

TITLE PAGE

TITLE: Valproic acid-induced liver injury: A case-control study from a prospective pharmacovigilance program in a tertiary hospital

RUNNING TITLE: A case-control study to assess the risk factors of DILI by valproic acid

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What is already known about this subject

- Valproic acid (VPA) therapy is known to cause liver injury in a small percentage of patients, with some fatal outcomes.
- Mechanisms associated with an altered mitochondrial β -oxidation pathway and excessive oxidative stress have been postulated.
- Certain risk factors (such as polytherapy and younger age) could determine the chance of developing hepatotoxicity.

What this study adds

- A greater characterisation of VPA hepatotoxicity (e.g., predominance of hepatocellular vs. cholestatic hepatitis).
- A number of other risk factors appear to be related to VPA-induced liver injury, such as alcohol consumption, seizures and increased baseline LDH levels.
- Considering these factors, patients at higher risk of liver injury should be identified and closely monitored.

ABSTRACT

Introduction: Valproic acid (VPA) is an antiepileptic drug extensively used for treating partial and generalised seizures, acute mania and as prophylaxis for bipolar disorder. Drug-induced liver injury (DILI) persists as a significant issue related to fatal outcomes by VPA. The aim of this study was to increase our knowledge about this condition and to better identify patients affected.

Methods: We conducted an observational retrospective case-control study that identified cases of DILI by VPA from the Pharmacovigilance Programme from our Laboratory Signals at La Paz University Hospital from January 2007 to December 2019. From the Therapeutic VPA Monitoring program, two control groups were assigned, VPA-tolerant patients and the other with patients who developed mild VPA-related hepatitis but who did not meet the DILI criteria, matched for date, age and sex.

Results: A total of 60 patients were included in the study: 15 cases of DILI, 30 VPA-tolerant controls and 15 controls with mild hepatitis. Mean age for the cases was 45.7 years, 4(26.7%) were women and 5(33.34%) were children under 18 years, of them 3(20%) were fatal. Polytherapy with other antiepileptic drugs ($p=0.047$) and alcohol consumption ($p<0.001$) were associated with a greater risk of developing DILI by VPA. A diagnosis of epileptic seizure was more frequently related to DILI when compared with the VPA-tolerant controls ($p<0.001$). The cases developed hepatocellular hepatitis ($p<0.001$), while the mild hepatitis controls had a higher rate of cholestatic hepatitis ($p<0.001$). The laboratory lactate dehydrogenase values were statistically higher (even at baseline) in patients with DILI than in both control groups ($p=0.033$ and $p=0.039$).

Conclusions: VPA hepatotoxicity remains a considerable problem. This study offers interesting findings for characterising VPA-induced liver injury and at-risk patients.

Valproic acid (VPA) therapy is known to cause liver injury (DILI). Polytherapy with other antiepileptic drugs, alcohol consumption, a diagnosis of epileptic seizure, and the lactase dehydrogenase high values were associated to DILI by VPA.

INTRODUCTION

Valproic acid (VPA) is a branched short-chain carboxylic acid, established as a first-line and widely used antiepileptic agent, with a broad spectrum of activity. VPA is employed to treat partial and generalised seizures in adults and children and is considered effective against generalised tonic-clonic absences, myoclonic and partial epileptic seizures, with or without secondary generalisation. Intravenous valproate has also been shown to be useful in treating status epilepticus. In addition, it can be considered for controlling acute mania and as prophylaxis for bipolar disorder and migraine headaches.^{1,2}

The clinical properties of VPA were discovered in 1962 when it was tested as a solvent for other compounds (khellin derivatives) that were being investigated for potential anticonvulsant activity. The first clinical trial on epilepsy using the sodium salt of valproic acid was reported in 1964. It was introduced into clinical practice in 1967 as *Depakine* in France, in 1973 in the United Kingdom and approved by the US Food and Drug Administration (FDA) in 1978.^{1,3,4,5}

A broad range of mechanisms has been reported to participate in the antiepileptic and mood-stabiliser mechanisms of VPA (**Fig. 1**); however, its precise mode of action has not been fully elucidated. Valproate increases the availability of synaptic gamma-aminobutyric acid (GABA) in both presynaptic and postsynaptic mechanisms. The inhibitory activity of GABA is thereby enhanced and facilitates GABA-mediated responses in specific brain regions thought to be involved in controlling seizure generation and propagation.^{3,6}

Other studies have shown that VPA reduces the glutamatergic N-methyl-D-aspartate (NMDA) and kainate-induced excitatory responses within the medial prefrontal cortex, suggesting that attenuation of NMDA receptor-mediated excitation is an essential mode of action for valproate's anticonvulsant effect.^{7,8,9}

