Novel molecular triggers underlie valproate-induced liver injury and its alleviation by the omega-3 fatty acid DHA: role of inflammation and apoptosis

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Abstract

Background/Aim: Hepatic injury is a hallmark adverse reaction to Valproate (VPA), a common used drug in the management of numerous CNS disorders, including epilepsy. DHA has a myriad of health benefits, including renal- and hepato-protective effects. Unfortunately, however, the underpinnings of such liver-pertinent VPA- and DHA-actions remain largely undefined. Accordingly, this study attempted to unveil the cellular and molecular triggers whereby VPA evokes, while DHA abates, hepatotoxicity.

Methods: We evaluated activity and/or expression of cellular markers of oxidative stress, inflammation, and apoptosis in rat liver, following treatment with VPA (500 mg/kg/day) with and without concurrent treatment with DHA (250 mg/kg/day) for two weeks.
**Results and conclusion:** VPA promoted hepatic oxidative stress as evidenced by enhancing activity/expression of NADPH-oxidase and its subunits, a ROS-generator, and by accumulation of lipid-peroxides. Moreover, VPA enhanced hepatic phosphorylation/activation of mitogen-activated protein kinase (MAPK), and expression of cyclooxygenase-2 (COX-2), as proinflammatory signals. Besides, VPA promoted hepatocellular apoptosis, as attested by enhanced expression of cleaved caspase-9 and increased number of TUNEL-positive hepatocytes. Lastly, VPA upregulated levels of hypoxia-inducible factor-1-alpha (HIF-1α), a multifaceted modulator of hepatocytic biology, and activity of its downstream antioxidant enzyme heme-oxygenase-1 (HO-1). These changes were significantly blunted by co-administration of DHA. Our findings demonstrate that VPA activated NADPH-oxidase and HIF-1α to induce oxidative-stress and hypoxia as initiators of hepatic injury. These changes were further aggravated by up-regulation of inflammatory (MAPK and COX-2) and apoptotic cascades, but could be partly lessened by HO-1 activation. Concurrent administration of DHA mitigated all VPA-induced anomalies.

Keywords: Biochemistry, Cell biology, Systems biology, Physiology, Medicine

1. **Introduction**

Liver injury is a major public health problem all over the world and it affects approximately 30% of the US population (Rinella, 2015). More than 900 drugs have been implicated in inducing liver injury as an adverse effect, a predominantly compelling reason for drug withdrawal from the market (Friedman et al., 2003). Valproate (VPA) is a widely used drug for treatment of epileptic seizures, bipolar and schizoaffective disorders, trigeminal neuralgia, social phobias, migraine pain and neuropathic pain (Johannessen and Johannessen, 2003). However, VPA has major adverse reactions, including gastrointestinal disorders (Jahromi et al., 2011), hemorrhagic pancreatitis, bone-marrow suppression and a more frequently hepatic injury (Gerstner et al., 2008). Hyperammonemia has been reported in patients using VPA therapy (Tseng et al., 2014). With chronic use of VPA, 44% of patients showed elevated serum liver enzymes and lipid peroxidation. Moreover, VPA therapy is associated with the development of fatty liver (Zimmerman and Ishak, 1982) that can progress to hepatic necrosis, apoptosis. While most of these reactions are VPA-dose- and time-dependent, some serious ones are not so, as they involve type-2 unpredictable (idiosyncratic) fatal reactions (Roma-Giannikou et al., 1999).

VPA is extensively metabolized in the liver via glucuronic acid conjugation, mitochondrial β-oxidation and cytosolic ω-oxidation to produce multiple metabolites; some of them are biologically active and could mediate VPA-induced hepatotoxicity. For instance, 4-ene-valproic (4-ene-VPA) is a more potent hepatotoxic than VPA and...
can trigger oxidative cascades that deplete reduced-glutathione, an indispensable antioxidant cellular protector (Ji et al., 2010). Moreover, valproate-induced activation of CYP2E1 and subsequently oxidative stress may provoke serious proteolytic reactions in hepatocytes, thereby damaging liver cell membranes and leaking out intracellular enzymes (Ji et al., 2010).

Docosahexaenoic acid/DHA is a biologically active polyunsaturated omega-3 fatty acid (ω-3 FA). DHA is found mainly in fish oil and the metabolic products of a third plant-derived ω-3 FA, alpha-linolenic acid (Li and Hu, 2009). DHA has numerous health benefits as it regulates membrane bound enzymes (Na⁺/K⁺-dependent ATPase) and plays a role in signal transduction by modulating inositol phosphates, diacylglycerol (DAG), and protein kinase C pathways (Abedi and Sahari, 2014). Moreover, DHA directly influences biosynthesis, signaling and uptake of serotonin, a CNS crucial neurotransmitter (Patrick and Ames, 2015). DHA also affects cell membrane structure and function (Wood et al., 2015) and plays an important role in controlling malignant, inflammatory, proliferative and vascular diseases (Simopoulos, 2003). Interestingly, this n-3FA is an approved OTC supplement/drug for both children and adults, thereby displaying a wide safety margin and high therapeutic index (El-Mowafy et al., 2011; Patrick and Ames, 2015).

