

## Comparison of Serum Total Valproic Acid Levels and %CDT Values in Chronic Alcohol Addictive Patients in an Italian Clinic: A Retrospective Study

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### Abstract

**Background** Valproate is a broad-spectrum anti-convulsant that is effective in the treatment of tonic-clonic, myoclonic and absence seizures as well as in partial seizures as a second-line drug. It has been widely demonstrated in the literature that the effect of valproate on type-A  $\gamma$ -aminobutyric acid (GABA-A) receptors may reduce relapse to ethanol abuse. This retrospective study evaluated a 3-year period in which 42 patients from the Department of Alcoholism and Substance Abuse (DASA) were treated with valproate.

**Objectives** We compared different serum total valproic acid (VPA) concentrations, and the effectiveness of this drug in maintaining alcohol abstinence was evaluated by percentage of carbohydrate deficient transferrin (%CDT) values.

**Method** CDT is a biochemical marker used for identifying regular high alcohol consumption and monitoring

abstinence in outpatients during treatment. Serum concentrations of valproate were divided into four groups: <10, 10–30, 31–50, and >50  $\mu\text{g}/\text{mL}$ .

**Results** This study shows that a mean serum total VPA concentration >30  $\mu\text{g}/\text{mL}$  is more effective in maintaining alcohol abstinence than a lower one ( $p < 0.05$ ). In this study, mean serum total VPA concentrations between 31 and 50  $\mu\text{g}/\text{mL}$  showed the same effectiveness as higher ones (>50  $\mu\text{g}/\text{mL}$ ); in fact, there was no significant difference in mean %CDT values between these two groups ( $p > 0.05$ ). After at least 12 months' treatment with valproate, mean platelet counts increased by  $12 \times 10^3/\mu\text{L}$  compared with baseline ( $254 \pm 63$  vs  $242 \times 10^3/\mu\text{L}$ ,  $p > 0.05$ , respectively) in patients with mean serum total VPA levels <10  $\mu\text{g}/\text{mL}$ ; increased by  $8 \times 10^3/\mu\text{L}$  from baseline ( $253 \pm 59$  vs  $245 \times 10^3/\mu\text{L}$ ,  $p > 0.05$ , respectively) in patients with levels between 10 and 30  $\mu\text{g}/\text{mL}$ ; decreased by  $2 \times 10^3/\mu\text{L}$  from baseline ( $265 \pm 63$  vs

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$267 \times 10^3/\mu\text{L}$ ,  $p > 0.05$ , respectively) in patients with levels between 31 and 50  $\mu\text{g/mL}$ , and decreased by  $48 \times 10^3/\mu\text{L}$  from baseline ( $215 \pm 56$  vs  $263 \times 10^3/\mu\text{L}$ ,  $p < 0.05$ , respectively) in patients with levels  $>50 \mu\text{g/mL}$ . **Conclusion** A mean serum total concentration lower than the currently accepted therapeutic level (50–100  $\mu\text{g/mL}$ ) may have the same effectiveness in maintaining alcohol abstinence with a lower risk of presenting side effects.

### Key Points

Mean total serum valproic acid levels between 31 and 50  $\mu\text{g/mL}$  appear to be as effective in maintaining alcohol abstinence as the currently accepted therapeutic range of 50–100  $\mu\text{g/mL}$ .

Patients with total serum valproic acid levels between 50 and 100  $\mu\text{g/mL}$  had significantly reduced platelet counts compared with patients with serum total valproic acid levels  $<30 \mu\text{g/mL}$ .

## 1 Introduction

Valproate is a broad-spectrum anticonvulsant that is effective in the treatment of tonic-clonic, myoclonic, tonic and absence seizures and also in partial seizures as a second-line drug [1, 2]. Valproate is currently the most frequently prescribed antiepileptic drug in the world [3]. It has been approved for the treatment of bipolar disorders and migraine [4–7], and is also administered as an anxiolytic compound [8–10]. The therapeutic mechanism of valproate is still unclear. Its pharmacological effects involve a variety of mechanisms including suppression of *N*-methyl-D-aspartate (NMDA)-evoked transient depolarization in the rat neocortex in vitro [11]; blockade of voltage-gated sodium channels and modulation of dopaminergic and serotonergic transmission [7]; and enhancement of type-A  $\gamma$ -aminobutyric acid (GABAA) receptor activation by increasing brain GABA levels. Furthermore, valproate activates the GABA-related, glutamic acid decarboxylase, and inhibits the GABA degradative enzymes GABA aminotransferase [12–14] and succinate semialdehyde dehydrogenase [15]. A study presented by Roy-Byrne et al. showed that some anticonvulsant drugs decrease ethanol withdrawal symptoms, and that anticonvulsant treatments may reduce the length of hospital stay for ethanol withdrawal [16]. In addition, it reduces the need for benzodiazepines in subgroups sensitive to benzodiazepine side effects, and enhances the efficacy of benzodiazepines when given in combination [17, 18]. A Hammer and Brady study

