemic activity at lower doses of VPA than those used in antiepileptic therapy, even in elderly patients (24).

Pancreatic cancer

After *in vitro* studies confirmed that VPA increased the anticancer effects of 5-fluorouracil in biliary tract and pancreatic cancer cell lines, the phase I and phase II clinical trials were performed to evaluate the efficacy and safety of a combined treatment with VPA and S-1 in patients with advanced pancreatobiliary tract cancers (S-1 is the mixture of three compounds including tegafur, a prodrug of 5-fluorouracil, gimeracil and oteracil potassium) (27, 28). It was found that this kind of therapy in patients with advanced pancreatic cancer provided high rate of tumor control (91.7 %) and was associated with acceptable toxicity (27).

Non-small cell lung cancer (NSCLC)

Due to epigenetic modifications observed in the tumorigenesis of lung cancer, it is suggested that VPA, as an HDI, especially when combined with other epigenetic modulating agents, could be a novel strategy in NSCLC treatment. Bearing in mind the observed earlier effects for combination of VPA and 5-AZA, *i.e.*, epigenetic modulation of nucleosome by DNA methylation and histone modifications, as well as nucleosome remodeling and tumor cell suppression, the synergistic effects were supposed. Unfortunately, this combined treatment was apparently limited by unacceptable side-effects, such as neurological symptoms, at relatively small doses, likely related to VPA (29).

VALPROIC ACID *VERSUS* OTHER HDIs IN THE CANCER TREATMENTS

In recent years, several HDIs of different chemical structures, *i.e.*, hydroxamic acids and cyclic peptides, were developed and evaluated in various preclinical and clinical studies to estimate both their effectiveness and safety. Moreover, some of these HDIs were approved by European Medicines Agency (EMA) and Food and Drug Administration (FDA). For instance, vorinostat was approved by FDA for cutaneous T-cell lymphoma in 2006, romidepsin for cutaneous T-cell lymphoma in 2009 and peripheral T-cell lymphoma in 2011, belinostat for peripheral T-cell lymphoma in 2014 and panobinostat for multiple myeloma in 2015 (9).

It was demonstrated that VPA, in comparison with other HDIs, such as vorinostat and romidepsin, showed significantly faster reduction of the expression of HDACs, more effective inhibition of migration and invasion of cancer cells, and better synergy with doxorubicin in triple-negative breast cancer cells (18).

OTHER SHORT-CHAIN FATTY ACIDS

Butyric, propionic and phenylbutyric acid

Sodium butyrate was shown to display an effective anticancer activity by inducing growth arrest, cell differentiation and inhibiting DNA synthesis in colon, prostate and breast cancer (30). *In vitro* study confirmed that it can reduce the BCL2 expression, leading to apoptosis and suppression of viability of MCF-7 breast cancer cells (31). It was also shown that the combination of sodium butyrate with proteasome inhibitors such as MG115, MG132, PS1 and PS2 presented synergistic pro-apoptotic activity in SW837 colon cancer cells (32). The study in Caco-2 colon cancer cells demonstrated that sodium butyrate, alone or in the combination with sodium propionate, induced apoptosis and G2-M cell cycle arrest (33). Synergistic action of sodium butyrate and panobinostat, leading to cell senescence by up-regulating the expression of miR-31 in breast cancer cells, has also been described (34).

Sodium phenylbutyrate was tested in a wide range of preclinical and clinical studies, mainly in central nervous system diseases, such as amyotrophic lateral sclerosis, Parkinson's disease and psychiatric disorders in which it displayed potent therapeutic effects (35). However, it was also characterized as a compound with potential anticancer activity (9). It was documented that it can be used as a sensitizing agent to overcome the resistance in NSCLC cells when combined with cisplatin, gefitinib or erlotinib (36). Another study demonstrated the synergistic pro-apoptotic activity of phenylbutyric acid (PBA) and platinum(IV) derivatives in pancreatic, cervical and colon cancer cells (37).