Valproate's action on neuronal firing is concentration and activity dependent. This effect, critical for the anticonvulsant activity, could be explained by the direct effect on excitable membranes, which is related to the modulation of sodium, calcium and potassium channels, especially with an use-dependent decrease in inward sodium currents. Specifically, VPA blocks both persistent and fast sodium currents.^{3,7} VPA has also demonstrated an increase in extracellular levels of serotonin or 5-hydroxytryptamine (5-HT) and dopamine (DA) in the hippocampus and striatum, a change that does not appear to be related to the antiepileptic effect. In contrast, the

modulation of serotonergic and dopaminergic complexes has revealed to be relevant for the antipsychotic and neuropsychiatric actions of VPA.^{3,10}

Although VPA therapy has an adequate safety profile, being effective and generally well tolerated, there is a small group of patients who experience hepatotoxic reactions to this therapy, reactions that have proved to be fatal, especially in those younger than 2 years of age and those undergoing polytherapy.^{11,12} Valproic acid hepatotoxicity has been associated with the formation of VPA-reactive metabolites, the inhibitory effect on the mitochondrial β -oxidation pathway, excessive oxidative stress and genetic variants of certain enzymes such as mitochondrial carbamoyl-phosphate synthase I (CPS1), mitochondrial enzyme polymerase gamma (POLG), glutathione S-transferase (GSTs), mitochondrial superoxide dismutase 2 (SOD2), uridine diphosphate-glucuronosyltransferase (UGTs) and cytochromes P450 (CYPs) genes. Genetic and congenital metabolic errors involving mitochondrial fatty acid oxidation or the electron transport chain can raise the risk of VPA-induced hepatotoxicity. In particular, acute liver failure and death from valproate-induced liver injury have been reported more frequently in patients with inherited neurometabolic syndromes caused by mutations in the gene encoding POLG (e.g., Alpers-Huttenlocher syndrome). The product summary for VPA formulations warns that POLG-related disorders should be suspected in patients with a family history or symptoms that indicate a POLG-related disorder, including but not limited to idiopathic encephalopathy, refractory epilepsy (focal, myoclonic), status epilepticus as the initial clinical picture, developmental delays, psychomotor regression, axonal sensorimotor neuropathy, myopathy, cerebellar ataxia, ophthalmoplegia and complicated migraine with occipital aura. POLG mutation testing should be performed in accordance with current clinical practice for the diagnostic evaluation of such disorders.⁵ Carnitine supplementation and antioxidant administration proved to be positive treatment strategies for VPA-induced hepatotoxicity.^{13,14} Despite these warnings, VPA hepatotoxicity continues to occur.

The Pharmacovigilance Programme from Laboratory Signals at a Hospital (PPLSH) is a programme based on the systematic detection of predefined abnormal laboratory values (automatic laboratory signals [ALS]), using the hospital's laboratory information system. PPLSH has proven to be useful for the early detection and evaluation of specific serious adverse drug reactions (SADRs) associated with increased morbidity, mortality and hospital stays, as well as for gathering the detailed information necessary to study the risk factors associated with these SADRs.¹⁵

The purpose of this study was to analyse patients who had already developed serious valproate-related hepatotoxicity detected by the PPLSH and compare them with VPA-tolerant subjects and with those patients who experienced a slight increase in transaminase levels but developed tolerance despite continuing the VPA therapy. Characterising these patients will provide more information on their clinical features, medical data and concomitant related drugs that could lead to an increased susceptibility to serious VPA hepatotoxicity and will specifically help identify those patients at risk when starting VPA treatment.

MATERIAL AND METHODS

Setting

We conducted an observational retrospective case-control study at the La Paz University Hospital in Madrid, Spain, a tertiary-care teaching facility that provides health care to a catchment area of 527,366 inhabitants in the north of Madrid. The Clinical Pharmacology Department performs all requests for therapeutic drug monitoring for the hospital and its healthcare area.

Definition of cases and controls

Cases were identified from the PPLSH of La Paz University Hospital from January 2007 to December 2019. The PPLSH is based on the proactive detection of abnormal laboratory parameters as possible indicators of SADRs. All recognised ALS are studied in order to determine their causality.