We have previously demonstrated the efficacy of DHA, or its analog (EPA) against VPA-induced hepatotoxicity, and doxorubicin-evoked renal injury (El-Mesery et al., 2009; El-Mowafy et al., 2011). Therefore, the current study was undertaken to extend our previous findings by investigating the molecular basis and mechanistic pathways whereby VPA evokes, while DHA may abate, hepatotoxicity. The impact and contribution of novel cellular components in VPA-induced hepatotoxicity that could exacerbate oxidative stress, hypoxia, inflammation and apoptosis were evaluated, then further challenged by co-administration of DHA.

2. Materials and methods

2.1. Drugs and chemicals

Sodium valproate was obtained from Cayman Chemical Company, USA, and was dissolved in distilled water. DHA was purchased from Swanson Health Product, North Dakota, USA, as capsules; each provides 250 mg of pure DHA. DHA was diluted in corn oil, with equivalent amounts of oil given to all animals in the control group.

2.2. Animal studies

All procedures with animals were performed in accordance with the Public Health Service Guide for the Care and Use of Laboratory Animals and Augusta University guidelines, and approved by the Institutional Animal Care and Use Committee of
Augusta University. Twelve week old male Sprague Dawley rats were purchased from Harlan Laboratory and used in the current study. Three group of rats were used in our study (n = 6–8 rats/group) as follows; Control-group received vehicle (corn oil daily) for 2 weeks, VPA-group received VPA alone (500 mg/kg PO, daily) for two weeks, VPA + DHA-group received VPA (500 mg/kg PO, daily), followed by DHA (250 mg/kg PO, daily) for two weeks. After two weeks of treatment, rats were terminated using sodium pentobarbital (50 mg/kg, IP) for liver collection. Liver was isolated, weighed, aliquoted in few tubes and snap frozen in liquid nitrogen. A 10% (w/v) liver homogenate was prepared in phosphate-buffered saline (PBS) (pH 7.4) for the assay of hepatic TBARs, NADPH-oxidase, and HO-1. Other frozen liver samples were homogenized in RIPA buffer for Western blotting (n = 4/group).

2.3. Biochemical determinations

Hepatic thiobarbituric reactive species (TBARs) were assessed spectrophotometrically according to manufacturer instructions (Cayman Chemical, Ann Arbor, MI) as a marker of oxidative stress. NADPH-oxidase activity was measured by lucigenin chemiluminescence (5 μmol/L) in the presence of 100 μmol/L NADPH as previously described (Pye et al., 2014). Briefly, the liver was homogenized in ice-cold buffer in the presence of protease inhibitors (Phenylmethylsulphonyl-fluoride 1 mM, Benzamidine-HCl 1 mM, Leupeptin, 0.5 μg/ml, Aprotinin 0.5 μg/ml, Pepstatin 1 mg/ml, NaF 50 mM). 50 μL of homogenate or buffer control was added to the wells of a 96-well micro-plate (OptiPlate-96, Perkin-Elmer, Waltham, MA) and incubated at 37 °C for 30 minutes in the presence of lucigenin (5 μmol/L) and NADPH, and total count per minute (cpm) was normalized to mg protein. Hepatic HO-1 activity, a marker of antioxidant pathway, was measured in liver samples using a commercially available ELISA according to manufacturer’s instructions (Enzo Life Sciences Inc., Farmingdale, NY).

2.4. Homogenization of the liver for protein expression using Western blotting

Liver samples were homogenized in RIPA buffer in the presence of protease inhibitors. Protein concentrations were determined by standard Bradford assay (Bio-Rad, Hercules, CA) using bovine serum albumin as the standard and liver homogenates were used to determine protein expression levels of hepatic P67, gp91 phox, HIF-1α, P-MAPK, COX-2, and cleaved caspase-9 using Western blotting. Thirty-fifty micrograms of liver samples was separated by SDS-PAGE then transferred onto PVDF membrane. Membranes were blocked in 5% milk in TBS and were incubated with primary antibodies overnight. The blots were then washed in TBS-0.1% Tween and incubated with the secondary antibody goat anti-rabbit or goat anti-mouse (1:5000) conjugated to horseradish peroxidase for 1 hour. Detection was accomplished using enhanced chemiluminescence. Band
intensity was measured densitometrically and the values were normalized to β-actin (Sigma, St Louis, MO) using NIH Image J software.

