Full Paper

A Theoretical Method for Normalizing Total Serum Valproic Acid Concentration in Hypoalbuminemic Patients

Jesús Hermida¹ and J. Carlos Tutor^{1,*}¹Central Laboratory, University Hospital Clinic, 15706 Santiago de Compostela, Spain

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Abstract. In patients with hypoalbuminemia, the total serum concentration of valproic acid may offer poor clinical information; however, very few clinical laboratories routinely analyze the free concentration of the drug. The aim of this study was to design a procedure to normalize the total concentration of valproic acid according to the level of serum albumin and using previously published free fraction values. In 121 adult patients, with albumin levels of 18–41 g/L, the total concentration of valproic acid was normalized using the derived equation: $C_N = \alpha_H C_H / 6.5$, where α_H is the free fraction of the drug corresponding to the patient's particular albuminemia and C_H is the total concentration of valproic acid. The value of 6.5 corresponds to the free fraction of the drug for a serum albumin of 42 g/L (percentile 50 of the reference range). For total concentrations lower than 75 mg/L, the predicted normalized valproic acid concentrations were reasonably concordant with the observed normalized concentrations calculated using the data from a protein-binding study. In a significant number of cases, subtherapeutic concentrations of the drug became therapeutic and even supratherapeutic when corrected according to the albumin levels. Furthermore, cases with therapeutic drug concentrations frequently became supratherapeutic when normalized. The limitations and clinical applications of the proposed formula for normalizing the total concentration of valproic acid are presented. It is concluded that it may be useful for the posological management of hypoalbuminemic patients when the free concentration of the drug is not available, and decisions have to be made based on the total serum concentration.

Keywords: valproic acid, hypoalbuminemia, dose adjustment, ultrafiltration

Introduction

In blood, most drugs are bound to serum proteins, principally albumin, α 1-acid glycoprotein and lipoproteins, to various degrees. The fraction bound to proteins is considered to be pharmacologically inactive, as only the unbound (free) fraction is available for diffusion out of the vascular system to sites for pharmacological action (1–4). The free fraction (α) represents the relationship between free and total drug concentration:

$$\alpha = \text{Free drug concentration} / \text{Total drug concentration (free + bound)}$$

In the case of drugs with high protein binding

($\alpha < 20\%$), it may be preferable to determine their free concentration; however, in clinical laboratories, the standard procedure is to monitor the total drug concentration (1–4), due to technical difficulties and a lack of established reference ranges for free drug concentrations (2, 4). The two antiepileptic drugs in which it may be clinically useful to determine the free drug concentration are phenytoin and valproic acid (5, 6). Free phenytoin is the most frequently requested free drug concentration by clinicians (3, 5), possibly because they are more familiar with it (5).

Valproic acid is extensively bound to albumin ($\alpha < 10\%$), and the drug-protein binding depends on both the serum albumin concentration and the drug concentration (2–7). As a result, the free fraction of this drug is subject to more variation than other highly protein-bound antiepileptic drugs (2). Studies published

*Corresponding author. FAX: +34 981 950403

E-mail: jocatuvva@hotmail.com, josecarlostutor@redfarma.org

by Gidal et al. (8) and Haraldson et al. (9) reported several cases demonstrating the clinical importance of monitoring free valproic acid, as well as the minimal utility of total drug concentration in patients with hypoalbuminemia. As early as 1980, Levy stressed that serious consideration should be given to the monitoring of free rather than total valproic acid concentration, which can be misleading (7); however, a recent survey by the College of American Pathologists revealed that only 2% of the laboratories which routinely performed total valproic acid determination offered free valproic acid assays (2). For some anticonvulsant drugs, under well-controlled and standardized conditions, the concentration in saliva bears a constant relationship to free serum concentration, but this is not the case for valproic acid (10).

The ability of equations using population mean binding parameters (association constant and total concentration of binding sites) to predict unbound valproic acid concentration was studied by Kodama et al. in pediatric and adult patients, whose albumin concentrations were assumed to be normal (11–13). However, because albumin levels directly influence the binding of the drug to plasma proteins, these methods may not accurately predict unbound valproic acid in patients with hypoalbuminemia. The relationship obtained by Parent et al. (14) between the free fraction of valproic acid (y) and serum albumin concentration (x): $y = Ae^{-Bx}$ ($A = 130.69$, $B = 4.96 \times 10^{-3}$, $r = -0.82$) would make it possible to estimate the free concentration of the drug. In our paper, a derived formula is proposed for normalizing the total concentration of valproic acid in hypoalbuminemic patients.