Case definition

For the detection of drug-induced liver injury (DILI), we considered an increase in alanine aminotransferase (ALT) levels 5 times the upper limit of normal (x5 ULN). Based on the 2011 recommended modified biochemical criteria for identifying DILIs by the International Serious Adverse Events Consortium (iSAEC), we defined DILI when any of the following items were met: (1) ALT levels ≥ 5 ULN; (2) alkaline phosphatase (ALP) levels ≥ 2 ULN, especially in patients with elevated 5'-nucleotidase or gamma-glutamyl transferase (GGT) levels and with no bone-disease-related increase in ALP levels; and (3) ALT levels ≥ 3 ULN and total bilirubin (TB) levels ≥ 2 ULN.^{16,17} The procedure for DILI detection, evaluation and classification has been described elsewhere.¹⁵ Briefly, in phase I, on-file laboratory data at admission or during the hospital stay were screened for an ALT signal 7 days a week, 24 hours per day. In phase II, the patients were identified to avoid duplicates, and electronic medical records were reviewed. In phase III, a case-by-case study was performed for the remaining cases. The cause and type of hepatitis were established according to the iSAEC consortium. The causality assessment of drugs was performed using the Roussel Uclaf Causality Assessment Method (RUCAM).^{18,19}

Control definition

Every identified case was matched with two VPA-tolerant controls, patients who underwent VPA therapy without developing valproate-related ADRs or any kind of previous liver disease. For this purpose, we selected individuals from the Therapeutic VPA Monitoring Programme (*Labtrack* program, TrackHealth, Woolloomooloo, Australia) where requests for serum valproate concentrations are registered and processed by the Clinical Pharmacology Department. These controls were paired by sex and age groups (newborn, <28 days; infant, 1–23 months; preschool, 2–4 years; infancy, 5–13 years; adolescence, 14–17 years; young adult, 18–35 years; adult, 36–65 years; and older adults, >65 years). Subjects should have received VPA therapy for at least one year.

Each case was also matched with one control patient who developed mild VPA-related ALT elevation (ALT levels >1–5 ULN). Subjects were also paired by sex and group age. The only exclusion criterion was the presence of liver disease from any cause other than VPA therapy.

A total of 168 variables were collected for each case and control, including clinical variables such as family and medical history, the reason for prescribing VPA or concomitant treatment, patient characteristics (sex, age, weight, etc.), laboratory data (ALT, aspartate transaminase, bilirubin (AST), prothrombin time, etc.) and serum VPA concentration.

For the case report form, onset date was defined as the first day on which the patient presented any abnormal liver value (AST, ALT, ALP or GGT). The baseline value was considered as the last value obtained prior to the onset date. The peak value was considered the maximum value reached after the onset date. The recovery value was the first laboratory value within the normal range after the onset date or the last available value.

Data analysis

Sample size calculation

Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a bilateral contrast, 15 cases and 45 controls are required to detect a minimum odds ratio of 8. It is assumed that the rate of exposed in the control group will be 0.35-0.4. A follow-up loss rate of 0% has been estimated. The POISSON approximation has been used.²⁰

Statistical analysis

Frequency results are expressed in absolute terms as percentages, and the continuous variables are presented as mean (standard deviation, SD) or median (range) according to the normality test (Kolmogorov-Smirnov test). To estimate the differences between variables, a chi-squared test was employed for the categorical variables and Student's t-test for the continuous parametric variables. In case of not following a normal distribution, non-parametric tests have been used (Mann-Whitney's U or Kruskal Wallis test, as appropriate).

We performed a multiple logistic regression based on 1000 bootstrap samples to estimate the standard error and confidence intervals for the coefficient of determination (pseudo-R²). Data was analyzed using the statistical analysis software SPSS 20.0 (IBM Corporation, Armonk, NY, USA).

Ethical statement

The study was approved by the research ethics committee of La Paz University Hospital of Madrid (PI-3970). The requirement for informed consent was waived due to retrospective data collection. The programme was conducted according to the Spanish Personal Data Protection Law.²¹

RESULTS

A total of 60 patients were included in the study: 15 cases of DILI, 30 VPA-tolerant controls and 15 controls with mild hepatitis. The mean (SD) age for the cases was 45.7 (30.8) years, out of them 66.7% were adults, and only 26.7% were women. There were no statistically significant differences in age, weight, height and body mass index (BMI) between any of the studied groups ([Table 1](#)).

In terms of family history, there was the interesting presence of an undiagnosed neurodegenerative disorder in a patient from the case group. Moreover, the presence of hydrocephalus (6.7% vs. 0%, $p = 0.012$), focal motor epilepsy (20% vs. 13.3%, $p = 0.05$) and Lennox-Gastaut syndrome (6.7% vs 0%, $p = 0.012$) was greater in the cases than in the mild hepatitis group. There was an increased presence of generalised epilepsy (26.7% vs. 40%, $p = 0.035$) and bipolar disorder (6.7% vs 13.3%, $p = 0.09$) in the patients with mild ALT elevation than in the cases. Compared with the cases, the VPA-tolerant control group had a higher prevalence of bipolar disorder (6.7% vs. 20%, $p = 0.003$) but a lower rate of focal motor epilepsy (20% vs 10%, $p = 0.047$) ([Table 1](#)).

