Differences between the Measured and Calculated Free Serum Phenytoin Concentrations in Epileptic Patients

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Purpose: The pharmacokinetics of phenytoin is complicated by genetic and environmental differences. It is, therefore, important to monitor the serum concentrations in patients who receive phenytoin. Because most of the phenytoin in serum is bound to proteins, the level of serum albumin influences the amount of free phenytoin. **Materials and Methods**: We compared the measured and calculated free phenytoin levels in epileptic patients who were taking phenytoin monotherapy, using the Sheiner-Tozer equation. A total of 49 patients (30 men and 19 women; age range, 15 - 87 years) were included in the study and their trough serum phenytoin and albumin concentrations were analyzed. **Results**: The linear correlation between free and total phenytoin concentrations was moderate (r = 0.822, $p < 0.001$). The mean difference between measured and calculated free phenytoin was large (0.65 ± 0.88 μg/mL; 95% confidence interval (CI), -1.11 to 2.41). After dividing the patients into groups by albumin concentration, hypoalbuminemic patients (< 3.5 g/dL) more often had a greater percent difference (≥ 20%) than observed in the normoalbuminemic (≥ 3.5 g/dL) group. **Conclusion**: In hypoalbuminemic patients, the measurement of free phenytoin level is necessary to properly evaluate the phenytoin level than that calculated from total phenytoin level.

Key Words : Phenytoin, epilepsy, hypoalbuminemia

INTRODUCTION

Phenytoin has become one of the most widely-used anti-epileptic drugs (AED) over the last several decades. The pharmacokinetics of phenytoin is nonlinear, and the drug has a narrow therapeutic range.[1] Small changes of dosage may greatly alter serum phenytoin levels and can lead to an overdose. Dosage changes must be carefully monitored as the therapeutic range is approached.[2] The results of several studies have suggested that increases in the unbound fraction of drugs that are highly bound to serum proteins increases the incidence of therapy-related toxic effects.[3,4] Only free, unbound phenytoin molecules can penetrate the blood-brain barrier and exert pharmacological effects;[5] therefore, these molecules are the ones responsible for the therapeutic and toxic effects. Measurement of the concentration of the free form of phenytoin is considered to better reflect clinical status than total concentration.[6] Total concentration is easily measured, and then free concentration is predicted using that value. The therapeutic range of the free phenytoin serum concentration is about 10% of that of the total phenytoin serum concentration and varies from 3.3 to 9.6 μmol/L (0.83 - 2.27 mg/L).[7,8] Only a few specialized laboratories offer to analyze the free phenytoin concentration because it is a more expensive and time-consuming test.

Phenytoin is primarily bound to serum albumin, although a smaller but significant portion is bound to α1-acid-glycoprotein (AAG).[9] The concentration

of albumin in serum is decreased with inflammation, chronic liver disease, or malnutrition. The presence of hypoalbuminemia is known to reduce the protein binding of phenytoin in plasma,[8,10] and both the ratio of free/total phenytoin concentration and the total phenytoin concentration may be altered. Therefore, it is difficult to accurately estimate the free phenytoin concentration from the total concentration.

Sheiner and Tozer proposed a correction formula based on serum albumin concentrations, and this is often used to estimate the free fraction of phenytoin using total concentration measurements.[11] This study compared the relationship between measured and calculated free phenytoin derived from the Sheiner-Tozer equation in order to assess the usefulness of monitoring the free concentration of phenytoin in epileptic patients.

MATERIALS AND METHODS

Subjects

Total and free phenytoin concentrations were measured in 49 epileptic patients (30 males and 19 females) receiving oral phenytoin for more than 7 days. We excluded patients who took other drugs that influence the metabolism of phenytoin such as other anti-epileptic drug, heparin, warfarin, aspirin, fatty acids, or tolbutamide. We also excluded subjects with pregnancy, end-stage renal disease or blood transfusion therapy. We collected trough levels of total and free phenytoin and serum albumin concentrations simultaneously.

Serum phenytoin level analysis

The serum total phenytoin level was analyzed using fluorescence polarization immunoassay (FPIA) technology (COBAS INTEGRA 800; Roche Diagnostics, Basel, Switzerland). Specimens were tested within 2 hours of collection. Free phenytoin levels were also analyzed using the same technology (TDx / TDxFLx; Abbott Laboratories, Abbott Park, IL, USA). Samples were ultrafiltered immediately after collection by centrifugation at $1000 - 2000 \times g$ for 15 - 20 minutes at $25 \pm 3°C$ with an assembled ultrafiltration device.

Corrected total phenytoin

Total phenytoin results were corrected for albumin concentration using the Sheiner-Tozer equation.