showed that valproate might be effective in the treatment of sedative-hypnotic withdrawal [19]. Moreover, the effect of valproate on GABAA receptors suggests that it may reduce relapse to ethanol and sedative use. Long-term GABAA receptor down-regulation in abstinent alcoholics and sedative abusers might explain ethanol and sedative craving and relapse. In fact, a case report from Brady et al. concerning ethanol relapse prevention suggests that valproate may reduce sedative-hypnotic relapse [20]. Thus, valproate, acting on GABA levels and GABAA receptor function, may have a role in the treatment of sedative-hypnotic withdrawal and relapse prevention.

Carbohydrate-deficient transferrin (CDT) is a biochemical marker used for identifying recent, regular high alcohol consumption and for monitoring abstinence during outpatient treatment [21]. Transferrins are a class of single-chain, iron-binding glycoproteins, classified and named depending on the number of terminal sialic acid residues on the N-linked oligosaccharide chains (glycans), which may be biantennary, triantennary, or even tetraantennary in structure [22]. Under physiological conditions, the major transferring glycoform contains two disialylated biantennary glycans and was named tetrasialotransferrin. In alcoholics, ethanol and acetaldehyde suppresses the activity of glycosyl transferase and increases the activity of sialidase [23, 24]. Moreover, individuals who have been excessively drinking over the past 2 or more weeks typically show increased relative amounts of disialotransferrin and, in cases of a high disialotransferrin level, of asialotransferrin [25]. The calculation of these forms with respect to total transferrin is indicated as a percentage of CDT (%CDT). %CDT showed a high sensitivity and specificity when compared with conventional markers of alcoholism such as GGT, AST, ALT and MCV [26, 27]. According to a comparative study by Legros et al. [28], we define 2.15 as the cut-off for these retrospective studies and for clinical diagnostic analysis in our laboratory.

In this retrospective study, we evaluated the effectiveness of valproate in maintaining alcohol abstinence based on %CDT values. We also associated different levels of serum total valproic acid (VPA) concentrations in alcoholic patients with %CDT values in order to identify the best treatment and drug response with minimal side effects.

## 2 Methods

This retrospective study evaluated a period of 3 years in which 42 patients (aged 29–64 years;  $44.41 \pm 8.17$ ) from the Department of Alcoholism and Substance Abuse were treated with valproate. Patients treated with psychotropic drugs for psychiatric syndromes were excluded from the study. For each patient, three measurements of serum total

VPA and %CDT at monthly intervals were analyzed. Sampling time was in the morning prior to valproate consumption. Platelet counts were analyzed before starting valproate treatment and monthly during treatment for 3 consecutive months. Each patient was treated with valproate for at least 1 year at the time of analysis.

We stratified the patients into the following four groups based on mean serum total VPA concentrations; the group with the highest concentration ( $>50 \mu\text{g/mL}$ ) corresponding to the maximum concentration ( $C_{\text{max}}$ ) with area under the concentration–time curve from 0 to 24 h ( $\text{AUC}_{(0-24\text{h})}$ ) of  $850 \pm 150 \mu\text{g} \times \text{h/mL}$  as reported by several pharmacokinetic studies [29]:

1.  $<10 \mu\text{g/mL}$  corresponding to approximately 10 % of  $\text{AUC}_{(0-24\text{h})}$
2.  $10\text{--}30 \mu\text{g/mL}$  corresponding to approximately 20 % of  $\text{AUC}_{(0-24\text{h})}$
3.  $31\text{--}50 \mu\text{g/mL}$  corresponding to approximately 50 % of  $\text{AUC}_{(0-24\text{h})}$
4.  $>50 \mu\text{g/mL}$  corresponding to  $\text{AUC}_{(0-24\text{h})}$  and  $C_{\text{max}}$  ( $45 \pm 10 \mu\text{g/mL}$ )

We compared the different serum concentrations of total VPA and the effectiveness in maintaining alcohol abstinence evaluated by %CDT values. We evaluated baseline platelet count, ethanol consumption prior to VPA assumption and the number of patients with a mean %CDT value  $>2.15$  in each of the groups.

