



Determinants of free serum valproate concentration: A prospective study in patients on divalproex sodium monotherapy



Wassim Nasreddine¹, Maya Dirani¹, Samir Atweh, Achraf Makki, Ahmad Beydoun*

Department of Neurology, American University of Beirut Medical Center, Beirut, Lebanon

ARTICLE INFO

Article history:

Received 24 August 2017

Received in revised form 9 April 2018

Accepted 17 April 2018

Keywords:

Antiepileptic drugs

Valproate

Therapeutic drug monitoring

Free level

Free fraction

ABSTRACT

Purpose: To evaluate variables affecting the valproate (VPA) free fraction and develop an equation for computing free VPA concentration from total VPA concentration.

Methods: Trough total and free VPA concentrations were collected from patients who participated in a prospective VPA monotherapy trial. All available paired data of trough total and free VPA concentrations were included. Significant variables from the univariate analysis were evaluated in a multivariate model. **Results:** A total of 902 concomitant total and free VPA concentrations were available. Multivariate analysis showed that total VPA concentration, age and gender were significantly associated with VPA free fraction. However, the effect size of total VPA concentration was substantially higher than that of gender and age. VPA free fraction remained stable at around 10% for total VPA concentration between 20 and 60 $\mu\text{g}/\text{mL}$ with subsequent linear increases for higher concentration. A scatter plot correlating total and free VPA concentrations showed that a quadratic equation best fitted the data, accounting for 88% of the free VPA concentration variance.

Conclusions: An increase in the total VPA concentration results in corresponding linear and non-linear rise in the VPA free fraction and free VPA concentration, respectively. The total daily dose of VPA should be increased in smaller increments whenever a total VPA concentration of 60 $\mu\text{g}/\text{mL}$ is reached. When drug monitoring is needed, we recommend measuring the free VPA concentration. If this test is unavailable, and for patients with normal albumin levels, it can be predicted from the total VPA concentration using the generated equation.

© 2018 British Epilepsy Association. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Therapeutic drug monitoring (TDM) for antiepileptic drugs (AEDs) is recommended in specific clinical situations to assess compliance, ensure therapeutic drug level, assist in the diagnosis of clinical toxicity, and guide dosage adjustment [1]. The reference range for trough total valproate serum concentration has traditionally been recognized to be between 50 $\mu\text{g}/\text{mL}$ and 100 $\mu\text{g}/\text{mL}$ (350 $\mu\text{mol}/\text{L}$ to 700 $\mu\text{mol}/\text{L}$) based on the results of studies enrolling small number of patients maintained on VPA as part of a polytherapy regimen [2,3]. More recent data suggests that the total VPA concentration needs to be individualized with higher serum levels required to control focal onset seizures compared to primarily generalized tonic-clonic seizures [4]. On the other hand, higher total VPA concentration were found to be associated with more frequent and more severe adverse events [5] including a

significant inverse linear correlation between total VPA concentration and platelet counts [6].

VPA has a complex pharmacokinetic profile. It is highly albumin bound [7,8] and the biologically active free VPA serum concentration varies non-linearly with increases in total VPA concentration [9–11]. It has therefore been suggested that measurement of the free VPA concentration is a better guide than total VPA concentration to reduce adverse events, improve seizure control, and avoid unwarranted dose adjustments [12,13].

The primary aim of this study was to evaluate the contribution and effect size of variables that could affect the VPA free fraction namely total VPA concentration, age and gender using data from a large cohort of patients who participated in a prospective randomized, double-blind, VPA monotherapy concentration-response design trial [5]. A secondary aim was to develop an equation that would allow computation of the free VPA concentration from the total VPA concentration.

2. Materials and methods

Patients 10 to 75 years of age, with a diagnosis of a localization related epilepsy and a documented history of at least two complex

* Corresponding author at: American University of Beirut Medical Center, PO BOX 11-0236, Riad El-Solh, 1107 2020, Beirut, Lebanon.

E-mail address: ab29@aub.edu.lb (A. Beydoun).

