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Review Article

Pharmacological modulation of cell functional activity with valproic acid and erythropoietin

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Abstract

Introduction: Valproic acid (VA) is carboxylic acid with a branched chain, which is used as an antiepileptic drug.

Valproic acid influence on cells *in vivo*: VA, which is an antiepileptic drug, is also a teratogen, which causes defects of a neural tube and an axial skeleton, although the mechanisms are not yet fully clear.

Valproic acid influence on mesenchymal stem cells (MSC) *in vitro*: It is shown that valproic acid reduces the intracellular level of oxygen active forms.

Valproic acid effect on tumor cells: VA inhibits tumor growth through several mechanisms, including the cell cycle stop, differentiation induction and inhibition of growth of tumor vessels.

Valproic acid influence on enzymes: It affects mainly GSK-3.

Valproic acid influence on animals' cells: It is shown that VA can significantly improve an ability to develop *in vitro* and improve nuclear reprogramming of embryos.

Erythropoietin (EPO): Is an hypoxia-induced hormone and a cytokine, which is necessary for normal erythropoiesis. EPO is widely used in *in vitro* experiments.

Conclusion: Thus, the influence of VA and EPO on cells can be used in cell technologies.

Keywords

biomedical cellular products, valproic acid, cell technologies, mesenchymal stem cells, erythropoietin.

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Introduction

Currently one of the most important tasks of medicine is using biomedical cellular products (BMCP) and understanding mechanisms of how cell therapy works. It is done in order to develop new efficient methods for treating many diseases using cells and the products of their life activity. Cells and their metabolites are powerful agents, but it is not enough for their being used in therapy. To improve the efficiency of cell use, including mesenchymal stem cells (MSC), it is necessary to search for their capacity modifiers *in vitro*.

Glycogen synthase kinase (GSK-3), in addition to taking part in glucose metabolism, is known to be involved in several diseases, including type II diabetes, Alzheimer's disease, inflammation, cancer, schizophrenia and bipolar disorder. It is also shown that GSK-3 regulates immune and migratory cellular processes – it participates in several signalling pathways of an innate immune response, including interleukin secretion (Jope et al. 2007). GSK-3 inhibition can have a therapeutic effect on certain types of cancer, for example, on human pancreatic cancer (Marchand et al. 2012).

Valproic acid (2-propylvaleric acid, VA) is carboxylic acid with a branched chain, which is a glycogen synthase kinase 3 (GSK-3) inhibitor. It is used as an antiepileptic drug. Valproates when dissociated into ions block the sodium channel conduction like diphenine and block calcium channels like ethosuximide;, and activate potassium channels. They also promote GABA accumulation in brain synapses. It is shown that cell migration improves when adding valproic acid and lithium to the culture.

Erythropoietin (EPO) is a hypoxia-induced hormone and cytokine, which is necessary for normal erythropoiesis. EPO interaction with its receptor leads to the activation of various intracellular pathways (IP3, Ras/MAPK, NF-kв), a change of the intracellular calcium level, a reduction in apoptosis and an increase in cell survivability. The maximum number of receptors to EPO is on erythroid cells. Megakaryocytes, skeletal myoblasts, neurocytes, microglia, astrocytes, endothelial cells, cardiomyocytes, ovaries and testicles also have receptors. Therefore, EPO fulfils several functions other than a hemopoietic one.

Valproic acid (VA) is fatty acid with a branched chain, a histone deacetylase inhibitor (Salerno et al. 2016), which improves the efficiency of somatic murine embryonic fibroblasts reprogramming (approximately 100-fold), activating pluripotency genes and repressing lineage differentiation genes (Luo et al. 2013). VA intensifies the induction of induced pluripotent stem cells (IPSC) from human bone marrow cells. In this case, cell proliferation at the early stages of the reprogramming improved by facilitating cell transition from G2 into M-phase (Chen et al. 2016). Thus, VA is a part of the compound, including low-molecular compositions, used for the production of induced pluripotent cells. VA also promotes IPSC differentiation into hepatocytes (Kondo et al. 2014, Raut and Khanna 2016).

Valproic acid influence on cells *in vivo*

VA, which is an antiepileptic drug, is also a teratogen, which causes defects of a neural tube and an axial skeleton, although the mechanisms are not yet fully clear. It is assumed that VA works through the retinoic acid receptor (Li and Marikawa 2016).

The main effect of VA on osseous metabolism consists in the decreased proliferation of osteoblasts, a change in collagen synthesis and the induction of vitamin D catabolism. In addition to these direct effects of VA in bones, there are indirect effects influencing other endocrine organs and promoting VA-induced bone mass loss. The chronic injection of VA affects the hypothalamic-pituitary-gonadal axis, predominantly in men (Verrotti et al. 2009). In women, long-term treatment with VA causes polycystic ovarian syndrome, menstrual disorders, hyperandrogenism (Pitetzis et al. 2017). By means of carnitine metabolism, VA indirectly affects the spermatozoa mobility (Roste et al. 2005). VA-induced carnitine deficit, especially in newborns, also has an indirect negative effect on the bone tissue metabolism (Rauchenzauner et al. 2009, Um et al. 2017). Therapy with VA also influences thyroid gland (Lossius 2009).

Valproic acid influence on mesenchymal stem cells (MSCs) *in vitro*

MSCs demonstrate a high potential for the treatment of several human diseases; but efficiency of MSC transplantation was hampered by the relatively low migratory ability of these cells towards disease areas. It is a fact that cell migration improves when adding VA and lithium. Short-term (for 3 hours) exposure of a relatively high VA concentration (2.5 mM) on MSCs noticeably increased the level of protein transcription and synthesis of CXC4 chemokine receptor (CXCR4). At the same time, histone deacetylase (HDAC) was inhibited, including HDAC1 isoform (Linares et al. 2016, Tsai et al. 2010). Valproic acid improves migration of MSCs from umbilical cord blood by increasing expression of CXCR4, CXCR7 and MMP-2 (Marquez-Curtis et al. 2014).

It is shown that stem cells from human amniotic fluid in the first trimester of pregnancy can be fully reprogrammed to the pluripotency in the culture on Matrigel in the human embryonic stem cells (hESC) medium with the addition of histone deacetylase inhibitor (HDACi) and VA to the medium (Moschidou et al. 2012).

VA promotes transformation of hUC-MSC cells into hepatocytes by increasing the expression of endodermal genes, including CXCR4, SOX17, FOXA1, FOXA2, GSC, c-MET, EOMES and HNF-1β, by activating AKT and ERK (An et al. 2014).

It is shown that preliminary treatment of human bone marrow cells with VA after their incubation with neuronal inductive media efficiently promotes MSC differentiation in the neuronal direction (Almutawaa et al 2014, Jeong et al. 2013). After treating the placenta MSCs with VA, there was an increase in the number of axons and markers specific for neural lineages compared with cells, grown only in the differentiation medium (Talwadekar et al. 2017). At the same time, the neuroprotective effect of VA is not proven. In the experiments, rat's embryonic motor neurons cultivated on a feeder layer consisting of Schwann cells were used (Ragancokova et al. 2009).

VA protects human bone mesenchymal stromal cells (hBM-MSCs) from oxidative stress and improves their migratory ability by increasing trophic factor secretion. This suggests that VA can be used for improving the stem cell functioning, though the molecular mechanisms are not known yet. It is assumed that valproic acid reduces the intracellular level of oxygen active forms by means of modulation of KRIT1 protein family, including FoxO1, SOD2 and cyclin D1 (Jung et al. 2015).