Materials and Methods

Total serum concentrations of valproic acid were determined for 121 adult patients who were receiving anticonvulsant treatment with this drug, all of who had a concentration of serum albumin lower than 42 g/L (mean 35.5 ± 4.6 g/L, range 18–41 g/L), corresponding to percentile 50 of the reference range for individuals between the ages of 25–55 years (15). In 53 patients, with a mean serum albumin concentration of 36.9 ± 5.0 g/L (range 24–41 g/L) and normal levels of bilirubin, creatinine, and urea, who were treated in monotherapy ($n = 37$) or in polytherapy with phenytoin, carbamazepine, lamotrigine, or mysoline ($n = 16$), additionally to the total, the free concentrations of valproic acid were determined by ultrafiltration. Blood samples were taken immediately before the morning dose (trough level) once the state of equilibrium had been reached. The study was approved by the ethical

committee of the University of Santiago de Compostela Hospital Clinic, and all participants provided their consent to participate.

The determination of free and total valproic acid was carried out by fluorescence polarization immunoassay in a Cobas Integra 400 analyzer using reagents commercialized by Roche Diagnostics (Basel, Switzerland). The sensitivity of the free valproic acid assay is 1.3 mg/L and its determination was made in duplicate. Protein-binding of valproic acid was evaluated by ultrafiltration at 25°C using the Centrifree Micropartition System (Millipore Corporation, Bedford, MA, USA) for the unbound drug separation (16). Serum albumin was determined using bromocresol purple (17) in a Dade Dimension analyzer (Dade Behring, Liederbach, Germany).

In hypoalbuminemic patients, the free fraction of valproic acid (α_H) may be given by the equation: $\alpha_H = C_F/C_H$, where C_F is the free drug concentration and C_H is the total concentration of the drug. In conditions of normoalbuminemia, for the same free concentration of the drug (C_F), the free fraction (α_N) may be $\alpha_N = C_F/C_N$, where C_N is the total normalized concentration of valproic acid. Combining both equations: $\alpha_N C_N = \alpha_H C_H$ and $C_N = \alpha_H C_H / \alpha_N$. This formula may be used to determine the total drug concentration that would be expected if the patients' albumin concentrations were normal.

The normalized concentration of valproic acid for a serum albumin of 42 g/L is given by the formula: $C_N = \alpha_H C_H / 6.5$, where 6.5 is the free fraction of the drug for this albumin concentration (Table 1), in agreement with the results presented by Parent et al. (14). The total experimental concentrations of valproic acid for all of the patients studied were normalized using this formula.

Statistical analysis of the data was carried out using the Microsoft Excel (v.5.0) package, and the Kolmogorov-Smirnov test was used to check for normality. As the data had not a Gaussian distribution, the Spearman's correlation coefficient and Passing-Bablok regression method were used.

Results

In the 53 patients in which the protein-binding of valproic acid was evaluated, for total drug concentrations lower than 75 mg/L ($n = 42$), the means of the predicted (using Table 1) and observed free fractions were analogous ($10.6 \pm 4.8\%$ vs $10.1 \pm 6.3\%$). However, for total concentrations greater than 75 mg/L ($n = 11$), the predicted free fractions ($7.7 \pm 1.3\%$) were significantly lower ($P < 0.001$) than the observed free fractions ($13.8 \pm 3.2\%$). The scatter diagram of the unbound

Table 1. Relationship between free fraction of valproic acid (α) and serum albumin concentration (Ref. 14)

Albumin (g/L)	α (%)
42	6.5
41	6.8
40	7.3
39	7.9
38	8.5
37	9.1
36	9.8
35	10.5
34	11.3
33	12.1
32	13.0
31	14.0
30	15.0
29	16.2
28	17.4
27	18.7
26	20.1
25	21.6
24	23.2
23	24.9
22	26.8
21	28.9
20	31.0
19	33.3
18	35.8

valproic acid prediction error (difference between predicted and observed unbound concentrations) against total valproic acid concentrations, reveal that this error was ≤ 2.5 mg/L for total drug concentrations lower than approximately 75 mg/L (see Fig. 1); however, for greater total concentrations, the unbound prediction error increases significantly. Figure 2 indicates the relationship between predicted and observed normalized valproic acid concentrations, using the proposed formula and the predicted and observed free fractions, respectively, for total drug concentrations lower and greater than 75 mg/L.