2.5. TUNEL assay

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay was used for in situ detection of apoptotic cells with a commercially available TUNEL kit (Promega, Madison, USA). Propidium iodide (PI) 1 μg/ml was added as a nuclear counter stain. The number of TUNEL-positive hepatic cells was counted and average TUNEL positive cells per cross sectional area were recorded.

2.6. Statistical analyses

All data are presented as mean ± SEM and analyzed using one-way ANOVA followed by Tukey's post hoc test for multiple group comparisons. Analyses were performed using GraphPad Prism Version 4.0 software (GraphPad Software Inc., La Jolla, CA). For all comparisons, P < 0.05 was considered statistically significant.

3. Results

We have previously shown protective effects for DHA against VPA-induced hepatotoxicity. However, the molecular basis of such effects remains unclear. Accordingly, the current study was designed to assess the roles of oxidative stress, inflammation and apoptosis in VPA-evoked liver toxicity and their possible alleviation by DHA.

Because oxidative stress plays a role in the pathogenesis of liver injury, we first assessed whether VPA could elevate oxidative stress in the rat liver. Accordingly, we measured hepatic TBARs levels as a marker of lipid peroxidation. Fig. 1A and Table 1 show that hepatic TBARs levels were significantly elevated in VPA-treated animals and concurrent administration of DHA abated such VPA-evoked lipid peroxidation (P < 0.05). Besides, the increase in lipid peroxidation was associated with activation of NADPH oxidase, the main source of reactive-oxygen species in the vasculature, in VPA treated rats (Fig. 1B and Table 1). Moreover, VPA up-regulated experssional levels of the membrane-bound NADPH oxidase catalytic subunit GP91 phox (NOX2) as well as cytoplasmic P67 subunit versus control (Fig. 1C,D). Combined administration of DHA reduced VPA-induced NADPH oxidase activation and up-regulation of its subunits. To further gain acumen into the hepatic nuclear changes occurring in VPA-treated rats due to the elevation in oxidative stress, we assessed protein expression levels of HIF-1α and subsequent activation of downstream HO-1 enzyme in the liver (Fig. 2 and Table 1). We also detected a concurrent upregulation of Nrf-2, an upstream factor from HO-1 (data not shown).
The elevation in oxidative stress in the livers of VPA-treated rats was associated with upregulation of HIF-1α, an important modulator of transcription in cellular response to variations in systemic oxygen, which displays a multifaceted modulating potential in the mammalian liver (Fig. 2A and Table 1). Likewise, overexpression of HIF-1α was accompanied by an increase in the hepatic redox-sensitive antioxidant HO-1 level (Fig. 2B and Table 1). Interestingly, DHA treatment reduced the elevation in expression of hepatic HIF-1α and HO-1 level in VPA-treated rats (Fig. 2).

Inflammation plays a crucial role in the pathogenesis of hepatic injury. Thus, we presently assessed the impact of VPA on phosphorylation/activation of MAPK and

![Figure 1](http://dx.doi.org/10.1016/j.heliyon.2016.e00130)
subsequent downstream inflammatory enzymes, such as COX-2 in rat liver. As shown in Fig. 3, VPA increased hepatic phosphorylation of MAPK (Fig. 3A) and induced the expression of its downstream inflammatory enzyme COX-2 (Fig. 3B and Table 1) and these changes were reduced following concurrent administration of DHA.

Furthermore, to determine whether apoptosis is involved in VPA-induced hepatotoxicity, we assessed the DNA-fragmentation, a terminal response in (apoptotic cells) in rat liver using TUNEL assay, and further determined the

Table 1. Detailed raw data for biochemical assessment of NADPH oxidase, TBARs and HO-1 levels as well as Western blotting densitometric analysis for HIF-1α and COX-2 in control, VPA and VPA plus DHA treated rats.

|                     | Control | VPA      | VPA + DHA |
|---------------------|---------|----------|-----------|
| Hepatic TBARs (nmol/mg protein) | 0.8 ± 0.02 | 1.1 ± 0.06 * | 0.9 ± 0.08 |
| Hepatic NADPH oxidase (OD/mg protein) | 26.0 ±0.80 | 33.0 ± 0.60 * | 27.0 ± 1.20 # |
| Hepatic HIF-1α | 1.4 ± 0.07 | 2.0 ± 0.04 * | 1.5 ± 0.08 # |
| Hepatic HO-1 (ng/mg protein) | 0.98 ± 0.07 | 1.2 ± 0.05 * | 0.9 ± 0.05 # |
| Hepatic COX-2 (DU) | 0.7 ± 0.06 | 1.0 ± 0.07 * | 0.74 ± 0.06 |

Data are expressed as means ± SE of the mean. (*P < 0.05 vs. control and #P < 0.05 vs. VPA-treated rats, n = 4/group for Western blotting and n = 6 for hepatic TBARs and NADPH-oxidase).