The patients who developed mild ALT elevation showed more previous drug allergies (6.7% vs. 26.6%, $p < 0.001$), while there was no statistically significant difference between the cases and the healthy control group (6.7% vs 10%, $p = 0.3$). There were also no statistically significant differences in the number of concomitant drugs in any of the study groups. There was a higher prevalence of alcoholism among the patients who developed DILI compared with those with mild hepatitis or no ADR (53.3% vs. 13.3% vs. 3.3%, $p < 0.01$) ([Table 1](#)).

The indication for VPA therapy, showed a major presence of seizures among the cases than in the VPA-tolerant controls (86.7% vs 66.7%, $p < 0.01$), but there were no differences between the cases and the mild hepatitis controls. Drug interactions were also more severe (red: 46.7% vs 13.3% vs. 13.3, $p < 0.01$) in the medicines prescription for the patients with DILI than in the patients with mild ALT elevation and the VPA-tolerant group ([Table 1](#)).

There were found disparities in the type of hepatitis, with hepatocellular hepatitis more common among the cases and cholestatic hepatitis more frequent among the patients who developed mild hepatitis. There were no differences in the mixed type of hepatitis. The time between the start of treatment with VPA and the onset of hepatitis was significantly shorter in the cases (61.6 vs. 304 days, $p < 0.01$), and the DILI duration until the recovery of the laboratory

values was also shorter in the cases (18.7 vs. 204.4 days, $p = 0.02$). The hepatitis outcomes also showed statistically significant differences; as expected, there were more deaths in the cases group than in the mild hepatitis control group ([Table 1](#)).

Regarding the laboratory variables, there was a higher elevation of lactate dehydrogenase (LDH) levels in all phases (baseline, onset, peak and recovery) in the cases compared with the mild hepatitis group ([Table 1](#)).

Finally, the multiple logistic regression ([Table 2](#)) showed that, among the toxics, alcohol achieved a statistically significant difference ($p < 0.001$; confidence interval, 6.17–8.44). The use of concomitant medication was also significant ($p = 0.001$), especially the use of concomitant antiepileptics ($p < 0.001$). There was a statistically significant difference in the major indication of VPA therapy for epilepsy ($p = 0.002$). The LDH elevation in the cases versus the control groups showed a confidence interval of 1.18–1.64 ($p = 0.02$).

DISCUSSION

This study presents the data reported during the last 13 years from La Paz University Hospital, a total of 15 cases of VPA-induced DILI, covering all ages.

The importance of polytherapy in the development of DILI observed in the study should be considered for all patients undergoing VPA therapy. This correlation between liver damage caused by VPA and co-medication was corroborated by the chi-squared test and the multiple logistic regression and is consistent with the available literature.^{22,23}

There is conflicting information regarding the sex distribution of DILI reported in the literature, with a number of articles indicating a slight female predominance (58.47% and 53%),^{21,23} while other studies have observed a propensity for the male sex (54%).²³ This study found a greater incidence of liver injury in the male population (73.3%). Although another published article reported similar data (75%),²⁵ these observations could be explained by simple chance when employing a small sample size. If we look at series with large numbers of collected cases, the sex ratio tends to be more balanced (58.47% and 46% of female patients).^{22,23}

A strong association between alcohol consumption and VPA-induced hepatitis is exposed in the study. This observation has been mentioned in some published articles related with hyperammonaemia^{13,26} but it is not usually described in published case reports,^{24,25,27} even in larges series.^{22,23} Given the known mechanism of hepatotoxicity of alcohol, this association seems biologically plausible, although further studies should be needed in order to confirm the relevance of this finding.

In our study, there was no correspondence between BMI and DILI predisposition. The cases presented an in-range BMI (23.1), similar to those with mild ADR (22.7) and the VPA-tolerant controls (24.7), with no statistically significant differences. Based on these observations, the patients with abnormal BMI (underweight or overweight) should not be considered at greater risk of VPA-induced DILI.

The liver damage duration was found to be shorter in the cases than in the mild ADR controls, which could be explained by the discontinuation of VPA treatment in all of the cases due to increased severity, while most of the control patients continued VPA therapy until tolerance was developed.