Figure 3 shows the relationship between predicted normalized and total valproic acid concentrations in the group of 121 patients. In these patients, 48 cases had subtherapeutic total valproic acid concentrations, with 47.9% of these cases reaching therapeutic levels and 10.4% supratherapeutic levels after being normalized for albumin concentrations. Similarly, in the 65 patients that have therapeutic total concentrations of the drug, 47.7% reached supratherapeutic levels after normalization.

Fig. 1. Scatter diagram of unbound valproic acid prediction error against total valproic acid concentration. **Fig. 2.** Relationship between predicted and observed normalized valproic acid concentrations for total valproic acid concentrations lower (open circles) and higher (closed circles) than 75 mg/L.

Discussion

The relationship indicated by Parent et al. between the free fraction of the valproic acid and serum albumin concentration, in the absence of renal failure, jaundice, or a high total drug concentration, would make it

Fig. 3. Scatter diagram of predicted normalized valproic acid concentrations against total valproic acid concentrations. The dashed lines correspond to the limits of therapeutic range.

possible to estimate the free drug concentration with an error of ≤ 2.5 mg/L (14). Our results appear to confirm this aspect for total valproic acid concentrations ≤ 75 mg/L (see Fig. 1). Consequently, the predicted and observed normalized concentrations were reasonably concordant for total valproic acid concentrations lower than 75 mg/L (Fig. 2), although a greater unbound prediction error would be produced for total concentrations around this cut-off value in very severe cases of hypoalbuminemia. For a greater total concentration (80–120 mg/L), the underestimation of the predicted normalized concentration is evident (Fig. 2); however, in this case, given the discrete dispersion of the values, the predicted normalized concentration may be corrected using the corresponding linear regression equation. The higher dispersion found between the values of predicted and observed normalized concentrations for total valproic acid concentrations lower than 75 mg/L, may be due to a higher imprecision in the experimental determination of lower free drug concentrations.

As may be seen from the results indicated above (Fig. 3), normalization of the total concentration of valproic acid in patients with hypoalbuminemia may have a significant influence on adjusting the dose of the drug. In patients with subtherapeutic total concentrations, after correcting these according to the albumin concentration, they frequently became therapeutic and even supratherapeutic. Similarly, therapeutic levels frequently became supratherapeutic after normalization.

In cases of neurotoxicity previously described in hypoalbuminemic patients, with high free concentrations of valproic acid (8, 9), normalization of the total concentration of the drug would have offered clinically useful information ($C_N > 100$ mg/L), suggesting a decrease of the doses.

The valproic acid free fractions used for the calculation of the normalized concentrations (Table 1), are reasonably concordant with the *in vitro* values recently obtained by Bailey and Briggs for a total drug concentration of 75.0 mg/L (18), and the only criteria used to select the 121 patients was that they presented a serum albumin level of less than 42 g/L. Changes in α_1 -acid glycoprotein concentrations had little effect (18); however, other factors apart from the albumin concentration may affect the free fraction of valproic acid. The protein binding is also drug-concentration-dependent, with a free fraction increasing from approximately 5% at a total valproic acid concentration of 10–60 mg/L to 10% at 50–100 mg/L and 20–30% at 145–160 mg/L (3, 4, 7), exhibiting a saturable protein binding at the upper end of the therapeutic range (2, 5). As a result, there is an unpredictable free fraction at high total drug concentrations. However, it is important to take into account that it is the normalization of total subtherapeutic and therapeutic concentrations of valproic acid that would be of real clinical interest, in which the imprecision of the free drug concentration determination is greater.

The most widely-used antiepileptic drugs (phenytoin, phenobarbital, and carbamazepine) have no effect on valproic acid binding to albumin (1), and salicylate is the only acidic drug known to effectively compete with valproic acid for albumin binding (4, 5). However, endogenous substances such as free fatty acids, uremic compounds and bilirubin would displace the bound drug from its albumin binding sites (1). Therefore, in situations of jaundice or uremia, which may produce an increase of the free drug fraction, the application of the derived formula could lead to an underestimation of the normalized concentration of valproic acid.

The clinicians are much more familiar with the use of the total than free serum drug concentrations. Evidently, in the best of cases the predicted normalized total valproic acid concentrations offer approximate results. However, despite its obvious limitations, the proposed formula may be of clinical use for dealing with hypoalbuminemic patients in centres where the free valproic acid concentration is not available, and posological decisions have to be made based on the total concentration of the drug. An evaluation of its clinical performance is currently underway.

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