Fig. 2. Hepatic HIF-1α expression relative to β-actin (A), hemeoxygenase-1 (HO-1) levels (B) in control and VPA-treated rats with or without DHA daily treatment for two weeks. HIF-1α levels and HO-1 activity were enhanced in VPA-treated versus control rats, in a DHA-sensitive manner (*P < 0.05 vs. control and #P < 0.05 vs. VPA-treated rats, n = 6–8/group for HO-1 and n = 4/group for HIF-1α Western blotting). Please see Figs. 5S–6S in the Supplementary Material for full blot images.
expression levels of the pro-apoptotic cleaved caspase-9, in the presence and absence of DHA. VPA caused a significant increase in TUNEL positive cells (Fig. 4A), while also increased cleaved caspase-9 expression levels. Both responses to VPA were significantly reduced with DHA administration (Fig. 4B).

It is noteworthy to mention that we also treated a rat group with DHA alone; however, all results with DHA group were not different from those of control group, thus these data were not presented.

4. Discussion

We previously demonstrated that DHA can ameliorate VPA-induced hepatotoxicity; however, the underlying molecular mechanisms remain to be delineated. This study targeted the role of key molecular regulators of VPA-induced hepatotoxicity, and further sought the capacity of DHA to counterbalance such signals. Our findings demonstrate that VPA up-regulates NADPH oxidase, ERK1/2 and HIF-1α, as upstream triggers of oxidative stress, hypoxia, inflammation and apoptosis, thereby concluding its profound liver injury. Besides, DHA counteracted VPA-induced hepatotoxicity, by showing anti-oxidant, anti-inflammatory and anti-apoptotic effects. The anti-oxidant/protective profiles of DHA have been demonstrated in human neutrophils and retina (Liu et al., 2014).

Valproic acid (VPA) is widely prescribed for children with newly diagnosed epilepsy; however, it can induce marked hepatotoxicity which could progress to fatal idiosyncratic reactions (Farinelli et al., 2015). VPA-mediated hepatotoxicity
is characterized by steatosis, necrosis, depletion of endogenous antioxidants, and disruption of mitochondrial membranes (Spiller et al., 2000).

HIF-1α is a transcription factor that plays a crucial role in cellular response to alterations in systemic oxygen levels in mammals. Accordingly, HIF-1α regulates transactivation of over 60 genes, such as VEGF and erythropoietin, which assist in promoting oxygen delivery to hypoxic regions. VPA modulates HIF-1α expression in diverse tissues, yet in an equivocal directions. In all cases, however, these effects were entailed to the known activity of VPA as a Histone deacetylase (HDAC) inhibitor (Mottamal et al., 2015). Thus, HIF-1α repression by VPA was established in human renal cancer cells and in rat gut mucosa. Conversely, in a rat brain stroke model, VPA up-regulated HIF-1α and its downstream proangiogenic factor, vascular endothelial growth factor (VEGF), (HO-1), thereby enhancing angiogenesis and functional recovery from ischemia (Wang et al., 2012). In the liver, HIF-1α status has never been verified in response to VPA treatment. Currently, we showed up-regulation of HIF-1α following VPA treatment. In the liver, HIF-1α is reportedly induced to aide steatosis, apoptosis and inflammatory/necrotic reactions in response to drug-induced toxicity or environmental insults (Cursio et al., 2008; van Swelm et al., 2012; Zhu et al., 2005). Sagaciously, because VPA presently evoked similar hepatic insults, while also induced HIF-1α, similar causalities and implications are warranted. Interestingly,
however, we also found that HO-1, a downstream antioxidant signal to HIF-1α, was jointly activated, presumably to oppose/counterbalance oxidative stress. Thus, together, these observations imply that HIF-1α currently stands as a multifaceted modulator of liver cell integrity and viability in response to VPA treatment. With the recent mounting interest in defining therapeutic utility to HIF-1α for an array of liver anomalies (Wilson et al., 2014), further specific/detailed investigations are required to delineate the exact scenario whereby HIF-1α modulates VPA-induced liver injury.

We reported that VPA upregulated inflammatory mediators such as COX-2 in the current study. Furthermore, VPA augmented the abundance of hepatic TUNEL positive cell and cleaved caspase-9 expression, indicating induction of apoptosis via hepatic mitochondrial dysfunction. In gastric cancer cell line, the VPA-induced apoptosis was ascribed to acetylation of histone and tubulin (Yagi et al., 2010).