Valproic acid effect on tumor cells

It is also known that VA inhibits tumor growth through several mechanisms, including the cell cycle stop, differentiation induction and inhibition of growth of tumor vessels (Sidana et al. 2012). VA has a cytotoxic effect on neuroendocrine tumor cells (NETs) of the intestinal or pancreatic origin. There are several mechanisms by which VA kills NET cells, which suggests the possibility of combination therapy. In this study, VA was found to induce dose-dependent inhibition of NET cells growth *in vitro*, which is mainly connected with the apoptosis activation. VA induced the main transcriptional response by changing expression of 16-19% of protein-encoding genes in NET cell lines. For example, TGF-β1, FOXO3, p53 signal transmission was activated, and MYC signal transmission was inhibited (Arvidsson et al. 2016).

The experiment with the transplantation of glioblastoma multiforme, which had been treated with VA cells three times, demonstrated that animals' survivability increased unlike the control (transplantation of untreated tumor cells to mice) (Hosein et al. 2015). Using PCR, it was shown that a combination of VA and cytarabine significantly increased Bax gene expression, suppressed leukemia cell proliferation and led to pathological cells apoptosis (Liu et al. 2016). Treatment with using VA also leads to the cell cycle stop in G1-phase by decreasing cyclin D1 (Fortunati et al. 2008, Ma et al. 2007) and can induce autophagy in certain types of cancer, such as prostate cancer and some kinds of lymphoma, by activating adenosine monophosphate kinase (AMPK) and inhibiting mTOR (Ji et al. 2005, Xia et al. 2016, Zhang et al. 2017).

The study showed that VA inhibits migration of hormone-sensitive breast cancer (Travaglini et al. 2009). VA is an efficient drug, which blocks tumor-stromal paracrine interactions and potentiates the doxorubicin effect through inhibiting the NF-kB transcription factor (Barneh et al. 2018). The anti-tumour activity of the combination of MSCs from bone marrow and VA is mediated by the increased cell apoptosis in glioma (Ryu et al. 2012). VA induces death of breast cancer cells (Aztopal et al. 2018). Some scientists suppose involvement of oxygen active forms in a VA toxicity mechanism and, probably, reactive metabolites as the main cause of cytotoxicity in the case of acetaminophen in the *in vitro* model (Tabatabaei and Abbot 1999).

VA in therapeutic doses for epilepsy in the experiment causes cell death in primary CD138-positive myeloma cells, as well as in myeloma cell lines, but not in CD138-negative bone marrow cells. VA suppresses osteoclastogenesis, as well as the osteoclast-mediated growth of myeloma cells. VA also inhibited the vessels formation, intensified by co-culturing myeloma cells and osteoclasts with thalidomide. In addition, VA induces caspase-dependent and caspase-independent death of myeloma cells and increases the antimyeloma effect of melphalan and dexamethasone. VA may have the multi-factor antimyeloma effect and can serve as a safe adjuvant for being included into antimyeloma chemotherapy (Kitazoe et al. 2009).

However, VA concentration and incubation time should be carefully controlled to minimize any harmful effects on the culture. It was shown that with acid concentration of 8 mM, cell morphology changes, and their proliferative activity decreases (Lee et al. 2018).

A high dose of VA (~10 mM) by activating the p21 CIP1/WAF1 transcription stops a cell at the G2/M phase of the cell cycle (Lee et al. 2009). It is important to note that the addition of VA and bioactive lipid sphingosine-1-phosphate (S1P) to MSC also intensifies several important cellular features of MSC, such as self-renewal and migratory activity. It is important for the development of positive results of cell therapy (Lim et al. 2017).

Studies of epithelial differentiation of human adipose mesenchymal stem cells by inhibiting glycogen synthase kinase 3 (GSK-3) and transforming growth factor beta (TGFb) showed positive results. In the study of ADSCs, at the second passage, cells were treated with CHIR99021 (GSK-3 inhibitor), E-616452 (TGFb1 kinase inhibitor), A-83-01 (TGFb1 receptor inhibitor), and VA (histone deacetylase inhibitor) (Setiawan et al. 2017). It is also shown that induction of the MSC differentiation into hepatocytes is possible using only GSK3 inhibitor (Huang et al. 2017).

Valproates reduce the concentration of two key osseous peptides, procollagen I and osteonectin, both in skin fibroblasts and in cultivated osteoblast-like cells (Wilson et al. 2016).

Valproic acid influence on enzymes

GSK-3 is a serine/threonine protein kinase that mediates the addition of phosphate molecules onto serine and threonine amino acid residues. For the first time, GSK-3 was discovered in 1980 as a regulating kinase for glycogen

synthase (Embi et al. 1980). GSK-3 is identified as a kinase for more than forty different proteins in different metabolic pathways (Jope and Johnson 2004). In mammalians, GSK-3 is encoded by two genes – GSK-3 alpha (GSK3A) and GSK-3 beta (GSK3B). GSK-3 is involved in several diseases, including several diseases, including type II diabetes, Alzheimer's disease, inflammation, cancer, schizophrenia and bipolar disorder. The enzyme participates in glucose metabolism including phosphorylation of IRS1 receptor (Liberman and Eldar-Finkelman 2005) and gluconeogenic enzymes –phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (Lochhead et al. 2001). But these interactions were not corroborated, because these pathways can be suppressed without GSK-3 regulation (Rayasam et al. 2009).

It is also shown that GSK-3 regulates immune and migratory cellular processes (it participates in several signalling pathways taking part in an innate immune response, including interleukin secretion). GSK-3β inactivation by different protein kinases also influences an adaptive immune response inducing proliferation and production of cytokines in naive and CD4+ memory T-cells. It is shown that GSK-3 inhibition plays controversial roles in cell migration and inflammatory reactions. Because local inhibition promotes mobility, while global GSK-3 inhibition suppresses cell migration (Jope et al. 2007, Wang et al. 2011). GSK-3β signal transmission promotes migration of hemopoietic stem and progenitor cells (HSPC) through regulating the microtubule rearrangement ,including CXCL12-induced polarization and actin polymerization (Lapid et al. 2013).

GSK-3 is also connected with the cell proliferation and apoptosis pathways. It was shown that GSK-3 phosphorylates β-catenin, promoting its degradation (Mills et al. 2011). Thus, GSK-3 is a part of the β-catenin/Wnt pathway, which signals to the cell about division and proliferation. GSK-3 participates in several apoptotic sygnal pathways through the phosphorylation of transcriptional factors, which regulate apoptosis (Jope and Johnson 2004). GSK-3 can promote apoptosis through activating p53 proapoptotic factor (Watcharasit et al. 2002) and inactivating factors stimulating the survival by means of their phosphorylation (Grimes and Jope 2001).

However, the role of GSK-3 in the apoptosis regulation is controversial, because some studies showed that mice with GSK-3β knockout were oversensitized to apoptosis and die at the embryonic stage. Other studies showed that GSK-3 overexpressing can induce apoptosis (Kotliarova et al. 2008). In general, GSK-3 stimulates and inhibits apoptosis, and this regulation varies depending on a concrete molecular and cellular context (Jacobs et al. 2012).

It is currently shown that lithium, which is used to treat bipolar disorder, acts as the mood stabilizer through selective inhibition of kinase. GSK-3 is thought to directly stimulate amyloid production , which leads to the neurofibrillary deposition in Alzheimer's disease (Jope et al. 2007, Jope and Johnson 2004). Consequently, GSK-3 inhibitors can have a positive theraupeutic effect on patients with Alzheimer's disease (Hu et al. 2009). Similarly, targeted inhibition of GSK-3 can have a theraupeutic effect on certain cancers. Although, as shown, GSK-3 promotes apoptosis in some cases; it is also reportedly a key factor in tumorigenesis in some cancers (Wang et al. 2008). GSK-3 inhibitors induces apoptosis in gliomas and pancreas cancer cells (Kotliarova et al. 2008, Marchand et al. 2012).