¹ Contributed equally to the manuscript.

partial seizures per month while maintained on one AED (carbamazepine, phenytoin, primidone or phenobarbital) at therapeutic serum concentrations were eligible to participate in this study. Patients previously exposed to divalproex sodium (DVPX) and who failed to respond at serum VPA concentrations greater than 40 $\mu\text{g/mL}$ (280 $\mu\text{mol/L}$) or with a history of intolerance to the drug were excluded. Also excluded were pregnant women, women of child bearing potential not practicing adequate birth control, patients with generalized seizures in the previous two years and those with major medical illnesses, psychiatric illnesses, or a history of pseudoseizures or non-compliance.

This was a randomized, double-blind, parallel group, multicenter, concentration-response design clinical trial that compared the safety and efficacy of two concentration ranges of DVPX [5]. All patients were administered Depakote^R, a delayed release formulation consisting of an oligomeric complex composed of sodium valproate and valproic acid in a 1:1 molar ratio twice daily. The Institutional Review Board at each center approved the study protocol, which was conducted in compliance with the US Food and Drug Administration regulations. More details about the trial design were previously published [5].

Patients were randomly assigned in a 1:1 ratio at each center into the high (80 to 150 $\mu\text{g/mL}$; 555 $\mu\text{mol/L}$ to 1040 $\mu\text{mol/L}$) or low (25 to 50 $\mu\text{g/mL}$; 175 to 345 $\mu\text{mol/L}$) target trough serum VPA concentration groups. The study consisted of a baseline phase lasting eight to 12 weeks followed by a 24-week double-blind experimental phase. The experimental phase was divided into a dosage adjustment period (first eight weeks) followed by a 16-week dosage maintenance period. During the dosage adjustment period, the baseline AED was tapered and treatment with DVPX was initiated. DVPX dosage was gradually titrated upward to achieve the maximum tolerated serum concentration within the targeted range for each patient. To enter the 16-week maintenance period, patients had to be completely withdrawn from their baseline AED and be treated with DVPX as monotherapy. During this phase of the protocol, DVPX dosage was adjusted based on efficacy and tolerability, while maintaining trough serum VPA concentration within the targeted ranges.

Trough total and free VPA concentrations were determined from analysis of blood samples collected either eight to 15 h after the last DVPX dose, or less than one hour after the first dose of the day (taken before noon). All laboratory values were analyzed at a central laboratory, using a commercially available fluorescent polarized immunoassay. The minimum detectable assay limit of the apparatus was 13.0 $\mu\text{g/mL}$ (90 $\mu\text{mol/L}$).

For each patient maintained on DVPX monotherapy we included all available paired data of trough total VPA concentration and free VPA concentration. The VPA free fraction was calculated as free VPA concentration divided by the corresponding total VPA concentration, and expressed as a percentage. Since many statistical models requires the assumption of linearity [14] and a non-linear association was previously reported between total VPA concentration and free VPA concentration [9,10], we initially performed the analysis using VPA free fraction as the dependent variable.

Descriptive statistics were reported as means with ranges for continuous variables and as frequencies for categorical variables.

Univariate linear regression analyses between age and total VPA concentration as the independent variables and VPA free fraction as the dependent variable were performed. A comparison between the means of VPA free fraction in men and women was performed using a two-sided *t*-test.

Significant variables from the univariate analysis ($p < 0.1$) were entered into a multiple regression analysis with VPA free fraction as the dependent variable. Values of significance were set at

$P < 0.05$. The effect size (standardized coefficient) for the individual variables found to be significant in the multivariate model were calculated [15,16].

3. Results

During the 16-week dose maintenance phase (DVPX monotherapy phase), a total of 902 total VPA concentrations and concomitant free VPA concentrations were available for 228 (86%) out of the 265 patients enrolled in the double blind phase of the trial. There were 124 women and 104 men with a mean age of 35.1 years (range: 10–77 years). The mean values of total VPA concentration, free VPA concentration and VPA free fraction were 92.6 $\mu\text{g/mL}$ (640 $\mu\text{mol/L}$), 18.3 $\mu\text{g/mL}$ (130 $\mu\text{mol/L}$), and 17.0%, respectively.