The indication for VPA therapy differed among the cases and controls. Bipolar disorder was more frequently the indication in the VPA-tolerant group, while epilepsy was more recurrent in the cases than in both control groups, presumably due to the disease's nature, lower need for co-medication and lower dosages employed in bipolar therapy. Patients with seizures undergoing VPA treatment should therefore be closely monitored.

Among the laboratory values, LDH elevation and VPA concentrations were of special interest. There was a statistically significant difference in VPA concentrations between certain measurements in each group, although this difference should not be considered clinically relevant. All of the mean VPA blood concentrations in this study were within the classical therapeutic range of 50–100 ug/mL,^{28,29,30} which suggests that although high VPA levels could be involved in the development of acute hepatitis, personal predisposing factors are significantly involved in hepatitis, with minor effect of VPA blood concentration levels. Therefore, the identification of these factors takes on major importance.

The LDH measurements showed constantly higher levels (even at baseline) in the cases with DILI than in both control groups. Given that LDH is considered a good indicator of cellular damage and necrosis in many cell lines,^{31,32} this increase could be due to a greater predisposition for cell injury and destruction of any nature in patients who developed drug-induced hepatitis and might be an interesting marker for DILI susceptibility.

The study's limitations included the small sample size used compared with other large case series registered, such as the series by Schmid et al. (2012) that collected all cases (n=132) of serious VPA-related hepatic adverse effects reported to the German Federal Institute for Drugs and Medical Devices between 1993 and 2009.²² There is also the publication of the individual case safety reports (ICSRs) to the World Health Organisation's global database *VigiBase*, which recorded 268 ICSRs related to VPA and fatal outcomes in children 17 years or younger, reported from 25 countries since 1977 to June 2013, with a total of 156 hepatotoxicity-related deaths.²³ These studies contribute to the characterisation of ADRs caused by VPA, whereas most other articles in the literature are limited to case reports. Our article could offer greater comprehension of the subject by presenting a case-control study with two different control groups.

The study's methodological limits are our sources of case data. It is possible that the PPLSH lost some DILI cases during the data collection process. Although this number should be low, given that the alternative causes for DILI presented are well known, we cannot rule out a possible

underestimation of cases. As a pharmacovigilance study, it is retrospective which could affect to the quality of the data, as it was based on previously registered information susceptible of biases. The single-centre design was another limitation; however, La Paz University Hospital is an important reference centre for pharmacovigilance in Spain.

CONCLUSIONS

Based on the findings and limitations of this observational retrospective study, certain considerations should be considered before starting VPA therapy to prevent the onset of hepatitis. In-range VPA blood concentrations and the absence of BMI-related risk factors are not sufficient determinants for preventing liver damage. Co-medication, seizure indication, known alcohol consumption and increased baseline LDH levels are important factors that should be closely monitored to improve the safety profile of this antiepileptic drug.

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Disclosure

The authors declare that they have no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author's contributors

- Participated in research design: ER, ESM.
- Data curation: MUE, AMB.
- Participated in data analysis: ER, MUE.
- Participated in the writing of the paper: ESM.
- Writing – review and editing: AMB, ER.