Scientists demonstrated that GSK3 inhibition causes JNK-cJUN-dependent apoptosis in human pancreas cancer cells. However, a full-fledged picture of the functions regulated by GSK-3 is still not clear. In this case, GSK-3 inhibition promotes increased activity of autophagy/lysosomic networks (Marchand et al. 2015).

Studies showed that injection of GSK-3 competitive inhibitors can increase glucose tolerance in diabetic mice (Jope et al. 2007). GSK-3 inhibitors can also have a theraupeutic effect after an acute ischaemic stroke (Wang et al. 2016).

It is shown that GSK-3 α/β activity increases in fetal cells in the case of premature birth. The pharmacological blockade of kinase significantly reduces pro-inflammatory mediators in the fetal tissues and myometrium, providing a possible target for the premature birth treatment (Lim and Lappas 2015).

It is also shown that GSK-3 gene deletion significantly increases precursor cell proliferation and at the same time suppresses neuron differentiation. In the Wnt canonical pathway, as mentioned above, GSK-3 inhibition is crucial to the β-catenin stabilization and transfer into the nucleus for stimulating T-cell factor (TCF), which leads to the subsequent activation of T-cell factor 4 (TCF4)-dependent gene transcription. Although the mechanism of regulation is not clear, GSK-3 is a known PI3K signal pathway effector (phosphatidyl inositol-3-kinase), which gives an explanation of the FGF-signaling disorders in the brain, when Gsk-3 genes are knocked out (Kim et al. 2009). Notch protein was stabilized by the GSK-3-mediated phosphorylation, while in another study the Notch protein transcription was intensified by the GSK-3 inhibition (Hur and Zhou 2010).

The treatment of human dermal papilla cells with VA led to an increased β-catenin level andGSK-3β inhibition through phosphorylation. In addition, the treatment with this acid accelerated induction of anagenic hairs (in an active growth phase) in C57BL/6 female mice at the age of 7 weeks. That is, VA intensifies the human hair growth by means of increased β-catenin , and, thus, can serve as an alternative therapeutic option for alopecia (Jo et al. 2013).

As a histone deacetylase (HDAC) inhibitor, VA influences the pericyte proliferation, their viability, migration and differentiation. The research results show that HDAC inhibition by VA *in vitro* causes inhibition of pericyte proliferation and migration without an influence on cell viability (Karén et al. 2011). Treating cells with HDAC inhibitors leads to an increase in the total number of hemopoietic cells compared with a cytokine-stimulated sample. In doing so, cells start to express genes associated with leukaemia (Lam et al. 2017).

VA promotes the osteoblast differentiation in the presence of type 1 collagen *in vitro* (Hatakeyama et al. 2011). Trichostatin A (TSA), which is an HDAC inhibitor, accelerates matrix mineralization and expression of osteoblast genes, type 1 collagen, osteopontin, bone sialoprotein and osteocalcin in MC3T3-E1 cells (osteoblast culture). Concentrations of HDAC inhibitors, which caused H3 histone hyperacetylation, promote a short-term increase of the osteoblast proliferation and viability, but do not change cell cycle profiles (Schroeder and Westendorf 2005). It is shown that HDAC inhibitors regulate expression of genes promoting the differentiation and maturationof osteoblasts (Schroeder et al. 2007).

HDAC inhibitors – trichostatin A and sodium butyrate – powerfully inhibit the cartilage degradation in an explant *in vitro*. These compounds decrease a level of collagenolytic enzymes in the culture and also inactivate these enzymes.. In the cell culture, these effects are explained by an ability of HDAC inhibitors to block induction of the key metalloproteinases (for example, MMP-1 and MMP-13) (Young et al. 2005).

Therefore, HDAC inhibitors are a potential new class of osseous and cartilaginous anabolic agents, which can be useful in the treatment of diseases associated with the bone mass loss, such as osteoporosis and cancer.

Valproic acid influence on animals' cells

It is shown that VA can significantly improve an ability to develop *in vitro* (frequency of blastocyst apoptosis decreases) and improve nuclear reprogramming of the cattle embryos (Xu et al. 2012).

VA decreases the inhibiting effect of glucocorticoids on BM-MSC proliferation and osteogenesis through inhibiting apoptosis and increasing expression of proteins associated with osteogenesis, which can promote prevention of glucocorticoid-induced necrosis of femoral head in rats (Zhou et al. 2018).

Erythropoietin (EPO)

Erythropoietin is a cytokine required for normal erythropoiesis. Reduction of the oxygen concentration in tissues is accompanied by an increased content of the EPO and its receptor (EpoR). EPO is approved by FDA (Food and Drag Administration) for the anaemia treatment, but it has prospects in the treatment of Alzheimer's disease, Parkinson's disease, immune system dysfunction, neuroprotection, cardiovascular diseases (acute myocardial infarction, ischemia/reperfusion, ischemic and uremic cardiomyopathy, chronic heart failure) therapy, spinal cord injuries, brain swelling, shock, infection, diseases of kidneys, lungs, eyes, gastrointestinal tract, metabolism, and fertility disorders (Mangileva 2014).

EPO does not only stimulate erythropoiesis in response to hypoxia, but it is also a cytokine with an antiapoptotic activity, which has neuroprotective and cardioprotective effects. It is involved in angiogenesis, neurogenesis and immune response (Lombardero et al. 2011;Maiese et al. 2008a). With its pronounced cytoprotective action, EPO increases the cell survival rate in the case of ischemia, reperfusion, infections, and a free radical affect. It decreases cytokine (IL-6, TNF-β) production by endothelial cells and the microglia activity. EPO supports communication and function of endothelial cells, stimulates angiogenesis, influencing the endothelial cell proliferation and migration, and also the release of their precursors from bone marrow. EPO stimulates angiogenesis in the myocardium, uterus, brain, and kidneys. Its stimulating effect on the vessel growth is comparable with the effect of VEGF (Mangileva 2014).

It is shown that an EPO neuroprotective activity is observed as early as in the developing brain; besides that, induction of EPO and its receptor by hypoxia can promote cell survival in the brain (Yu et al. 2002). It is shown *in vivo* that EPO is neuroprotective for animal models of brain ischemia (hypoxia induces production of EPO and its receptor) (Chen et al. 2006). Hypoxia promotes differentiation of embryonic neural progenitor cells with EPO participating in differentiation, while the number of EPO receptors does not increase (Giese et al. 2010). In a developing human embryo, EpoR expression is first detected as early as within 7-8 weeks in neurons and astrocytes of the spine cord and brain (Ostrowski and Heinrich 2018). Mice with this receptor knock-out demonstrate a decrease in the total number of neuronal cells and the reduced neurogenesis (Tsai 2006), as well as of heart defects accompanied by a decreased number of cardiomyocytes and endothelial cells (Ostrowski and Heinrich 2018). EPO has a powerful potential for preventing Schwann cells apoptosis; it may be connected with reducing oxidative stress and regulating protein factors associated with apoptosis (Zhang and Shi 2017). It is assumed that signals transmission through EpoR plays a crucial role in the normal development of the brain and other organs.

The transduction of EPO gene in MSC induces secretion of the hormone and various trophic factors, which can have a neuroprotective effect both in *in vitro* and *in vivo* animal models of ischemic stroke (Cho et al. 2010).

In vivo, EPO also stimulates neovascularization and mobilizes endothelial progenitor cells from bone marrow (Chen et al. 2006), increasing producing of vasodilator factor NO by them (Chin et al. 2000).