In order to compare valproate consumption with the mean %CDT corresponding to each treatment group we applied computed statistical analysis to better define the application of significance.

For the statistical analysis, data are expressed as means  $\pm$  SEM. For evaluation of different parameters between the four groups, ANOVA followed by the appropriate post-hoc test was used.

In addition, we observed the number of cases in which the value of %CDT was over the cut-off level (2.15 %). In this case, a chi-squared test for the statistical analysis was

used. A  $p$  value of  $p < 0.05$  was considered statistically significant.

Serum concentration of total VPA was evaluated by a Siemens Dimension EXL immunochemical method. %CDT was measured by the HPLC method (Bio-Rad Variant Hplc).

We also excluded patients with genetic variants of transferrin, specifically C and D variants.

### 3 Results

Regarding the features of the study participants, there were no significant differences between the four groups in age, sex, baseline ethanol consumption or baseline platelet count (Table 1).

After at least 12 months' treatment with valproate, mean platelet counts increased by  $12 \times 10^3/\mu\text{L}$  compared with baseline ( $254 \pm 63$  vs  $242 \times 10^3/\mu\text{L}$ ,  $p > 0.05$ , respectively) in patients with mean serum total VPA levels  $<10 \mu\text{g/mL}$ ; increased by  $8 \times 10^3/\mu\text{L}$  from baseline ( $253 \pm 59$  vs  $245 \times 10^3/\mu\text{L}$ ,  $p > 0.05$ , respectively) in patients with levels between 10 and  $30 \mu\text{g/mL}$ ; decreased by  $2 \times 10^3/\mu\text{L}$  from baseline ( $265 \pm 63$  vs  $267 \times 10^3/\mu\text{L}$ ,  $p > 0.05$ , respectively) in patients with levels between 31 and  $50 \mu\text{g/mL}$ ; and decreased by  $48 \times 10^3/\mu\text{L}$  from baseline ( $215 \pm 563$  vs  $263 \times 10^3/\mu\text{L}$ ,  $p < 0.05$ , respectively) in patients with levels  $>50 \mu\text{g/mL}$ .

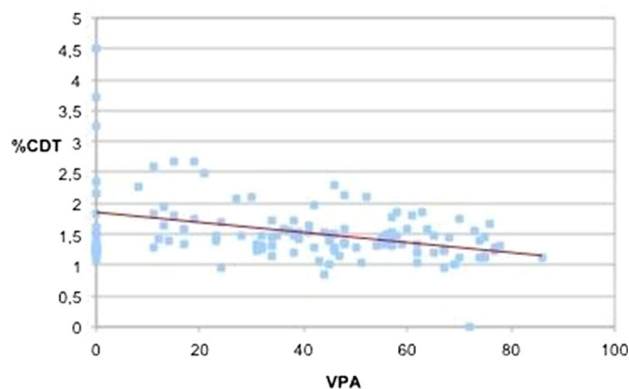
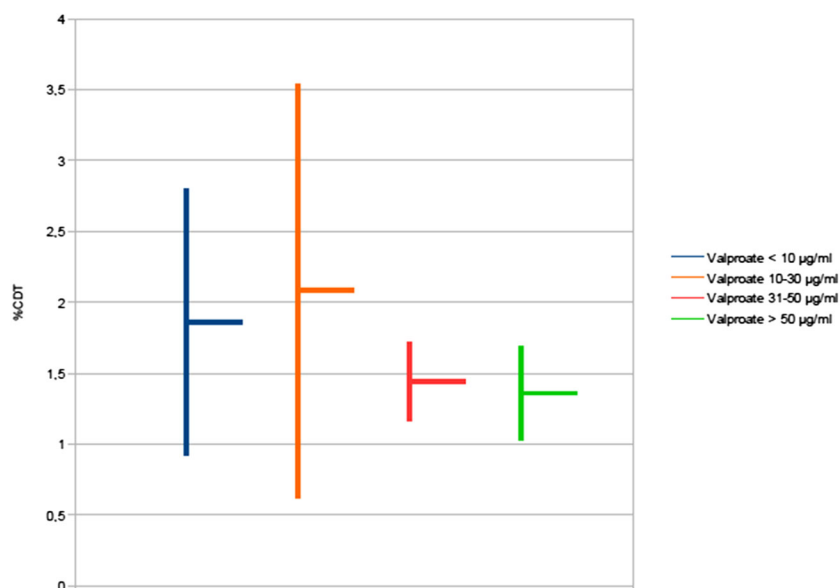
Patients with mean serum total VPA levels  $<10 \mu\text{g/mL}$  had a mean %CDT of  $1.86 \pm 0.94$ , patients with levels between 10 and  $30 \mu\text{g/mL}$  had a mean %CDT of  $2.08 \pm 1.46$ , patients with levels between 31 and  $50 \mu\text{g/mL}$  had a mean %CDT of  $1.44 \pm 0.28$ , patients with levels  $>50 \mu\text{g/mL}$  had a mean %CDT of  $1.36 \pm 0.36$ .