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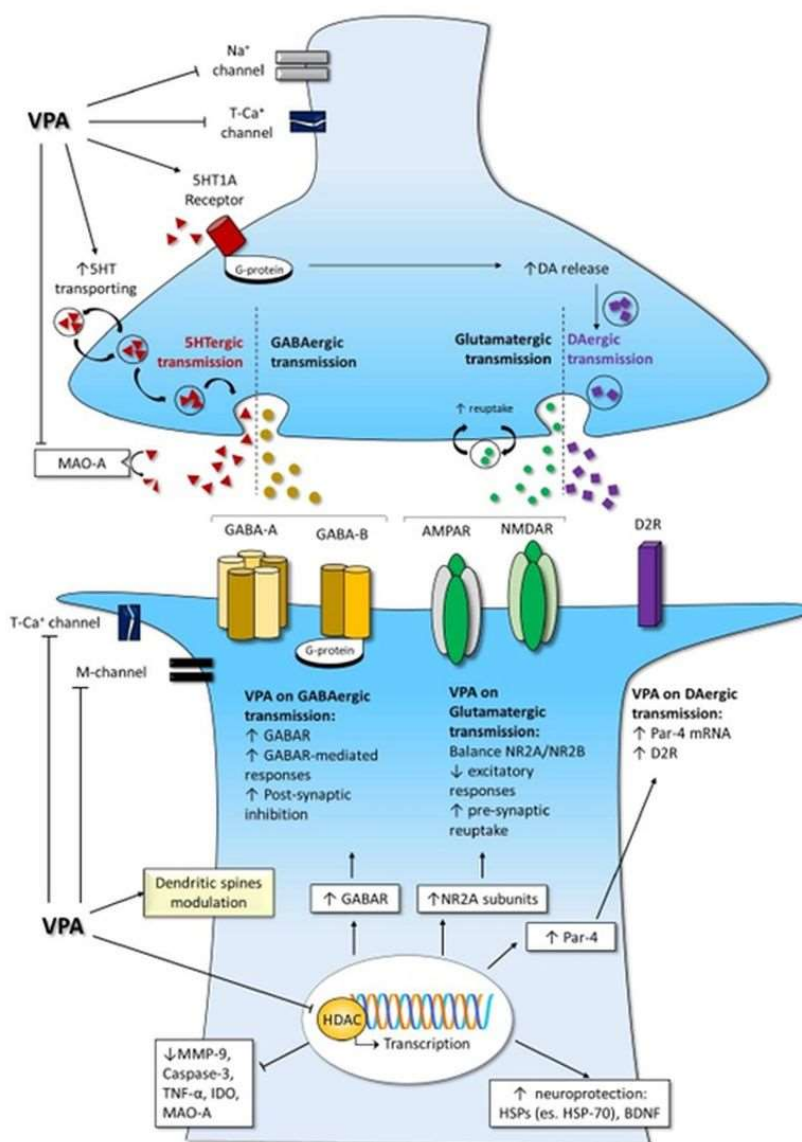
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Figure. Valproate effects on synaptic cleft and intracellular pathways.

Reprinted from Romoli M et al (2019).³ Legend: Valproate effects on synaptic cleft and intracellular pathways. The increase in GABAergic transmission pairs with a modulation of other aminergic systems; the excitatory ionic channels, such as calcium and sodium channels, are negatively modulated, while potassium currents are enhanced. Moreover, epigenetic mechanisms influence receptor expression and neuroprotective pathways. Abbreviations: AMPAR, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid –AMPA- receptor; BDNF, brain-derived neurotrophic factor; DA, dopamine; D2R, dopamine D2 receptor; GABA, gamma aminobutyric acid; GABAR, GABA receptors; GABA-A, GABA receptor type A; GABA-B, GABA receptor type B; HSPs, heat shock proteins; HSP-70, heat shock protein 70; K⁺, potassium; IDO, indoleamine 2,3 dioxxygenase; MAO-A, monoamines oxidase type A; M-channel, low-threshold noninactivating voltage-gated potassium current; MMP-9, metalloproteinases-9; Na⁺, sodium; NMDAR, N-methyl-D-aspartate -NMDA- receptor; NR2A, 2A subunit of NMDAR; NR2B, 2B subunit of NMDAR; Par-4, prostate apoptosis response-4; T-Ca⁺ channel, low-threshold T-calcium channel; TNF- α , tumour necrosis factor α ; VPA, valproate; 5HT, serotonin.



Tables

Table 1. Characteristics of cases DILI by VPA compared with mild hepatitis controls and with VPA-tolerant controls.

	Cases DILI (n=15)	Mild hepatitis controls (n=15)	Cases vs. mild hepatitis controls p value	VPA-tolerant controls (n=30)	Cases vs. VPA-tolerant controls p value
Age, years*	45.73 (30.8)	46.2 (31.1)	0.967	44.70 (29.8)	0.840
Adults, n (%)	10 (66.7)	10 (66.7)	1.00	20 (66.7)	1.00
Median age, years (range)	68 (27–85)	69 (19–80)	0.977	68.5 (32–77)	0.890
Children, n (%)	5 (33.39)	5 (33.39)	1.00	10 (33.3)	1.00
Median age, years (range)	9 (2–17)	9 (3–17)	0.977	9 (2–16)	0.89
Women, n (%)*	4 (26.7)	4 (26.7)	1.00	8 (26.7)	1.00
Weight, kg	58.1 (25.4)	54 (25)	0.662	58.0 (23.3)	0.580
Height, cm	153 (32)	143.7 (24.2)	0.647	155.5 (11.2)	0.18
BMI, kg/m ²	23.1 (6.8)	22.7 (5.6)	0.918	24.7 (4.2)	0.16
Family history					
Neurodegenerative disorder without diagnosis	1 (6.7)	0	0.012	0	0.012
Medical history, n (%)					
CNS mass	4 (26.7)	5 (33.3)	0.28	7 (23.3)	0.623
Hydrocephalus	1 (6.7)	0	0.012		
Dravet syndrome	1 (6.7)	1 (6.7)	1		
Generalised epilepsy	4 (26.7)	6 (40.0)	0.035	8 (26.7)	1
Focal motor epilepsy	3 (20)	1 (6.7)	0.003	3 (10)	0.047
Bipolar disorder	1 (6.7)	2 (13.3)	0.091	6 (20)	0.003
Lennox Gastaut syndrome	1 (6.7)	0	0.012	1 (3.3)	0.31