Univariate linear regressions showed that both total VPA concentration ($R = 0.764$) and age ($R = 0.094$) were significantly associated with VPA free fraction. In addition, there was a gender difference with a significantly higher mean VPA free fraction in women (means = 17.6%) compared to that in men (16.2%; $p = 0.004$).

A multiple regression analysis showed that all three independent variables (total VPA concentration, age and gender) were significantly associated with the VPA free fraction ($R = 0.782$ and adjusted $R\text{-squared} = 0.61$). The effect size (standardized coefficient) for total VPA concentration, age, and gender were 0.771, 0.122, and 0.103, respectively. This implies that total VPA concentration was the most clinically relevant variable. Therefore, a plot of the mean VPA free fraction (with 95% confidence intervals) versus total VPA concentration at increments of 20 $\mu\text{g/mL}$ (140 $\mu\text{mol/L}$) was performed (Fig. 1). As can be appreciated from this Figure, the mean VPA free fraction remains relatively constant at 10% for total VPA concentrations ranging between 20 and 60 $\mu\text{g/mL}$ (140 $\mu\text{mol/L}$ and 415 $\mu\text{mol/L}$), with a subsequent linear increase in the mean VPA free fraction for total VPA concentration above 60 $\mu\text{g/mL}$ (415 $\mu\text{mol/L}$). For instance a rise in total VPA concentration from 70 $\mu\text{g/mL}$ (485 $\mu\text{mol/L}$) to 110 $\mu\text{g/mL}$ (765 $\mu\text{g/mL}$) (representing a 57.1% relative increase) was associated with an increase in the VPA free fraction from 12.5% to 18.9% (representing a 51.2% relative increase; Fig. 1).

Since the free VPA concentration equals the product of total VPA concentration and VPA free fraction, the increase in total VPA concentration is associated with a non-linear increase in the biologically active free VPA concentration. In the previous example

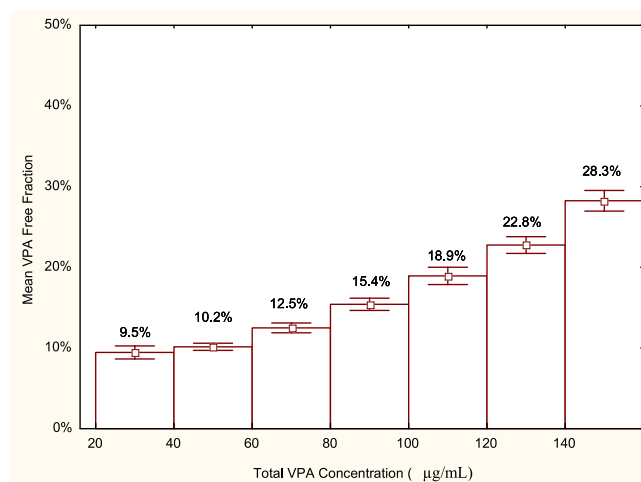


Fig. 1. Mean VPA free fraction (with 95% confidence intervals) within specific total VPA concentration ranges.

a rise in total VPA concentration from 70 $\mu\text{g/mL}$ (485 $\mu\text{mol/L}$) to 110 $\mu\text{g/mL}$ (765 $\mu\text{mol/L}$) (representing a 57.1% relative increase) would be associated with an increase in the free VPA concentration from 8.75 $\mu\text{g/mL}$ (60 $\mu\text{mol/L}$) to 20.79 $\mu\text{g/mL}$ (145 $\mu\text{mol/L}$) (representing a 137.6% relative increase in the concentration of the biologically active drug).

To assess the non-linear relationship between total and free VPA concentrations, a scatter plot correlating those two variables showed that a second degree polynomial equation best fitted the data ($y = (0.0016 * x^2) + (0.012 * x) + 0.4314$, $R = 0.937$ and adjusted $R\text{-squared} = 0.878$) (Fig. 2). The predicted free VPA concentration values from this equation for total VPA concentration of 70 $\mu\text{g/mL}$ (485 $\mu\text{mol/L}$) and 110 $\mu\text{g/mL}$ (765 $\mu\text{mol/L}$) were 9.09 $\mu\text{g/mL}$ (65 $\mu\text{mol/L}$) and 21.09 $\mu\text{g/mL}$ (145 $\mu\text{mol/L}$), respectively, results very similar to those derived from Fig. 1.