Mean %CDT levels were significantly higher (indicating increased alcohol consumption) in patients with mean serum total VPA levels  $<10 \mu\text{g/mL}$  compared with patients with levels between 31 and 50 ( $p < 0.05$ ) or  $>50 \mu\text{g/mL}$  ( $p < 0.05$ ). Patients with mean serum total VPA levels

**Table 1** Baseline characteristics of chronic alcohol-addicted patients in an Italian clinic stratified by mean serum total valproic acid concentration

Participant characteristics	Mean valproic acid serum level				ANOVA $p$
	$<10 \mu\text{g/mL}$ ( $n = 10$ )	$10\text{--}30 \mu\text{g/mL}$ ( $n = 10$ )	$31\text{--}50 \mu\text{g/mL}$ ( $n = 11$ )	$>50 \mu\text{g/mL}$ ( $n = 11$ )	
Age (years)	$42.77 \pm 4.34$	$46.64 \pm 8.86$	$40.84 \pm 9.29$	$41.79 \pm 5.87$	$>0.05$
Sex (M/F)	7/3	7/3	8/3	8/3	$>0.05$
Ethanol consumption prior to cessation (g/day)	$55.3 \pm 7.7$	$53.2 \pm 7.4$	$50.5 \pm 5.3$	$54.6 \pm 6.5$	$>0.05$
Baseline platelet count ( $\times 10^3/\mu\text{L}$ )	$242 \pm 62$	$245 \pm 57$	$267 \pm 60$	$263 \pm 58$	$>0.05$

**Fig. 1** Mean %CDT values stratified by mean serum total valproic acid concentrations in chronic alcohol-addicted patients in an Italian clinic. Mean %CDT levels were significantly higher in patients with mean serum total VPA levels <10 µg/mL and between 10 and 30 µg/mL compared with values between 31 and 50 (p < 0.05) or >50 µg/mL (p < 0.05), respectively. There was no significant difference in mean %CDT between patients with mean serum total VPA levels between 31 and 50 µg/mL and those with levels >50 µg/mL. CDT carbohydrate-deficient transferrin



**Fig. 2** Correlation between mean serum total valproic acid concentrations and %CDT in chronic alcohol-addicted patients in an Italian clinic. Linear regression analysis showed an inverse relation between these two parameters ( $r = -0.4$ ). CDT carbohydrate-deficient transferrin, VPA mean serum total valproic acid levels

between 10 and 30 µg/mL also had significantly higher %CDT values when compared with patients with higher VPA levels ( $p < 0.05$  vs 31–50 µg/mL and >50 µg/mL). However, there was no significant difference in mean %CDT values between patients with mean serum total VPA levels between 31 and 50 µg/mL and those with levels >50 µg/mL (Fig. 1).

Linear regression analysis showed an inverse relation between total VPA concentration and %CDT (Fig. 2,  $r = -0.4$ ). Thus, with increasing serum total VPA concentration, the %CDT decreased.

Overall, this study shows 12 cases with %CDT over the threshold ( $\geq 2.15$  %), six cases with serum total VPA <10 µg/mL (60 %), five cases with serum total VPA between 10 and 30 µg/mL (50 %), one case with a serum total VPA level between 31 and 50 µg/mL (9 %) and no cases with serum total VPA levels >50 µg/mL (Table 2).

**Table 2** Serum total valproic acid concentrations of chronic alcoholic patients with %CDT values over 2.15 %<sup>a</sup>

Mean serum total valproic acid level	Number of patients n (%)	Patients with %CDT >2.15 n (%)	p value
<10 µg/mL	10 (24)	6 (60)	NS (vs 10–30 µg/mL) <0.005 (vs 31–50 µg/mL) <0.001 (vs >50 µg/mL)
10–30 µg/mL	10 (24)	5 (50)	<0.05 (vs 31–50 µg/mL) 0.005 (vs >50 µg/mL)
31–50 µg/mL	11 (26)	1 (9)	NS (vs >50 µg/mL)
>50 µg/mL	11 (26)	0	Reference
Total	42 (100)		

CDT carbohydrate-deficient transferrin, NS non-significant

<sup>a</sup> %CDT >2.15 % indicates recent, regular high alcohol consumption

## 4 Discussion

Valproate has been extensively used as a pharmaceutical drug for prevention of relapse in chronic alcohol addictive patients. The effectiveness of treatment with valproate was evaluated by measuring the values of %CDT, which represents the desialylated isoforms induced by alcohol and has been adopted as the most sensitive marker for monitoring alcohol abuse [28].