In addition to its regulatory function in the vertebrates' erythropoiesis, EPO promotes useful functions in various non-haemopoietic tissues including the nervous system. EPO protects cells from apoptosis, reduces inflammatory reactions and maintains function recovery through stimulating the cells migration and differentiation. An EPO function is also widely expressed in the nervous system. EPO promotes myelination through increasing the proliferation of oligodendrocytes which survived after ische-

mia. In the brain, EPO and EpoR mRNAs are widely expressed throughout the whole development of neurons, astrocytes and endothelial cells (Cho et al. 2012).

In vitro, EPO has a protective effect in hyperglycemia, when it maintains Wnt1 protein expression required for proliferation and survival of neurons, cardiomyocytes, erythrocytes, endothelial and adipose cells; it prevents DNA degradation, and maintains mitochondrial membrane potential. Its cytoprotective effect is realized through NF-kB, which attaches itself to DNA, activating transcription of genes inhibiting apoptosis proteins (Baksheev and Kolomoec 2008, Maiese 2009, Raddino et al. 2008).

However, along with a positive effect, EPO has several side effects preventing the hormone introduction in clinical practice. It can reduce apoptosis of cancer cells, improve tumor vascularization, promote metastasis progression; increase a risk of thrombosis (including the development of acute myocardial infarction) and thromboembolisms through increasing the blood viscosity and activating platelets and increase ABP level as a result of activating humoral and hemodynamic mechanisms (Lombardero et al. 2011, Maiese et al. 2008b)

EPO use can be extended by means of using its non-erythropoietic effects, whose presence is adequately demonstrated in the experiment, but in order to introduce it into routine clinical practice, what is needed is drugs with minimum number of side effects, whicih are currently being worked on by many scientists(Maiese et al. 2008a).

It is shown that an EPO injection suppresses apoptosis of MSCs and increases their survivability when transplanted into a heart after infarction. EPO reduces apoptosis induced by H_2O_2 in the culture of human adipose tissue-derived MSCs (hAT-MSC) (Ercan et al. 2014).

MSCs transplantation with simultaneous EPO infusion can improve cardiac function acting through PI3-K/Akt pathway (Zhang et al. 2006). EPO increases MSC survivability in the case of their combined injection in the Alzheimer's disease treatment (Khairallah et al. 2014).

EPO stimulates MSC proliferation from adipose tissue, but it does not influence their migratory activity (Bondarenko et al. 2016). EPO can enhance proliferation (Zeng et al. 2008) and differentiation of MSCs obtained from bone marrow (mBM-MSC) but decreases BAS release by them (Liu et al. 2012). EPO in combination with G-CSF enhances MMP-2 expression in MSC and promotes cell migration (Yu et al. 2014).

In one of the experiments, BM-MSCs treated with EPO (4 IU/ml) within 24 and 48 hours were studied. Such cells express more hepatocytes growth factor (HGF) than control cells cultivated in usual conditions. The study showed that EPO could induce migration of hemopoietic stem cells (HSC) through an HGF-dependent pathway. EPO-treated MSCs can be the main source of HGF production. Therefore, HGF can be used in the cultivation as a factor stimulating migration and as a mobilizing agent for HSC (Tari et al. 2017).

MSCs activated by EPO can promote efficient healing of diabetic foot ulcers. EPO can reduce inflammatory microenvironment of diabetic foot ulcers. The mechanism involves inhibiting the release of proinflammatory cytokine TNF-α by cells, decreasing monocyte migration into the focus of inflammation (Lu et al. 2016).

It is discovered that EPO and bone morphogenetic protein-2 (BMP-2) cause MSC osteogenic differentiation *in vitro* (Nair et al. 2013).

EPO reduces expansion of human naive CD4+ T-cells after their transplantation into NOD-mice (Cravedi et al. 2014). EPO injection *in vivo* decreased VCAM1 and Cxcl12 expression in endothelial cells of bone marrow (Ito et al. 2017) and mobilized mesenchymal stem cells from bone marrow into peripheral blood (Gilevich et al. 2017).

Conclusion

Thus, VA and EPO can be used not only as drugs for treating epilepsy and hemopoiesis disorders, but also in cell technologies. These compounds have a variety of useful effects on cell cultures, which can be used in combination with other chemical compounds.

Conflict of interests

The authors and peer reviewers of this paper report no conflicts of interest.

References

- An SY, Han J, Lim HJ, Park SY, Kim JH, Do BR, Kim JH (2014) Valproic acid promotes differentiation of hepatocyte-like cells from whole human umbilical cord-derived mesenchymal stem cells. Tissue and Cell 46(2): 127–135. https://doi.org/10.1016/j.tice.2013.12.006 [PubMed]
- Almutawaa W, Kang NH, Pan Y, Niles LP (2014) Induction of neurotrophic and differentiation factors in neural stem cells by valproic acid. Basic and Clinical Pharmacology and Toxicology 115(2): 216–221. https://doi.org/10.1111/bcpt.12201 [PubMed]
- Arvidsson Y, Johanson V, Pfragner R, Wängberg Bet, Nilsson O (2016) Cytotoxic effects of valproic acid on neuroendocrine tumour cells. Neuroendocrinology 103(5): 578–591. https://doi.org/10.1159/000441849 [PubMed]
- Aztopal N, Erkisa M, Erturk E, Ulukaya E, Tokullugil, AH, Ari F (2018) Valproic acid, a histone deacetylase inhibitor, induces apoptosis in breast cancer stem cells. Chemico-Biological Interactions 280: 51–58. https://doi.org/10.1016/j.cbi.2017.12.003 [PubMed]