The MAPK (ERK) pathway can play a role in cell growth and survival, or alternatively stimulates inflammatory cascades. In the CNS, the ERK pathway is recruited by neurotrophic factors to maintain neuronal growth, differentiation and survival in the hippocampus and frontal cortex. This pathway, when disrupted, can be restored by drugs intended in mood stabilization, depressive mania and bipolar disorders, like valproate (Einat et al., 2003; Engel et al., 2009). In support, chronic treatment of rats with valproate promoted levels of MAPK in relevant neurons (Hao et al., 2004). Likewise, MAPK ablation disrupted animal behavior and evoked excitements similar to those by psychostimulants such as amphetamine. These responses to ERK ablation were reversed by VPA and olanzapine, thereby substantiating the role of ERK signaling as a target in inducing behavioral anomalies, and for their alleviation by anti-manic drugs (Engel et al., 2009). Notably as well, HDAC inhibitors were ineffective on such VPA neuronal responses, implying that VPA can undertake multifaceted triggers to activate the neuronal ERK cascade (Hao et al., 2004). In contrast to the CNS, a paucity of information has been available to link VPA with ERK-activity in the liver. Thus, by far, only one in vitro study reported that VPA activates ERK phosphorylation in primary human hepatocytes, a response that was mimicked by HDAC inhibitor, trichostatin-A (Bitman et al., 2014), implying that HDAC inhibition is a primary stimulus for ERK in human hepatocytes. However, this study has not looked at role of oxidative stress or VPA metabolites, possibly due to limited biologic windows within a cell culture setup.

Currently, in the liver, and following a 2 week of VPA treatment, ERK is up-regulated, yet appears to play a pathogenic role culminating into liver injury. We show here for the first time that VPA up-regulates NADPH oxidase (NOX), as a prime generator of ROS and contributor to oxidative stress. Notably, NOX, in the liver, has been shown as a crucial mediator of diverse deleterious reactions, including inflammation, mitogenesis, fibrosis, apoptosis and steatosis (Li et al.,
2013; Moon et al., 2010). In such latter redox-sensitive anomalies, both ERK and NOX appear inevitably involved so as to shape responses to toxins, infections, or malignancies (Chen et al., 2007). Not surprisingly, cross talk activation between NADPH-oxidase (ROS) and ERK signaling was evident to further recruit additional factors including HIF-1α, as typically shown in mammary cancer cells (Moon et al., 2010). Our current results indicate a similar hepatic scenario among the three latter effectors, and further implicate COX-2 as an inflammatory co-player, in response to VPA treatment. Congruent to our latest liver finding with VPA, the association between ERK and COX-2 was revealed with other stimuli like phorbol-esters and cytokines in mammary cancer cells and murine auditory cells (Hong and Jang, 2014; Jeong et al., 2007).

VPA is a risk factor for developing serious liver injury in patients with Alpers-Huttenlocher syndrome (AHS), a neuro-metabolic disorder that can develop into fatal hepatotoxicity in stressful conditions. Thus, with VPA, this erupts due to disruption of liver cells, which involves a caspase-9-mediated apoptosis (Li et al., 2015). It is noteworthy that we have earlier demonstrated, via histopathologic analyses, that apoptosis occurs in the rat liver after similar VPA treatments (Abdel-Dayem et al., 2014). Therefore, our present study, sought the impact of VPA on expression of cleaved caspase-9 in normal liver in absence of other stressful risk factors, and further attempted to confirm this by an end-point checker for DNA fragmentation, using the TUNEL assay. Thus, VPA markedly augmented cleaved caspase-9 levels to induce apoptosis, as confirmed by the risen abundance of TUNEL-positive hepatocytes. This has been shown to VPA in other non-hepatic tissues, especially cancer cells, and in neural tissues in neonate-animals (Yochum et al., 2008). The present apoptotic reactions were sensitive to DHA treatment, which is consistent with previous observations that DHA supplementation reduces apoptosis in livers from high fat-fed animals (Schmocker et al., 2007) and necrosis in response to the cytokine TNF-α (Pacheco et al., 2014).

Caspase-9 has repeatedly been shown as a downstream apoptotic trigger from upstream stimuli like NOX/ROS, ERK1/2 or COX-2 (Zhang et al., 2003). These notions prompted us to check for the status of caspase-9, especially where the activation of the aforementioned effectors has presently occurred with VPA treatment. Thus, caspase-9 upregulation was evident with VPA, and further reinforced with TUNEL-assay of hepatocytes. Accordingly, multiple cellular pathways and molecular effectors accumulate, likely in an inter-dependent fashion, to orchestrate hepatic injury in response to VPA. With no rival in seen, hepatic oxidative stress stands as a likely initiator and coordinator of such events. Not surprisingly, strong antioxidants or their combinations have demonstrated considerable efficacy against VPA-evoked liver injury (Baran et al., 2006; Chang and Abbott, 2006). Currently, DHA was a superb antioxidant in the rat liver. However, beyond that, omega-3 fatty-acids displayed an array of favorable
molecular responses including suppression of prostaglandin formation, and activation of peroxisome proliferator-activated receptors (PPARs), members of the nuclear hormone receptor superfamily of ligand-dependent transcription factors, which suppress cytokine-mediated inflammation. This was documented in cell culture setups by using inhibitors of PPAR-gamma, which abolished the anti-inflammatory response to DHA and EPA fatty acids (Li et al., 2005). DHA provides anti-inflammatory effect by favoring the formation of anti-inflammatory prostaglandin E3, at the expense of inflammatory prostaglandin E2. Prostaglandin E3 also inhibits the synthesis of TNF-α and IL-1β (Maroon et al., 2010). Furthermore, Chen and colleagues also showed that DHA down-regulated MAPK, thereby alleviating rodent cholestatic liver injury (Menendez et al., 2004), a consistent observation with our current results which, altogether, substantiate an inflammatory role for ERK in liver cells in response to insults of diverse origin.