4. Discussion

This is the largest prospective study demonstrating that VPA exhibits a concentration-dependent protein binding and that increases in the total VPA concentration results in corresponding linear and non-linear rise in the VPA free fraction and free VPA concentration, respectively.

Since total VPA concentration increases linearly with the daily VPA dose [4], a progressive saturation of the VPA binding sites and competition by free fatty acids for these binding sites have been proposed to explain the linear increase in the VPA free fraction with higher total VPA concentration [17,9]. Our results indicate that the VPA free fraction remains stable at around 10% for total VPA concentration ranging between 20 $\mu\text{g/mL}$ and 60 $\mu\text{g/mL}$ (140 $\mu\text{mol/L}$ and 415 $\mu\text{mol/L}$) with progressive linear increases above this level. This finding, which suggests that the VPA binding sites become saturated at a total VPA concentration of approximately 60 $\mu\text{g/mL}$ (415 $\mu\text{mol/L}$) is consistent with *in vitro* [18] and animal [18,9] studies that documented that the total VPA concentration threshold for binding sites saturation becomes evident at 70 $\mu\text{g/mL}$ (485 $\mu\text{mol/L}$) and 80 $\mu\text{g/mL}$ (555 $\mu\text{mol/L}$), respectively. A recent study evaluating the protein binding of 25 AEDs found that the VPA binding is saturable within the clinically relevant concentration range. More specifically, when the free and total VPA concentrations were measured in 20 patients with epilepsy receiving multiple AEDs that were not highly protein-bound, the free fraction was found to be highly variable with

substantial interpatient variability and ranged between 7.3% and 26.1% for total VPA concentrations between 38.2–128.2 $\mu\text{g/mL}$ [11].

In addition to the total VPA concentration, we found that age and gender were also significantly associated with VPA free fraction in a multivariate analysis. However, the effect size, which measures the strength of a relationship, was substantially higher for total VPA concentration ($\beta = 0.771$) compared to age ($\beta = 0.122$) or gender ($\beta = 0.103$). This small effect size for age and gender indicates that these variables are hardly of any clinical relevance. Two previous studies evaluating the effect of age on VPA free fraction reported results similar to ours. The first, which evaluated 70 adult patients maintained on VPA monotherapy [19], found that the VPA free fraction was significantly higher in patients 45 years and older (mean VPA free fraction of 9.8% at a mean total VPA concentration of 55.2 $\mu\text{g/mL}$ (383 $\mu\text{mol/L}$) compared to those younger than 45 years (mean VPA free fraction of 7.5% at a mean total VPA concentration of 55.4 $\mu\text{g/mL}$ (384 $\mu\text{mol/L}$). The other study, which evaluated the kinetics of a single dose of VPA in 6 elderly and 6 young healthy volunteers, reported a higher free VPA concentration in the elderly group [20]. Possible explanations for this age related finding include a difference in the binding characteristics of VPA to serum proteins in various age groups [19] or a reduction in albumin serum levels in older patients [20].

We also documented a gender difference in the free fraction of VPA, with a higher mean VPA free fraction in women compared to men. This finding is consistent with a previous study that documented a higher VPA free fraction in women (mean free fraction of 9.0% at a mean total VPA concentration of 59.4 $\mu\text{g/mL}$) compared to men (mean free fraction of 7.7% at a mean total VPA concentration of 51.3 $\mu\text{g/mL}$) [19]. This gender difference remains unexplained.

In addition, our results demonstrated that there is a quadratic increase in the biologically active free VPA concentration at total VPA concentration higher than 60 $\mu\text{g/mL}$ (415 $\mu\text{mol/L}$). This finding is concordant with a study that evaluated 25 patients maintained on VPA monotherapy and who had blood drawn at various time intervals post VPA administration [9]. However, no confidence intervals for the means of the VPA free fractions were reported in that study nor were other potential variables affecting this relationship analyzed. This non linearity in VPA pharmacokinetics is reminiscent of that of phenytoin which exhibits an exponential rise in serum concentration with dose increase following liver enzyme saturation [21]. From a clinical perspective, our findings indicate that the total daily dose of VPA should be increased in smaller increments whenever a total VPA concentration of 60 $\mu\text{g/mL}$ (415 $\mu\text{mol/L}$) or higher is reached. This would likely reduce side effects since a substantial increase in total VPA concentration related frequency and severity of adverse events was previously documented [5].