- Baksheev VI, Kolomoec NM (2008) Erythropoietin in clinical practice: past, present and future. Clinical Medicine [Klinicheskaya meditsina] 85(9): 30–37. [in Russian]
- Barneh F, Salimi M, Goshadrou F, Ashtiani M, Mirzaie M, Zali H, Jafari M (2018) Valproic acid inhibits the protective effects of stromal cells against chemotherapy in breast cancer: Insights from proteomics and systems biology. Journal of Cellular Biochemistry 119(11): 9270–9283. https://doi.org/10.1002/jcb.27196 [PubMed]
- Bondarenko N, Nikonorova Y, Surovtseva M, Lykov A, Poveshchenko O, Poveshchenko A, Pokushalov E, Romanov A, Konenkov V (2016) Effect of vascular endothelial growth factor and erythropoietin on functional activity of fibroblasts and multipotent mesenchymal stromal cells. Bulletin of Experimental Biology and Medicine 160(4): 498–501. https://doi.org/10.1007/s10517-016-3206-8 [PubMed]
- Chen X, Zhai Y, Yu D, Cui J, Hu, JF, Li W (2016) Valproic acid enhances iPSC induction from human bone marrow derived cells through the suppression of reprogramming induced senescence. Journal of Cellular Physiology 231(8): 1719–1727. https://doi.org/10.1002/jcp.25270 [PubMed]
- Chen ZY, Warin R, Noguchi CT (2006) Erythropoietin and normal brain development: receptor expression determines multi-tissue response. Neurodegenerative Diseases 3(1–2): 68–75. https://doi.org/10.1159/000092096
- Chin K, Yu X, Beleslin-Cokic B, Liu C, Shen, K, Mohrenweiser HW, Noguchi CT (2000) Production and processing of erythropoietin receptor transcripts in brain, Molecular Brain Research 81(1–2): 29–42. https://doi.org/10.1016/S0169-328X(00)00157-1 [PubMed]
- Cho GW, Koh SH, Kim MH, Yoo AR, Noh MY, Oh S, Kim SH (2010) The neuroprotective effect of erythropoietin-transduced human mesenchymal stromal cells in an animal model of ischemic stroke. Brain Research 1353: 1–13. https://doi.org/10.1016/j.brainres.2010.06.013 [PubMed]
- Cho YK, Kim G, Park S, Sim JH, Won YJ, Hwang CH, Yoo JY, Hong HN (2012) Erythropoietin promotes oligodendrogenesis and myelin repair following lysolecithin-induced injury in spinal cord slice culture. Biochemical and Biophysical Research Communications 417(2): 753–759. https://doi.org/10.1016/j.bbrc.2011.12.029 [PubMed]
- Cravedi P, Manrique J, Hanlon KE, Reid-Adam J, Brody J, Prathuangsuk P, Mehrotra A, Heeger PS (2014) Immunosuppressive effects of erythropoietin on human alloreactive T cells. Journal of the American Society of Nephrology 25(9): 2003–2015. https://doi.org/10.1681/ASN.2013090945 [PubMed] [PMC]
- Ercan E, Bagla AG, Aksoy A, Gacar G, Unal ZS, Asgun HF, Karaoz E (2014) In vitro protection of adipose tissue-derived mesenchymal stem cells by erythropoietin. Acta Histochemica 116(1): 117–125. https://doi.org/10.1016/j.acthis.2013.06.007 [PubMed]
- Embi N, Rylatt DB, Cohen P (1980) Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. European Journal of Biochemistry 107(2): 519–527. https://doi.org/10.1111/j.1432-1033.1980.tb06059.x [PubMed]
- Fortunati N, Bertino S, Costantino L, Bosco O, Vercellinatto I, Catalano MG, Boccuzzi G (2008) Valproic acid is a selective antiproliferative agent in estrogen sensitive breast cancer cells. Cancer Letters 259(2): 156–164. https://doi.org/10.1016/j.canlet.2007.10.006 [PubMed]
- Giese AK, Frahm J, Hübner R, Luo J, Wree A, Frech MJ, Rolfs A, Ortinau S (2010) Erythropoietin and the effect of oxygen during proliferation and differentiation of human neural progenitor cells. BMC Cell Biology 11: 94. https://doi.org/10.1186%2F1471-2121-11-94 [PubMed] [PMC]
- Gilevich IV, Fedorenko TV, Pashkova IA, Porkhanov VA, Chekhonin VP (2017) Effects of growth factors on mobilization of mesenchymal stem cells. Bulletin of Experimental Biology and Medicine 162(5): 684–686. https://doi.org/10.1007/s10517-017-3687-0 [PubMed]
- Grimes CA, Jope RS (2001) CREB DNA binding activity is inhibited by glycogen synthase kinase-3 beta and facilitated by lithium. Journal of Neurochemistry 78(6): 1219–1232. https://doi.org/10.1046/j.1471-4159.2001.00495.x [PubMed] [PMC]
- Hatakeyama Y, Hatakeyama J, Takahashi A, Oka K, Tsuruga E, Inai T, Sawa Y (2011) The effect of valproic acid on mesenchymal pluripotent cell proliferation and differentiation in extracellular matrices. Drug Target Insights 5: 1–9. https://doi.org/10.4137/DTI.S6534 [PubMed] [PMC]
- Hosein AN, Lim YC, Day B, Stringer B, Rose S, Head R, Cosgrove L, Sminia P, Fay M, Martin JH (2015) The effect of valproic acid in combination with irradiation and temozolomide on primary human glioblastoma cells. Journal of Neuro-Oncology 122(2): 263–271. https://doi.org/10.1007/s11060-014-1713-x [PubMed]
- Hu S, Begum AN, Jones MR, Oh MS, Beech WK, Beech BH, Yang F, Chen P, Ubeda OJ, Kim PC, Davies P, Ma Q, Cole GM, Frautschy SA (2009) GSK3 inhibitors show benefits in an Alzheimer's disease (AD) model of neurodegeneration, but adverse effects in control animals. Neurobiology of Disease 33(2): 193–206. https://doi.org/10.1016/j.nbd.2008.10.007 [PubMed] [PMC]
- Huang J, Guo X, Li W, Zhang Y (2017) Activation of Wnt/β-catenin signalling via GSK3 inhibitors direct differentiation of human adipose stem cells into functional hepatocytes. Scientific Reports 7: 40716. https://doi.org/10.1038/srep40716 [PubMed] [PMC]
- Hur EM, Zhou FQ (2010) GSK3 signalling in neural development. Nature Reviews Neuroscience 11(8): 539–551. https://doi.org/10.1038/nrn2870 [PubMed] [PMC]
- Ito T, Hamazaki Y, Takaori-Kondo A, Minato N (2017) Bone marrow endothelial cells induce immature and mature B cell egress in response to erythropoietin. Cell Structure and Function 42(2): 149–157. https://doi.org/10.1247/csf.17018 [PubMed]
- Jacobs KM, Bhave SR, Ferraro DJ, Jaboin JJ, Hallahan DE, Thotala D (2012) GSK-3β: a bifunctional role in cell death pathways. International Journal of Cell Biology 2012: 930710. https://doi.org/10.1155/2012/930710 [PMC]
- Jeong SG, Ohn T, Kim SH, Cho GW (2013) Valproic acid promotes neuronal differentiation by induction of neuroprogenitors in human bone-marrow mesenchymal stromal cells. Neuroscience Letters 554: 22–27. https://doi.org/10.1016/j.neulet.2013.08.059 [PubMed]
- Ji MM, Wang L, Zhan Q, Xue W, Zhao Y, Zhao X, Xu PP, Shen Y, Liu H, Janin A, Cheng S, Zhao WL (2005) Induction of autophagy by valproic acid enhanced lymphoma cell chemosensitivity through HDAC-independent and IP3-mediated PRKAA activation. Autophagy 11: 2160–2171. https://doi.org/10.1080/15548627.2015.1082024 [PubMed] [PMC]