5. Conclusion

VPA stimulation of molecular triggers of oxidative stress and inflammation such as NADPH oxidase, ERK and HIF-1α exacerbates hepatic injury. The generated oxidative stress, hypoxia and inflammation orchestrate further up-regulation of COX-2 and caspase-9, thereby accentuating hepatocellular apoptosis. HIF-1α apparently assumes a dual role in response to VPA that is not only deleterious. Thus, HIF-1α stimulation of HO-1 as a downstream antioxidant enzyme is only seemingly to lessen the severity of oxidative stress, as implied by the overall cellular outcomes. DHA appreciably abated all of the VPA-evoked liver injury and blunted its evoked hepatocellular apoptosis as well. The fact that DHA possesses anticonvulsant effects (El-Mowafy et al., 2011), and is also able to mitigate liver adverse reactions to VPA, could suggest a powerful drug-combination regimen in the future management of epilepsy.

Declarations

Author contribution statement

Abdalla M. El-Mowafy: Conceived and designed the experiments; Wrote the paper.

Mohamed M. Katary: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Chelsey Pye: Performed the experiments.

Ahmed S. Ibrahim: Performed the experiments; Analyzed and interpreted the data.

Ahmed A. Elmarakby: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.
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Competing interest statement

The authors declare no conflict of interest.

Additional information

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References

Abdel-Dayem, M.A., Elmarakby, A.A., Abdel-Aziz, A.A., Pye, C., Said, S.A., El-Mowafy, A.M., 2014. Valproate-induced liver injury: modulation by the omega-3 fatty acid DHA proposes a novel anticonvulsant regimen. Drugs R D 14, 85–94.

Abedi, E., Sahari, M.A., 2014. Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. Food Sci. Nutr. 2, 443–463.

Baran, O.P., Kervancioglu, P., Akkus, M., Nergiz, Y., 2006. Ultrastructural investigation of the protective role of folic acid and vitamin E against toxic effects of valproic acid on maternal liver tissue during period of gestation. Saudi Med. J. 27, 407–409.

Bitman, M., Vrzal, R., Dvorak, Z., Pavek, P., 2014. Valproate activates ERK signaling pathway in primary human hepatocytes. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. Repub. 158, 39–43.

Chang, T.K., Abbott, F.S., 2006. Oxidative stress as a mechanism of valproic acid-associated hepatotoxicity. Drug Metab. Rev. 38, 627–639.

Chen, J.X., Zeng, H., Tuo, Q.H., Yu, H., Meyrick, B., Aschner, J.L., 2007. NADPH oxidase modulates myocardial Akt, ERK1/2 activation, and angiogenesis after hypoxia-reoxygenation. Am. J. Physiol. Heart Circ. Physiol. 292, H1664–H1674.

Cursio, R., Miele, C., Filippa, N., Van Obberghen, E., Gugenheim, J., 2008. Liver HIF-1 alpha induction precedes apoptosis following normothermic ischemia-reperfusion in rats. Transplant. Proc. 40, 2042–2045.
Einat, H., Yuan, P., Gould, T.D., Li, J., Du, J., Zhang, L., et al., 2003. The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. J. Neurosci. 23, 7311–7316.

El-Mesery, M., Al-Gayyar, M., Salem, H., Darweish, M., El-Mowafy, A., 2009. Chemopreventive and renal protective effects for docosahexaenoic acid (DHA): implications of CRP and lipid peroxides. Cell Div. 4, 6.

El-Mowafy, A.M., Abdel-Dayem, M.A., Abdel-Aziz, A., El-Azab, M.F., Said, S. A., 2011. Eicosapentaenoic acid ablates valproate-induced liver oxidative stress and cellular derangement without altering its clearance rate: dynamic synergy and therapeutic utility. Biochim. Biophys. Acta 1811, 460–467.

Engel, S.R., Creson, T.K., Hao, Y., Shen, Y., Maeng, S., Nekrasova, T., et al., 2009. The extracellular signal-regulated kinase pathway contributes to the control of behavioral excitement. Mol. Psychiatry 14, 448–461.

Farinelli, E., Giampaoli, D., Cenciarini, A., Cercado, E., Verrotti, A., 2015. Valproic acid and nonalcoholic fatty liver disease: A possible association. World J. Hepatol. 7, 1251–1257.