Myoclonic epilepsy				1 (3.3)	
Schizoaffective disorder / schizophrenia				3 (10)	
Temporal seizures				1 (3.3)	
Previous drug allergies	1 (6.7)	4 (26.6)	<0.001	3 (10)	0.3
Indication of VPA therapy					
Seizures	13 (86.7)	14 (93.3)	0.111	20 (66.7)	<0.001
Bipolar disorder	2 (13.3)	1 (6.7)		10 (33.3)	
VPA dosage, mg/kg/day	26.4 (13.5)	24.5 (24.0)	0.795	21.7 (11.1)	0.213
Treatment time to ADR, days	61.6 (73.8)	304 (309)	<0.001	NA	
Toxics, n (%)					
Alcohol	8 (53.3)	2 (13.3)	<0.001	1 (3.3)	<0.001
Tobacco	2 (13.3)	2 (13.3)	1	6 (20)	0.18
Alcohol and Tobacco	1 (6.7)	1 (6.7)	1	1 (3.3)	0.31
Concomitant medication	4.27 (3.1)	4.2 (3.6)	0.957	3.6 (2.8)	0.759
Concomitant antiepileptics, median (range)	2 (0–3)	1 (0–2)	0.047	2 (0–3)	0.049
Interactions [#]					
Red	7 (46.7)	2 (13.3)	<0.001	4 (13.3)	<0.001
Yellow	9 (60)	2 (13.3)	<0.001	4 (13.3)	<0.001
Green	3 (20)	0	<0.001	0	<0.001
Hepatitis					
Hepatocellular	11 (73.3)	1 (6.7)	<0.001	NA	
Mixed	2 (13.3)	2 (13.3)	1	NA	
Cholestatic	1 (6.7)	8 (53.3)	<0.001	NA	
Severity					
Acute hepatitis	7 (46.6)	15 (100)	<0.001	NA	
Severe liver dysfunction (INR >1.5)	1 (6.7)	-		NA	
Acute liver failure (INR > 1.5) and...				NA	
Encephalopathy G1	6 (40)	-		NA	

Encephalopathy G2	0	-		NA	
Encephalopathy G3	1 (6.7)	-		NA	
Encephalopathy G4	0	-		NA	
Hepatitis duration, days	18.7 (13.9)	204.4 (241.5)	0.02	NA	
Outcome, n (%)					
Recovered	12 (80)	12 (80)	1	NA	
Death	3 (20)	1 (6.7)	0.003	NA	
Nor recovered	0	2 (13.3)	0.012		
Laboratory Data					
Baseline					
ALT, UI/L	23.9 (11.6)	23 (17.9)	0.877	16.8 (10.1)	0.706
AST, UI/L	22.92 (8.1)	28.7 (16.4)	0.28	21.14 (9.6)	0.637
GGT, UI/L	54.6 (57.1)	42 (32.5)	0.528	32.1 (35.0)	0.041
ALP, UI/L	85.4 (54.3)	94.1 (38.2)	0.7	91.27 (57.5)	0.737
TB, mg/dL	0.6 (0.3)	0.5 (0.3)	0.537	1.7 (5.9)	0.214
PA, %	82.5 (17.5)	76.8 (33.9)	0.766	84.7 (26.3)	0.43
LDH, UI/L	332.8 (54.7)	226.5 (26.0)	0.033	202.2 (66.6)	0.039
Cr, mg/dL	0.79 (0.79)	1.0 (0.62)	0.21	0.71 (0.30)	0.108
Albumin, g/dL	4.4 (1.4)	9.5 (17.0)	0.409	3.7 (0.7)	0.444
Protein, g/dL	5.7 (2.0)	6.8 (0.9)	0.922	11.2 (16.1)	0.121
Ammonium, µmol/L	58 (-)	-	-	-	-
Onset					
ALT, UI/L	435 (347.3)	199.9 (386.6)	0.025	NA	
AST, UI/L	445.8 (332.7)	163.3 (404.5)	0.009	NA	
GGT, UI/L	207.2 (94.9)	77 (87.3)	0.042	NA	
ALP, UI/L	166 (193.4)	146.9 (103.2)	0.824	NA	
TB, mg/dL	1.6 (1.8)	0.6 (0.2)	0.036	NA	
PA, %	74.4 (28.7)	85.3 (16.0)	0.37	NA	