- Jo SJ, Choi SJ, Yoon SY, Lee JY, Park WS, Park PJ, Kim KH, Eun HC, Kwon O (2013) Valproic acid promotes human hair growth in in vitro culture model. Journal of Dermatological Science 72(1): 16–24. https://doi.org/10.1016/j.jdermsci.2013.05.007 [PubMed]
- Jope RS, Johnson GV (2004) The glamour and gloom of glycogen synthase kinase-3. Trends in Biochemical Sciences 29(2): 95–102. https://doi.org/10.1016/j.tibs.2003.12.004 [PubMed]
- Jope RS, Yuskaitis CJ, Beurel E (2007) Glycogen synthase kinase-3 (GSK3): inflammation, diseases, and therapeutics. Neurochemical Research 32(4–5): 577–595. https://doi.org/10.1007/s11064-006-9128-5 [PubMed] [PMC]
- Jung KH, Han DM, Jeong SG, Choi MR, Chai YG, Cho GW (2015) Proteomic analysis reveals KRIT1 as a modulator for the antioxidant effects of valproic acid in human bone-marrow mesenchymal stromal cells. Drug and Chemical Toxicology 38(3): 286–292. https://doi.org/10.3109/01480545.2014.951762 [PubMed]
- Karén J, Rodriguez A, Friman T, Dencker L, Sundberg C, Scholz B (2011) Effects of the histone deacetylase inhibitor valproic acid on human pericytes in vitro. PLoS One 6(9): e24954. https://doi.org/10.1371/journal.pone.0024954 [PubMed] [PMC]
- Khairallah MI, Kassem LA, Yassin NA, El Din MA, Zekri M, Attia M (2014) The hematopoietic growth factor erythropoietin” enhances the therapeutic effect of mesenchymal stem cells in Alzheimer’s disease. Pakistan Journal of Biological Sciences 17(1): 9–21. https://doi.org/10.3923/pjbs.2014.9.21 [PubMed]
- Kim WY, Wang X, Wu Y, Double BW, Patel S, Woodgett JR, Snider WD (2009) GSK-3 is a master regulator of neural progenitor homeostasis. Nature Neuroscience 12: 1390–1397. https://doi.org/10.1038/nn.2408 [PubMed] [PMC]
- Kitazoe K, Abe M, Hiasa M, Oda A Amou H, Harada T, Nakano A, Takeuchi K, Hashimoto T, Ozaki S, Matsumoto T (2009) Valproic acid exerts anti-tumor as well as anti-angiogenic effects on myeloma. International Journal of Hematology 89(1): 45–57. https://doi.org/10.1007/s12185-008-0226-9 [PubMed]
- Kondo Y, Iwao T, Yoshihashi S, Mimoriet K, Ogihara R, Nagata K, Kurose K, Saito M, Niwa T, Suzuki T, Miyata N, Ohmori S, Nakamura K, Matsunaga T (2014) Histone deacetylase inhibitor valproic acid promotes the differentiation of human induced pluripotent stem cells into hepatocyte-like cells. PloS One 9(8): e104010. https://doi.org/10.1371/journal.pone.0104010 [PMC]
- Kotliarova S, Pastorino S, Kovell LC, Kotliarov Y, Song H, Zhang W, Bailey R, Maric D, Zenklusen JC, Lee J, Fine HA (2008) Glycogen synthase kinase-3 inhibition induces glioma cell death through c-MYC, nuclear factor-kappaB, and glucose regulation. Cancer Research 68(16): 6643–6651. https://doi.org/10.1158/0008-5472.CAN-08-0850 [PubMed] [PMC]
- Lam YM, Chan YF, Chan LC, NG RK (2017) Histone deacetylase inhibitors induce leukemia gene expression in cord blood hematopoietic stem cells expanded ex vivo. International Journal of Hematology 105(1): 37–43. https://doi.org/10.1007/s12185-016-2075-2
- Lapid K, Itkin T, D’Uva G, Ovadya Y, Ludin A, Caglio G, Kalinkovich A, Golan K, Porat Z, Zollo M, Lapidot T (2013) GSK3β regulates physiological migration of stem/progenitor cells via cytoskeletal rearrangement. The Journal of Clinical Investigation 123(4): 1705–1717. https://doi.org/10.1172/JCI64149 [PubMed] [PMC]
- Lee H, Lee JY, Ha DH, Jeong JH, Park JB (2018) Effects of valproic acid on morphology, proliferation, and differentiation of mesenchymal stem cells derived from human gingival tissue. Implant Dentistry 27(1): 33–42. https://doi.org/10.1097/ID.0000000000000711 [PubMed]
- Lee S, Park JR, Seo MS, Roh KH, Park SB, Hwang JW, Sun B, Seo K, Lee YS, Kang SK, Jung JW, Kang KS (2009) Histone deacetylase inhibitors decrease proliferation potential and multilineage differentiation capability of human mesenchymal stem cells. Cell Proliferation 42(6): 711–720. https://doi.org/10.1111/j.1365-2184.2009.00633.x [PubMed]
- Li ASW, Marikawa Y (2016) Adverse effect of valproic acid on an in vitro gastrulation model entails activation of retinoic acid signaling. Reproductive Toxicology 66: 68–83. https://doi.org/10.1016/j.reprotox.2016.09.015 [PubMed]
- Liberman Z, Eldar-Finkelman H (2005) Serine 332 phosphorylation of insulin receptor substrate-1 by glycogen synthase kinase-3 attenuates insulin signaling. The Journal of Biological Chemistry 280(6): 4422–4428. https://doi.org/10.1074/jbc.M410610200 [PubMed]
- Lim R, Lappas M (2015) A novel role for GSK3 in the regulation of the processes of human labour. Reproduction 149(2): 189–202. https://doi.org/10.1530/REP-14-0493 [PubMed]
- Lim J, Lee S, Ju H, Kim Y, Heo J, Lee HY, Choi KC, Son J, Oh YM, Kim IG, Shin DM (2017) Valproic acid enforces the priming effect of sphingosine-1 phosphate on human mesenchymal stem cells. International Journal of Molecular Medicine 40(3): 739–747. https://doi.org/10.3892/ijmm.2017.3053 [PubMed] [PMC]
- Linares GR, Chiu CT, Scheuing L, Leng Y, Liao HM, Maric D, Chuang DM (2016) Preconditioning mesenchymal stem cells with the mood stabilizers lithium and valproic acid enhances therapeutic efficacy in a mouse model of Huntington’s disease. Experimental Neurology 281: 81–92. https://doi.org/10.1016/j.expneurol.2016.04.003 [PubMed]
- Liu NM, Tian J, Wang WW, Han GF, Cheng J, Huang J, Zhang JY (2012) Effects of erythropoietin on mesenchymal stem cells’ function of differentiation and secretion cultured under acute kidney injury microenvironment. Genetics and Molecular Research 12(4): 6477–6487. https://doi.org/10.4238/2013.February.28.14 [PubMed]
- Liu N, Wang C, Wang L, Gao L, Cheng H, Tang G, Hu X, Wang J (2016) Valproic acid enhances the antileukemic effect of cytarabine by triggering cell apoptosis. International Journal of Molecular Medicine 37(6): 1686–1696. https://doi.org/10.3892/ijmm.2016.2552 [PubMed]
- Lochhead PA, Coghlan M, Rice SQ, Sutherland C (2001) Inhibition of GSK-3 selectively reduces glucose-6-phosphatase and phosphatase and phosphoenolypyruvate carboxykinase gene expression. Diabetes 50(5): 937–946. https://doi.org/10.2337/diabetes.50.5.937
- Lombardero M, Kovaes K, Schethauer BW (2011) Erythropoietin: a hormone with multiple functions. Pathobiology 78(1): 41–53. https://doi.org/10.1159/000322975 [PubMed]
- Lossius MI, Tauboll E, Mowinckel P, Gjerstad L (2009) Reversible effects of antiepileptic drugs on thyroid hormones in men and women with epilepsy: a prospective randomized double-blind withdrawal study. Epilepsy and Behavior 16(1): 64–68. https://doi.org/10.1016/j.yebeh.2009.07.014 [PubMed]
- Lu H, Wu X, Wang Z, Li L, Chen W, Yang M, Huo D, Zeng W, Zhu C (2016) Erythropoietin-activated mesenchymal stem cells promote healing ulcers by improving microenvironment. Journal of Surgical Research 205(2): 464–473. https://doi.org/10.1016/j.jss.2016.06.086 [PubMed]
- Luo M, Ling T, Xie W, Sun H, Zhou Y, Zhu Q, Shen M, Zong L, Lyu G, Zhao Y, Ye T, Gu J, Tao W, Lu Z, Grummt I (2013) NuRD

blocks reprogramming of mouse somatic cells into pluripotent stem cells. Stem Cells (Dayton, Ohio) 31(7): 1278-1286 https://doi.org/10.1002/stem.1374 [PubMed]