Gerstner, T., Bell, N., Konig, S., 2008. Oral valproic acid for epilepsy—long-term experience in therapy and side effects. Expert Opin. Pharmacother. 9, 285–292.

Hao, Y., Creson, T., Zhang, L., Li, P., Du, F., Yuan, P., et al., 2004. Mood stabilizer valproate promotes ERK pathway-dependent cortical neuronal growth and neurogenesis. J. Neurosci. 24, 6590–6599.

Hong, H., Jang, B.C., 2014. Prednisone inhibits the IL-1beta-induced expression of COX-2 in HEI-OC1 murine auditory cells through the inhibition of ERK-1/2, JNK-1 and AP-1 activity. Int. J. Mol. Med. 34, 1640–1646.

Jahromi, S.R., Togha, M., Fesharaki, S.H., Najafi, M., Moghadam, N.B., Kheradmand, J.A., et al., 2011. Gastrointestinal adverse effects of antiepileptic drugs in intractable epileptic patients. Seizure 20, 343–346.

Jeong, M.A., Lee, K.W., Yoon, D.Y., Lee, H.J., 2007. Jaceosidin, a pharmacologically active flavone derived from Artemisia argyi, inhibits phorbol-ester-induced upregulation of COX-2 and MMP-9 by blocking phosphorylation of ERK-1 and −2 in cultured human mammary epithelial cells. Ann. NY Acad. Sci. 1095, 458–466.
Ji, Q., Shi, X., Lin, R., Mao, Y., Zhai, X., Lin, Q., et al., 2010. Participation of lipid transport and fatty acid metabolism in valproate sodium-induced hepatotoxicity in HepG2 cells. Toxicol. In Vitro 24, 1086–1091.

Johannessen, C.U., Johannessen, S.I., 2003. Valproate past, present, and future. CNS Drug Rev. 9, 199–216.

Li, D., Hu, X., 2009. Fish and its multiple human health effects in times of threat to sustainability and affordability: are there alternatives? Asia Pac. J. Clin. Nutr. 18, 553–563.

Li, H., Ruan, X.Z., Powis, S.H., Fernando, R., Mon, W.Y., Wheeler, D.C., et al., 2005. EPA and DHA reduce LPS-induced inflammation responses in HK-2 cells: evidence for a PPAR-gamma-dependent mechanism. Kidney Int. 67, 867–874.

Li, Q., Fu, G.B., Zheng, J.T., He, J., Niu, X.B., Chen, Q.D., et al., 2013. NADPH oxidase subunit p22(phox)-mediated reactive oxygen species contribute to angiogenesis and tumor growth through AKT and ERK1/2 signaling pathways in prostate cancer. Biochim. Biophys. Acta 1833, 3375–3385.

Li, S., Guo, J., Ying, Z., Chen, S., Yang, L., Chen, K., et al., 2015. Valproic acid-induced hepatotoxicity in Alpers syndrome is associated with mitochondrial permeability transition pore opening-dependent apoptotic sensitivity in an induced pluripotent stem cell mode. Hepatology (Baltimore, Md) 61, 1730–1739.

Liu, M., Boussetta, T., Makni-Maalej, K., Fay, M., Driss, F., El-Benna, J., et al., 2014. Protectin DX a double lipoxygenase product of DHA, inhibits both ROS production in human neutrophils and cyclooxygenase activities. Lipids 49, 49–57.

Maroon, J.C., Bost, J.W., Maroon, A., 2010. Natural anti-inflammatory agents for pain relief. Surg. Neurol. Int. 1, 80.

Menendez, J.A., Ropero, S., Mehmi, I., Atlas, E., Colomer, R., Lupu, R., 2004. Overexpression and hyperactivity of breast cancer-associated fatty acid synthase (oncogenic antigen-519) is insensitive to normal arachidonic fatty acid-induced suppression in lipogenic tissues but it is selectively inhibited by tumoricidal alphalinolenic and gamma-linolenic fatty acids: a novel mechanism by which dietary fat can alter mammary tumorigenesis. Int. J. Onkol. 24, 1369–1383.

Moon, E.J., Sonveaux, P., Porporato, P.E., Danhier, P., Gallez, B., Batinic-Haberle, I., et al., 2010. NADPH oxidase-mediated reactive oxygen species production activates hypoxia-inducible factor-1 (HIF-1) via the ERK pathway after hyperthermia treatment. Proc. Natl. Acad. Sci. USA 107, 20477–20482.