In the recent study, a series of new PBA derivatives showed cytotoxic activities against A549 (NSCLC), MDA-MB-231 (breast cancer) and SW1116 (colon cancer) cells. Among the synthesized compounds, B9 and B15 (Fig. 1) exhibited the highest cytotoxic activities. The

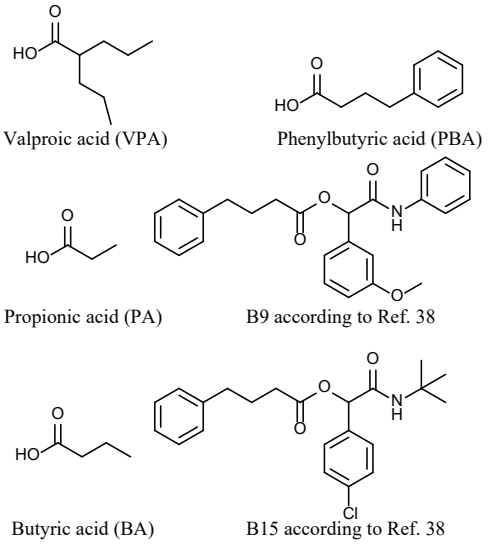

Fig. 1. Chemical structures of valproic acid (VPA) and other short-chain fatty acids (SCFAs).

molecular docking of these compounds on histone deacetylase complex confirmed their high affinity to the zinc-ion in the active site of the enzyme. Further results suggested that B9 can effectively induce apoptosis in breast cancer cells in a dose-dependent manner (38).

Short-chain fatty acids and histone post-translational modifications

Several studies suggest the association between SCFAs and newly identified histone post-translational modifications, named crotonylation, butyrylation and propionylation. All of these modifications are chemically related to histone acetylation and collectively termed as lysine acylations. *In vitro* observations suggest that histone crotonylation is highly regulated over the cell cycle and deregulation of this process may be connected with cancer development. As for SCFAs activity in this area, it was confirmed that butyric acid (BA) strongly promoted histone crotonylation in HCT116 colon cancer cells (39).

Short-chain fatty acids and human gut microbiota

Some SCFAs, including butyric and propionic acid, are produced by friendly bacteria in the human gut. This production mainly occurs in large intestine through multiple interactions between the gut microbiota and dietary compounds (37, 40). Recently, these SCFAs became the most thoroughly studied compounds among gut microbiota metabolites. Also, diversity and functions of gut microbiota, as well as its connection with dietary intake appeared an area of research. In consequence, opportunity for prevention and treatment of some life-threatening conditions, including cancer, were taken into account. Nevertheless, further studies are needed to evaluate how microbiota and SCFAs are linked as well as how this connection influences histone modifications over specific genes and cancer development, progression and therapy (39, 40).

CONCLUSIONS

Accumulated data indicates that epigenetic modifying agents, including VPA and other SCFAs, may be clinically relevant in anticancer treatments. They can affect different alterations of cancer cell epigenome by modulating multiple signaling pathways, such as histone acetylation, crotonylation, butyrylation and propionylation, as well as Notch and GPCR signaling. In addition, they exert positive side-effects profiles which hold promise for patients with different cancers. Furthermore, some SCFAs, as products of bacterial fermentation in the human gut, could be regulated to some extent, by relatively simple dietary interventions. Nevertheless, further studies are needed to evaluate the tumor-suppressive role of VPA and other SCFAs. Especially, their impact on Notch and GPCR signaling should be extensively studied, due to observed divergent effects in different cancer types.

Abbreviations, acronyms, symbols. – AML – acute myeloid leukemia, 5-AZA – 5-aza-2′-deoxycytidine, BA – butyric acid, FZD – frizzled proteins, GABA – γ-aminobutyric acid, GPCR – G protein-coupled receptor, HCC – hepatocellular carcinoma, HDAC – histone deacetylase, HDI – histone deacetylase inhibitor, MDS – myelodysplastic syndrome, NMDA – *N*-methyl-D-aspartate, NSCLC – non small cell lung cancer, PA – propionic acid, PBA – phenylbutyric acid, SCFA – short-chain fatty acid, SIRT – sirtuin, SMO – smoothened proteins, VPA – valproic acid

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