- Ma X-J, Wang Y-S, Gu W-P, Zhao X (2007) The role and possible molecular mechanism of valproic acid in the growth of MCF-7 breast cancer cells. Croatian Medical Journal 58(5): 349–357. [PubMed] [PMC]
- Mangileva TA (2014) Erythropoietin and its non-erythropoietic effects. Tavrida Bulletin of Medicine and Biology [Tavricheskij Mediko-biologicheskij Vestnik] 17(3): 111–115. [in Russian]
- Maiese K (2009) Triple play: promoting neurovascular longevity with nicotinamide, WNT and erythropoietin in diabetes mellitus. Biomedicine and Pharmacotherapy 62(4): 218–232. https://doi.org/10.1016/j.biopha.2008.01.009 [PubMed] [PMC]
- Maiese K, Chong ZZ, Hou J, Shang CS (2008a) Erythropoietin and oxidative stress. Current Neurovascular Research 5(2): 125–142. https://doi.org/10.2174/156720208784310231 [PubMed] [PMC]
- Maiese K, Chong ZZ, Li F (2008b) Erythropoietin: elucidating new cellular targets that broaden therapeutic strategies. Progress in Neurobiology 85(2): 194–213. https://doi.org/10.1016/j.pneurobio.2008.02.002 [PubMed] [PMC]
- Marchand B, Arsenault D, Raymond-Fleury A, Boisvert FM, Boucher MJ (2015) Glycogen synthase kinase-3 (GSK3) inhibition induces pro-survival autophagic signals in human pancreatic cancer cells. Journal of Biological Chemistry 290(9): 5592–5605. https://doi.org/10.1074/jbc.M114.616714 [PubMed] [PMC]
- Marchand B, Tremblay I, Cagnol S, Boucher MJ (2012) Inhibition of glycogen synthase kinase-3 activity triggers an apoptotic response in pancreatic cancer cells through JNK-dependent mechanisms. Carcinogenesis 33(3): 529–537. https://doi.org/10.1093/carcin/bgr309 [PubMed]
- Marquez-Curtis LA, Qiu Y, Xu A, Janowska-Wieczorek A (2014) Migration, proliferation, and differentiation of cord blood mesenchymal stromal cells treated with histone deacetylase inhibitor valproic acid. Stem Cells International 2014: 610495. https://doi.org/10.1155/2014/610495 [PubMed] [PMC]
- Mills CN, Nowsheen S, Bonner JA, Yang ES (2011) Emerging roles of glycogen synthase kinase 3 in the treatment of brain tumors. Frontiers in Molecular Neuroscience 4: 47. https://doi.org/10.3389/fnmol.2011.00047 [PubMed] [PMC]
- Moschidou D, Mukherjee S, Blundell MP, Drews K, Jones GN, Abdulrazzak H, Nowakowska B, Phoolchund A, Lay K, Ramasamy TS, Cananzi M, Nettersheim D, Sullivan M, Frost J, Moore G, Vermeesch JR, Fisk NM, Thrasher AJ, Atala A, Adjaye J, Schorle H, De Coppi P, Guillot PV (2012) Valproic acid confers functional pluripotency to human amniotic fluid stem cells in a transgene-free approach. Molecular Therapy 20(10): 1953–1967. https://doi.org/10.1038/mt.2012.117 [PubMed] [PMC]
- Nair AM, Tsai YT, Shah KM, Shen J, Weng H, Zhou J, Sun X, Saxena R, Borrelli Jr J, Tang L (2013) The effect of erythropoietin on autologous stem cell-mediated bone regeneration. Biomaterials 34(30): 7364–7371. https://doi.org/10.1016/j.biomaterials.2013.06.031 [PubMed] [PMC]
- Ostrowski D, Heinrich R (2018) Alternative erythropoietin receptors in the nervous system. Journal of Clinical Medicine 7(2): 24. https://doi.org/10.3390/jcm7020024 [PubMed] [PMC]
- Pitetzis DA, Spilioti MG, Yovos JG, Yavropoulou MP (2017) The effect of VPA on bone: From clinical studies to cell cultures – The molecular mechanisms revisited. Seizure 48: 36–43. https://doi.org/10.1016/j.seizure.2017.03.013 [PubMed]
- Raddino R, Robba D, Caretta V, Bonadei I, Teli M, Zanini G, Madureri A, Vizzardi E, Dei Cas L (2008) Erythropoietin: a new perspective in cardiovascular therapy. Monaldi Archives for Chest Disease 70(4): 206–213. https://doi.org/10.4081/monaldi.2008.414 [PubMed]
- Ragancokova D, Jahn K, Kotsiari A, Schlesinger F, Haastert K, Stangel M, Petri S, Krampfl K (2009) Analysis of neuroprotective effects of valproic acid on primary motor neurons in monoculture or co-cultures with astrocytes or Schwann cells. Cellular and Molecular Neurobiology 29(6–7): 1037–1043. https://doi.org/10.1007/s10571-009-9393-3 [PubMed]
- Rauchenzauner M, Bitsche G, Svalheim S, Tauboll E, Haberlandt E, Wildt L, Rostasy K, Luef G (2009) Effects of levetiracetam and valproic acid monotherapy on sex-steroid hormones in prepubertal children – results from a pilot study. Epilepsy Research 88(2–3): 264–268. https://doi.org/10.1016/j.eplepsyres.2009.11.003 [PubMed]
- Raut A, Khanna A (2016) Enhanced expression of hepatocyte-specific microRNAs in valproic acid mediated hepatic trans-differentiation of human umbilical cord derived mesenchymal stem cells. Experimental Cell Research 343(2): 237–247. https://doi.org/10.1016/j.yexcr.2016.03.015 [PubMed]
- Rayasam GV, Tulasi VK, Sodhi R, Davis JA, Ray A (2009) Glycogen synthase kinase 3: more than a namesake. British Journal of Pharmacology 156(6): 885–898. https://doi.org/10.1111/j.1476-5381.2008.00085.x [PubMed] [PMC]
- Roste LS, Tauboll E, Morkrid L, Bjornenak T, Saetre ER, Mørland T, Gjerstad L (2005) Antiepileptic drugs alter reproductive endocrine hormones in men with epilepsy. European Journal of Neurology 12(2): 118–124. https://doi.org/10.1111/j.1468-1331.2004.00899.x [PubMed]
- Ryu CH, Park KY, Kim SM, Jeong CH, Woo JS, Hou Y, Jeun SS (2012) Valproic acid enhances anti-tumor effect of mesenchymal stem cell mediated HSV-TK gene therapy in intracranial glioma. Biochemical and Biophysical Research Communications 421(3): 585–590. https://doi.org/10.1016/j.bbrc.2012.04.050 [PubMed]
- Salerno JC, Ngwa VM, Nowak SJ, Chrestensen CA, Healey AN, McMurry JL (2016) Novel cell-penetrating peptide-adaptors effect intracellular delivery and endosomal escape of protein cargos. Journal of Cell Science 129(5): 893–897. https://doi.org/10.1242/jcs.182113 [PubMed] [PMC]
- Schroeder TM, Nair AK, Staggs R, Lamblin AF, Westendorf JJ (2007) Gene profile analysis of osteoblast genes differentially regulated by histone deacetylase inhibitors. BMC Genomics 8(1): 362. https://doi.org/10.1186/1471-2164-8-362 [PubMed] [PMC]
- Schroeder TM, Westendorf JJ (2005) Histone deacetylase inhibitors promote osteoblast maturation. Journal of Bone and Mineral Research 20(12): 2254–2263. https://doi.org/10.1359/JBMR.050813 [PubMed]
- Setiawan M, Tan XW, Goh TW, Hin-Fai Yam G, Mehta JS (2017) Inhibiting glycogen synthase kinase-3 and transforming growth factor-β signaling to promote epithelial transition of human adipose mesenchymal stem cells. Biochemical and Biophysical Research Communications 490(4): 1381–1388. https://doi.org/10.1016/j.bbrc.2017.07.036 [PubMed]
- Sidana A, Wang M, Shabbeer S, Chowdhury WH, Netto G, Lupold SE, Carducci M, Rodriguez R (2012) Mechanism of growth inhibition of prostate cancer xenografts by valproic acid. BioMed Research International 2012: 180363. https://doi.org/10.1155/2012/180363 [PubMed] [PMC]