LDH, UI/L	580.0 (36.1)	427.6 (35.4)	0.009	NA	
Cr, mg/dL	0.82 (0.48)	0.75 (0.54)	0.668	NA	
Albumin, g/dL	3.4 (0.6)	2.8 (1.5)	0.374	NA	
Protein, g/dL	5.8 (0.5)	5.7 (2.1)	0.336	NA	
Ammonium, μmol/L	62 (20–92)**	201 (-)	0.013	NA	
Peak					
ALT, UI/L	731.9 (334.7)	231.79 (302.9)	0.005	NA	
AST, UI/L	811.1 (222.7)	162.46 (386.4)	0.023	NA	
GGT, UI/L	1083.7 (259.1)	140.1 (139.1)	<0.001	NA	
ALP, UI/L	194.5 (146.3)	146 (104.3)	0.385	NA	
TB, mg/dL	4.5 (7.7)	0.5 (0.5)	0.002	NA	
PA, %	64.88 (32.8)	81.3 (8.7)	0.147	NA	
LDH, UI/L	651.8 (49.7)	512.5 (31.9)	0.005	NA	
Cr, mg/dL	1.04 (0.67)	0.82 (0.55)	0.668	NA	
Albumin, g/dL	3.1 (0.6)	2.7 (1.3)	0.362	NA	
Protein, g/dL	5.2 (0.8)	6.0 (2.1)	0.245	NA	
Ammonium, μmol/L	142.5 (75–432)**	-	-	NA	
Recovery					
ALT, UI/L	77.4 (106.1)	57.7 (98.1)	0.64	NA	
AST, UI/L	56.3 (63.2)	30.4 (14.1)	0.219	NA	
GGT, UI/L	89.6 (88.7)	76.1 (126.9)	0.342	NA	
ALP, UI/L	109.6 (66.0)	71.8 (38.9)	0.222	NA	
TB, mg/dL	7.1 (17.1)	1.2 (2.2)	0.242	NA	
PA, %	84.8 (20.0)	95.3 (8.7)	0.154	NA	
LDH, UI/L	246.4 (106.7)	255.3 (76.7)	0.861	NA	
Cr, mg/dL	0.84 (0.45)	0.79 (0.30)	0.411	NA	
Albumin, g/dL	3.6 (0.6)	3.8 (0.5)	0.401	NA	
Protein, g/dL	6.4 (0.5)	6.2 (2.1)	0.893	NA	

Ammonium, $\mu\text{mol/L}$	101 (66–116)**	-	-	NA	
VPA concentration					
VPA 1, ug/mL	59.4 (28.3)	59.6 (20.6)	0.985	57.9 (20.8)	0.003
Time since onset, days	-38 (-12)	-49 (19)	0.122	-31 (12)	0.437
VPA 2, ug/mL	56.3 (22.2)	58.3 (27.5)	0.92	57.7 (26.5)	0.152
Time since onset, days	-1.17 (3.7)	10 (13)	0.097	9 (9)	0.897
VPA 3, ug/mL	51.0 (16.8)	76.3 (19.4)	0.018	61.6 (16.2)	0.014
Time since onset, days	5 (9.8)	44.8 (57.3)	0.004	34.6 (67.1)	0.09
Values are presented as mean (\pm standard deviation) unless otherwise noted. (*) Matching variables. (**) sig. $P=0.043$. (#) Ref. iDOctus v2.3.401 (1) ESGPr23401001, last update 2020/03/21, 21:46. Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate transaminase; BMI, body mass index; CNS, central nervous system; Cr, creatinine; GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; PA, prothrombin activity; TB, total bilirubin; VPA, valproic acid; SD, standard deviation; NA, not applicable.					

Table 2. Significant factors and covariates in the multiple logistic regression for drug-induced liver injury.

	Pseudo-OR*	95% BCa CI	P
Toxics (alcohol)	7.30	6.17 – 8.44	<0.001
Co-antiepileptics, n	4.33	2.57 – 6.09	<0.001
Indication (Epilepsy)	2.34	1.94 – 2.74	0.002
Co-medication, n	1.58	1.39 – 1.77	0.001
Lactate dehydrogenase, IU/L	1.41	1.18 – 1.64	0.02
Pseudo R ² = 0.908			

*Based on 1000 bootstrap samples; Abbreviations: BCa, bias corrected and accelerated; n, number; OR, odds ratio