Mottamal, M., Zheng, S., Huang, T.L., Wang, G., 2015. Histone deacetylase inhibitors in clinical studies as templates for new anticancer agents. Molecules (Basel Switzerland) 20, 3898–3941.
Pacheco, F.J., Almaguel, F.G., Evans, W., Rios-Colon, L., Filippov, V., Leoh, L.S., et al., 2014. Docosahexanoic acid antagonizes TNF-alpha-induced necroptosis by attenuating oxidative stress, ceramide production, lysosomal dysfunction, and autophagic features. Inflamm. Res. 63, 859–871.

Patrick, R.P., Ames, B.N., 2015. Vitamin D and the omega-3 fatty acids control serotonin synthesis and action, part 2: relevance for ADHD, bipolar disorder, schizophrenia, and impulsive behavior. FASEB J. 29, 2207–2222.

Pye, C., Elsherbyn, N.M., Ibrahim, A.S., Liou, G.I., Chadli, A., Al-Shabrawey, M., et al., 2014. Adenosine kinase inhibition protects the kidney against streptozotocin-induced diabetes through anti-inflammatory and anti-oxidant mechanisms. Pharmacol. Res. 85, 45–54.

Rinella, M.E., 2015. Nonalcoholic fatty liver disease: a systematic review. JAMA 313, 2263–2273.

Roma-Giannikou, E., Syriopoulou, V., Kairis, M., Pangali, A., Sarafidou, J., Constantopoulos, A., 1999. In vivo effect of sodium valproate on mouse liver. Cell. Mol. Life Sci. 56, 363–369.

Schmocker, C., Weylandt, K.H., Kahlke, L., Wang, J., Lobeck, H., Tiegs, G., et al., 2007. Omega-3 fatty acids alleviate chemically induced acute hepatitis by suppression of cytokine. Hepatology (Baltimore, Md) 45, 864–869.

Simopoulos, A.P., 2003. Essential fatty acids in health and chronic diseases. Forum Nutr. 56, 67–70.

Spiller, H.A., Krenzelok, E.P., Klein-Schwartz, W., Winter, M.L., Weber, J.A., Sollee, D.R., et al., 2000. Multicenter case series of valproic acid ingestion: serum concentrations and toxicity. J. Toxicol. Clin. Toxicol. 38, 755–760.

Tseng, Y.L., Huang, C.R., Lin, C.H., Lu, Y.T., Lu, C.H., Chen, N.C., et al., 2014. Risk factors of hyperammonemia in patients with epilepsy under valproic acid therapy. Medicine 93, e66.

van Swelm, R.P., Laarakkers, C.M., Blous, L., Peters, J.G., Blaney Davidson, E.N., van der Kraan, P.M., et al., 2012. Acute acetaminophen intoxication leads to hepatic iron loading by decreased hepcidin synthesis. Toxicol. Sci. 129, 225–233.

Wang, Z., Tsai, L.K., Munasinghe, J., Leng, Y., Fessler, E.B., Chibane, F., et al., 2012. Chronic valproate treatment enhances postischemic angiogenesis and promotes functional recovery in a rat model of ischemic stroke. Stroke 43, 2430–2436.
Wilson, G.K., Tennant, D.A., McKeating, J.A., 2014. Hypoxia inducible factors in liver disease and hepatocellular carcinoma: current understanding and future directions. J. Hepatol. 61, 1397–1406.

Wood, K.E., Mantzioris, E., Gibson, R.A., Ramsden, C.E., Muhlhausler, B.S., 2015. The effect of modifying dietary LA and ALA intakes on omega-3 long chain polyunsaturated fatty acid (n-3 LCPUFA) status in human adults: a systematic review and commentary. Prostaglandins Leukot. Essent. Fatty Acids 95, 47–55.

Yagi, Y., Fushida, S., Harada, S., Kinoshita, J., Makino, I., Oyama, K., et al., 2010. Effects of valproic acid on the cell cycle and apoptosis through acetylation of histone and tubulin in a scirrhous gastric cancer cell line. J. Exp. Clin. Canc. Res. 29, 149.

Yochum, C.L., Dowling, P., Reuhl, K.R., Wagner, G.C., Ming, X., 2008. VPA-induced apoptosis and behavioral deficits in neonatal mice. Brain Res. 1203, 126–132.

Zhang, X., Shan, P., Sasidhar, M., Chupp, G.L., Flavell, R.A., Choi, A.M., et al., 2003. Reactive oxygen species and extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase mediate hyperoxia-induced cell death in lung epithelium. Am. J. Respir. Cell Mol. Biol. 28, 305–315.

Zhu, H., Chen, X.P., Luo, S.F., Guan, J., Zhang, W.G., Zhang, B.X., 2005. Involvement of hypoxia-inducible factor-1-alpha in multidrug resistance induced by hypoxia in HepG2 cells. J. Exp. Clin. Canc. Res. 24, 565–574.

Zimmerman, H.J., Ishak, K.G., 1982. Valproate-induced hepatic injury: analyses of 23 fatal case. Hepatology (Baltimore, Md) 2, 591–597.