- Tabatabaei AR, Abbott FS (1999) Assessing the mechanism of metabolism-dependent valproic acid-induced in vitro cytotoxicity. Chemical Research in Toxicology 12(4): 323–330. https://doi.org/10.1021/tx9801864 [PubMed]
- Talwadekar M, Fernandes S, Kale V, Limaye L (2017) Valproic acid enhances the neural differentiation of human placenta derived mesenchymal stem cells in vitro. Journal of Tissue Engineering and Regenerative Medicine 11(11): 3111–3123. https://doi.org/10.1002/term.2219 [PubMed]
- Tari K, Atashi A, Kaviani S, AkhavanRahnama M, Anbarlou A, Mossahebi-Mohammadi M (2017) Erythropoietin induces production of hepatocyte growth factor from bone marrow mesenchymal stem cells in vitro. Biologicals 45: 15–19. https://doi.org/10.1016/j.biologicals.2016.10.010 [PubMed]
- Travaglini L, Vian L, Billi M, Grignani F, Nervi C (2009) Epigenetic reprogramming of breast cancer cells by valproic acid occurs regardless of estrogen receptor status. International Journal of Biochemistry and Cell Biology 41: 225–234. https://doi.org/10.1016/j.biocel.2008.08.019 [PubMed]
- Tsai PT (2006) A critical role of erythropoietin receptor in neurogenesis and post-stroke recovery. Journal of Neuroscience 26(4): 1269–1274. https://doi.org/10.1523/JNEUROSCI.4480-05.2006 [PubMed]
- Tsai LK, Leng Y, Wang Z, Leeds P, Chuang DM (2010) The mood stabilizers valproic acid and lithium enhance mesenchymal stem cell migration via distinct mechanisms. Neuropsychopharmacology 35(11): 2225–2237. https://doi.org/10.1038/npp.2010.97 [PubMed] [PMC]
- Um S, Lee H, Zhang, Kim HY, Lee JH, Seo BM (2017) Valproic acid modulates the multipotency in periodontal ligament stem cells via p53-mediated cell cycle. Tissue Engineering and Regenerative Medicine 14(2): 153–162. https://doi.org/10.1007/s13770-017-0027-4 [PMC]
- Verrotti A, Loiacono G, Laus M, Coppola G, Chiarelli F, Tiboni GM (2009) Hormonal and reproductive disturbances in epileptic male patients: emerging issues. Reproductive Toxicology 31(4): 519–527. https://doi.org/10.1016/j.reprotox.2011.02.002 [PubMed]
- Wang H, Brown J, Martin M (2011) Glycogen synthase kinase 3: a point of convergence for the host inflammatory response. Cytokine 53(2): 130–140. https://doi.org/10.1016/j.cyto.2010.10.009 [PubMed]
- Wang W, Li M, Wang Y, Li Q, Deng G, Wan J, Yang Q, Chen Q, Wang J (2016) GSK-3β inhibitor TWS119 attenuates rtPA-induced hemorrhagic transformation and activates the Wnt/β-catenin signaling pathway after acute ischemic stroke in rats. Molecular Neurobiology 53(10): 7028–7036. https://doi.org/10.1007/s12035-015-9607-2 [PubMed]
- Wang Z, Smith KS, Murphy M, Piloto O, Somervaille TC, Cleary ML (2008) Glycogen synthase kinase 3 in MLL leukaemia maintenance and targeted therapy. Nature 455(7217): 1205–1209. https://doi.org/10.1038/nature07284 [PubMed] [PMC]
- Watcharasit P, Bijur GN, Zmijewski JW, Song L, Zmijewska A, Chen X, Johnson GV, Jope RS (2002) Direct, activating interaction between glycogen synthase kinase-3beta and p53 after DNA damage. Proceedings of the National Academy of Sciences of the United States of America 99(12): 7951–7955. https://doi.org/10.1073/pnas.122062299 [PubMed] [PMC]
- Wilson EL, Garton M, Fuller HR (2016) Anti-epileptic drugs and bone loss: Phenytoin reduces pro-collagen I and alters the electrophoretic mobility of osteonectin in cultured bone cells. Epilepsy Research 122: 97–101. https://doi.org/10.1016/j.eplepsyres.2016.03.002 [PubMed]
- Xia Q, Zheng Y, Jiang W et al. (2016) Valproic acid induces autophagy by suppressing the Akt/mTOR pathway in human prostate cancer cells. Oncology Leters 12: 1826–1832. https://doi.org/10.3892/ol.2016.4880 [PubMed] [PMC]
- Xu W, Wang Y, Li Y, Wang L, Huang Z, Wang M, Rodriguez R, Jin X (2012) Valproic acid improves the in vitro development competence of bovine somatic cell nuclear transfer embryos. Cellular Reprogramming 14(2): 138–145. https://doi.org/10.1089/cell.2011.0084 [PubMed] [PMC]
- Young DA, Lakey RL, Pennington CJ, Jones D, Kevorkian L, Edwards DR, Cawston TE, Clark IM (2005) Histone deacetylase inhibitors modulate metalloproteinase gene expression in chondrocytes and block cartilage resorption. Arthritis Research and Therapy 7(3): R503-R512. https://doi.org/10.1186/ar1702 [PubMed] [PMC]
- Yu Q, Chen L, You Y, Zou C, Zhang Y, Liu Q, Cheng F (2014) Erythropoietin combined with granulocyte colony-stimulating factor enhances MMP-2 expression in mesenchymal stem cells and promotes cell migration. Molecular Medicine Reports 4(1): 31–36. https://doi.org/10.3892/mmr.2010.387 [PubMed]
- Yu X, Shacka JJ, Eells JB, Suarez-Quian C, Przygodzki RM, Beleslin-Cokic B, Lin CS, Nikodem VM, Hempstead B, Flanders KC, Costantini F, Noguchi CT (2002) Erythropoietin receptor signalling is required for normal brain development. Development 129(2): 505–516. [PubMed]
- Zhang X, He X, Li Q, Kong X Ou Z, Zhang L, Gong Z, Long D, Li J, Zhang M, Ji W, Zhang W, Xu L, Xuan A (2017) PI3K/AKT/mTOR signaling mediates valproic acid-induced neuronal differentiation of neural stem cells through epigenetic modifications. Stem Cell Reports 8(5): 1256–1269. https://doi.org/10.1016/j.stemcr.2017.04.006 [PubMed] [PMC]
- Zeng QB, Cheng FJ, Zhang WG, Tang JM, Chen L, Liu QH, Gao QP, Wang JN (2008) Erythropoietin promotes proliferation of human bone marrow mesenchymal stem cells in vitro. Journal of Experimental Hematology [in Chinese] 16(6): 1392–1397. [PubMed]
- Zhang DG, Zhang FM, Zhang YQ, Zhou F, Gao X, Li CF, Cao KJ (2006) Erythropoietin enhances the therapy potency of autologous bone marrow stromal cells in a rat heart infarction model via phosphatidylinositol-3-kinase/Akt pathway. Chinese Journal of Cardiology [in Chinese] 34(10): 912–916. [PubMed]
- Zhang S, Shi B (2017) Erythropoietin modification enhances the protection of mesenchymal stem cells on diabetic rat-derived Schwann cells: implications for diabetic neuropathy. BioMed Research International 2017: 6352858 https://doi.org/10.1155/2017/6352858 [PubMed] [PMC]
- Zhou D, Chen Y-X, Yin J-H, Tao S-C, Guo S-C et al. (2018) Valproic acid prevents glucocorticoid-induced osteonecrosis of the femoral head of rats. International Journal of Molecular Medicine 41(6): 3433–3447. https://doi.org/10.3892/ijmm.2018.3534 [PubMed] [PMC]

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