The most commonly reported adverse effects of valproate include gastrointestinal disturbances, tremor and bodyweight gain. Other notable adverse effects include encephalopathy symptoms (sometimes associated with hyperammonemia), platelet disorders, pancreatitis and liver toxicity [7]. This study shows that a serum concentration of total VPA  $>30$   $\mu\text{g/mL}$  is more effective in maintaining alcohol abstinence than a lower one. More interestingly, a total VPA serum level between 31 and 50  $\mu\text{g/mL}$  shows the same effectiveness as a higher one. Therefore, a serum concentration ranging from 30 to 50  $\mu\text{g/mL}$ , lower than the accepted therapeutic range for epilepsy (50–100  $\mu\text{g/mL}$ ), could have the same effectiveness but with a lower risk of adverse effects. In fact, when we reported the %CDT values among the four groups, we had evidence %CDT levels were significantly low corresponding to a total VPA concentration between 31 and 50  $\mu\text{g/mL}$  and  $>50$   $\mu\text{g/mL}$ . In addition, the number of relapses to alcohol addiction was lower in the groups with serum total VPA concentrations between 31 and 50  $\mu\text{g/mL}$  or  $>50$   $\mu\text{g/mL}$  (1 and 0 cases, respectively) compared with patients with mean serum total VPA concentrations  $<10$   $\mu\text{g/mL}$  or between 10 and 30  $\mu\text{g/mL}$  (six and five cases, respectively) and there are no statistically significant differences between the two groups with the highest serum total VPA levels.

Moreover, serum total VPA concentration but not %CDT levels correlated with modulation of platelet counts; as a matter of fact, in patients with serum total VPA levels  $>50$   $\mu\text{g/mL}$ , we found lower platelet counts with respect to the other groups. This finding is quite relevant since adoption of a standardized lower VPA concentration (ranging 30–50  $\mu\text{g/mL}$ ) may be sufficient to achieve the best therapeutic efficacy with a reduced risk of adverse effects.

It has been demonstrated that valproate is characterized by a hepatic metabolism and a pronounced inter-individual variability in the pharmacokinetics [30, 31]. This variability may be due to genetic polymorphisms that encode drug metabolizing enzymes as such cytochrome P450 2C9, 2C19 (CYP2C9, CYP2C19) and uridine diphosphate (UDP) glucuronosyltransferase (UGTs) involved in the hepatic metabolism of valproate. Thus, such polymorphisms, affecting the serum concentration of

valproate, may influence the effectiveness of this drug in maintaining alcohol abstinence. Moreover, testing these genetic variants prior to initiating drug treatment may indicate the best valproate administration to patients considering the possibility of titrating the valproate dosage to achieve a serum total VPA level of between 31 and 50  $\mu\text{g/mL}$  and could decrease the likelihood of side effects. Therefore, administering the appropriate dosage must be considered a key concept in the effort to optimize drug treatment.

## 5 Conclusions

Since we have demonstrated that the best therapeutic effectiveness of valproate on alcohol addictive patients correlates with serum concentration between 31 and 50  $\mu\text{g/mL}$ , it would be of clinical interest to test CYPs/UGTs genetic profile of patients and to titrate the valproate dosage to achieve the indicated level for the best therapeutic performance.

### Compliance with Ethical Standards

**Ethical approval** Informed consent was retrieved from patients whose retrospective data were used for this study. In addition, according to the guidelines of the Declaration of Helsinki for retrospective studies, we notified this project to the University Department where experiments were conducted and to the local Ethical Committee. However, internal procedures do not require an ethical committee formal approval.

**Conflict of interest** The following researchers do not declare any conflict of interests for the submitted paper: Dr. Vincenzo De Iuliis, Dr. Raimondo Gelormini, Dr. Mariarosaria Flacco, Dr. Giuseppe Moriello, Dr. Marika Caruso, Dr. Eugenia Barone, Dr. Maria Golato, Prof. Elena Toniato, Prof. Pio Conti, Prof. Stefano Martinotti.

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