The major weakness of our study is the lack of serum albumin levels which prevented us from assessing a potential association between albumin concentrations and free VPA concentration. Although the adjusted $R\text{-squared}$ of 88% in the model assessing the relationship between total and free VPA concentrations indicates that only 12% of the free VPA concentration variability is accounted for by other factors such as free fatty acids and/or genetic factors, the absence of concomitant serum albumin values precludes the extrapolation of our data to patients with abnormal albumin serum levels. This is especially relevant since this trial excluded patients with chronic medical illnesses [5], with a high likelihood that the majority of the enrolled patients had serum albumin levels within the normal range. In patients with hypoalbuminemia, the free VPA fraction will be higher than predicted by our results. For instance, a patient with mild hypoalbuminemia (albumin concentration = 3.3 g/dL) maintained on VPA monotherapy was admitted

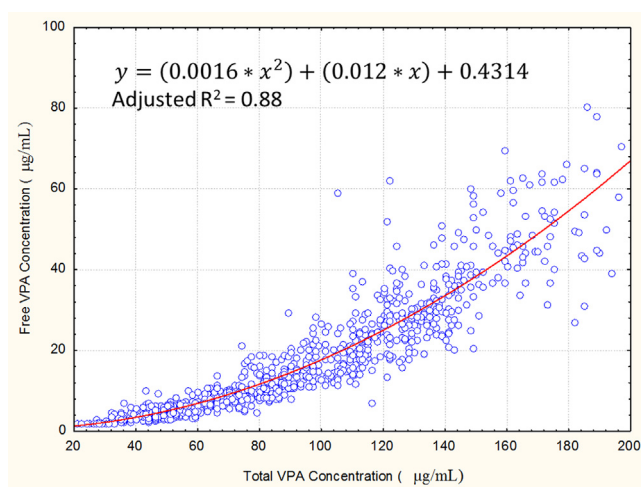


Fig. 2. Scatterplot of free VPA concentration as a function of total VPA concentration, showing the second degree polynomial equation that best fitted the data.

because of somnolence, dysarthria and difficulty walking. Although his total VPA concentration was 103 $\mu\text{g/mL}$, his free VPA concentration of 26.8 $\mu\text{g/mL}$ and corresponding VPA free fraction of 26% were higher than expected and likely the cause of his adverse events [22]. A more recent publication reported on a 53 years old woman with heart transplant and hypoalbuminemia who was found to have VPA free fractions of 69% and 23% with corresponding albumin concentrations of 2.9 g/dL and 3.3 g/dL, respectively. This patient who was admitted because of profound lethargy only improved when the VPA dose was adjusted based on the free rather than the total VPA concentration [23]. We therefore recommend measurement of the free VPA concentration for patients with hypoalbuminemia.

Our study has multiple strengths. It is the first double-blind trial that allowed the prospective evaluation of the relationship between the total VPA concentration and its free fraction over a wide range of trough serum concentrations performed at a centralized laboratory. In addition, the dataset was large enough to allow for the prediction of the VPA free fraction from the total VPA concentration with narrow confidence intervals.

5. Conclusion

Based on our results and as suggested by others [12,13] we recommend assessing the biological active free VPA concentration especially when clinical toxicity is suspected. If the free VPA concentration cannot be obtained, the equation that we generated can be used to predict the free VPA concentration from the total VPA concentration value for patients with normal albumin levels.

Conflicts of interest

None.

References

- [1] Patsalos P.N., Berry D.J., Bourgeois B.F., Cloyd J.C., Glauser T.A., Johannessen S.I., et al. Antiepileptic drugs—best practice guidelines for therapeutic drug monitoring: a position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies. *Epilepsia* 2008;49(7):1239–76.
- [2] Schobben F, Kleijn E, Gabreels F. Pharmacokinetics of di-n-propylacetate in epileptic patients. *Eur J Clin Pharmacol* 1975;8(2):97–105.
- [3] Gram L, Flachs H, Wurtz-Jørgensen A, Parnas J, Andersen B. Sodium valproate, serum level and clinical effect in epilepsy: a controlled study. *Epilepsia* 1979;20(3):303–11.
- [4] Turnbull D, Rawlins M, Weightman D, Chadwick D. Plasma concentrations of sodium valproate: their clinical value. *Ann Neurol* 1983;14(1):38–42.
- [5] Beydoun A, Sackellares J, Shu V. Safety and efficacy of divalproex sodium monotherapy in partial epilepsy a double-blind, concentration-response design clinical trial. *Neurology* 1997;48(1):182–8.
- [6] Nasreddine W, Beydoun A. Valproate-induced thrombocytopenia: a prospective monotherapy study. *Epilepsia* 2008;49(3):438–45.
- [7] Wulff K, Flachs H, Wurtz-Jørgensen A, Gram L. Clinical pharmacological aspects of valproate sodium. *Epilepsia* 1977;18(2):149–57.
- [8] Perucca E. Pharmacological and therapeutic properties of valproate. *CNS Drugs* 2002;16(10):695–714.
- [9] Cramer J.A., Mattson R.H., Bennett D.M., Swick C.T. Variable free and total valproic acid concentrations in sole-and multi-drug therapy. *Ther Drug Monit* 1986;8(4):411–5.
- [10] Bellver M, Sánchez M, Gonzalez A, Buelga D.S., Dominguez-Gil A. Plasma protein binding kinetics of valproic acid over a broad dosage range: therapeutic implications. *J Clin Pharm Ther* 1993;18(3):191–7.
- [11] Patsalos P.N., Zugman M, Lake C, James A, Ratnaraj N, Sander J.W. Serum protein binding of 25 antiepileptic drugs in a routine clinical setting: a comparison of free non-protein-bound concentrations. *Epilepsia* 2017;58(7):1234–43.
- [12] Lenn N.J., Robertson M. Clinical utility of unbound antiepileptic drug blood levels in the management of epilepsy. *Neurology* 1992;42(5) 988–988.
- [13] Dasgupta A. Usefulness of monitoring free (unbound) concentrations of therapeutic drugs in patient management. *Clin Chim Acta* 2007;377(1):1–13.
- [14] Field A. *Discovering statistics using IBM SPSS statistics*. Sage; 2013.
- [15] Fan X. Statistical significance and effect size in education research: two sides of a coin. *J Educ Res* 2001;94(5):275–82.
- [16] Nakagawa S, Cuthill J.C. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol Rev* 2007;82(4):591–605.
- [17] Patel I, Levy R.H. Valproic acid binding to human serum albumin and determination of free fraction in the presence of anticonvulsants and free fatty acids. *Epilepsia* 1979;20(1):85–90.
- [18] Löscher W. Serum protein binding and pharmacokinetics of valproate in man, dog, rat and mouse. *J Pharmacol Exp Ther* 1978;204(2):255–61.
- [19] Kodama Y, Kodama H, Kuranari M, Tsutsumi K, Ono S, Yukawa E, et al. Gender- or age-related binding characteristics of valproic acid to serum proteins in adult patients with epilepsy. *Eur J Pharm Biopharm* 2001;52(1):57–63.
- [20] Perucca E, Grimaldi R, Gatti G, Pirracchio S, Crema F, Frigo G. Pharmacokinetics of valproic acid in the elderly. *Br J Clin Pharmacol* 1984;17(6):665–9.
- [21] Richens A, Dunlop A. Serum-phenytoin levels in management of epilepsy. *Lancet* 1975;306(7928):247–8.
- [22] Gidal B.E., Collins D.M., Beinlich B.R. Apparent valproic acid neurotoxicity in a hypoalbuminemic patient. *Ann Pharmacother* 1993;27(1):32–5.
- [23] Haroldson J.A., Kramer L.E., Wolff D.L., Lake K.D. Elevated free fractions of valproic acid in a heart transplant patient with hypoalbuminemia. *Ann Pharmacother* 2000;34(2):183–7.