The relationship between the plasma drug and protein concentrations can be expressed as follows, and this equation can be used to estimate the degree to which an altered plasma protein concentration will affect the desired therapeutic drug concentration. Cp' represents the patient's plasma drug concentration and P' is the plasma protein concentration. $Cp_{normal\ binding}$ is the plasma drug concentration that would be expected if the patient's plasma protein concentration were normal (P_{NL}).

$$Cp'/Cp_{normal\ binding} = (1-\alpha)[P'/P_{NL}] + \alpha \text{ (Equation 1)}$$

Placing the corresponding values for a normal plasma albumin results as follows:

Corrected total phenytoin = [total phenytoin (μg/mL)] / {[albumin (g/dL) / 4.4] $\times$ 0.9 + 0.1} (Equation 2)

Based on the corrected concentration of total phenytoin, the calculated free phenytoin concentration can be obtained by multiplying by 0.1.[12]

Statistical analysis

The Pearson product-moment correlation coefficient (r) was used to measure linear association between variables. The mean difference between actual free phenytoin and calculated free phenytoin by the Sheiner-Tozer equation was determined by regression analysis with 95% confidence intervals for the mean difference defined as 2D. The F test, in the analysis of variance, was used to compare the ratio of free to total phenytoin concentrations between three different serum albumin intervals with a *post hoc* Bonferroni adjustment for multiple group comparisons. All statistical analyses were performed with the assistance of computer software (SPSS version 11.5). Two-tailed values of $p < 0.05$ were considered statistically significant with Bonferroni adjustment as appropriate.

RESULTS

The mean age of the patients was 52.7 ± 17.8 years (range, 15 to 87 years), and the mean weight was 59.3 ± 13.1 kg. The mean serum albumin concentration was 3.3 ± 0.8 g/dL, (range 1.3 to 4.9 g/dL). Hypoalbuminemia (< 3.5 g/dL) was present in 34 patients and normoalbuminemia (≥ 3.5 g/dL) in 15 patients.

The linear correlation between free and total phenytoin concentrations was moderate (Pearson r = 0.822, $p < 0.001$)
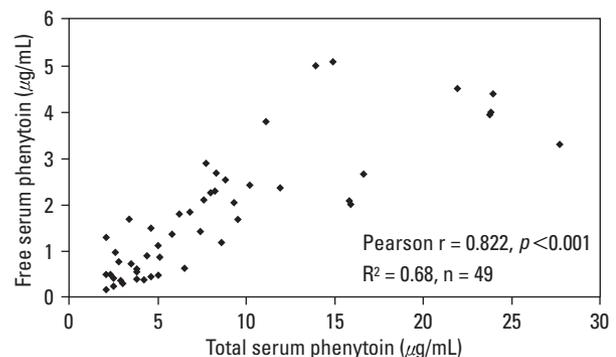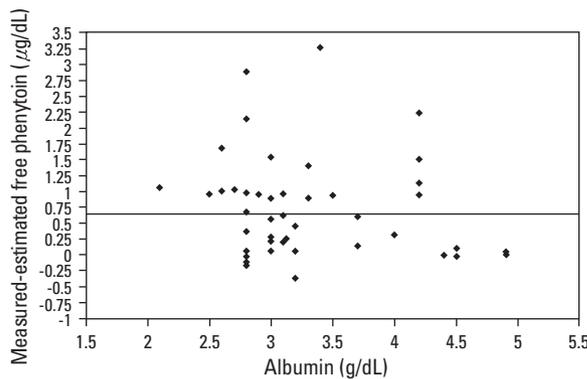

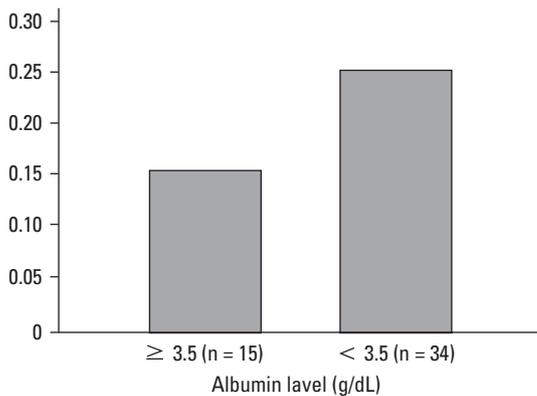**Fig. 1.** Distribution of free and total serum phenytoin concentrations.

(Fig. 1). The coefficient of determination between free and total phenytoin concentrations ($R^2 = 0.68$) indicated that 68% of the variation in free levels is explained by total phenytoin concentration ($p < 0.001$).

After correction using the Sheiner-Tozer equation, the correlation between free and total phenytoin concentration was not improved (Pearson r = 0.762, $R^2 = 0.58$), and the mean difference between measured and calculated free phenytoin (one tenth of the Sheiner-Tozer corrected value) remained high (0.65 ± 0.88 µg/mL; 95% confidence interval, -1.11 to 2.41). There was also substantial scatter in this relationship (Fig. 2).

A negative correlation was observed between albumin and free phenytoin fraction, demonstrating that lower serum albumin levels were associated with higher free fractions. Interestingly, the binding ratio observed with hypoalbuminemia was elevated to 0.25 (25.3% free phenytoin) (Fig. 3).

We also compared the percent difference between the calculated and measured free phenytoin levels of the hypoalbuminemic (< 3.5 g/dL) and normoalbuminemic (≥ 3.5 g/dL) groups. By cross tabulation analysis, hypoalbuminemic patients more often had a greater percent difference (≥ 20%) than observed in the normoalbuminemic group ($p = 0.029$) (Table 1).

DISCUSSION

There has been debate about whether the measurement of free serum phenytoin is necessary to ensure that therapeutic levels of the phenytoin are not exceeded in epileptic patients. A number of previous studies have drawn attention to the fact that the use of total phenytoin concentrations in certain patient groups may misguide therapy. Dutkiewicz et al.[13] showed that in hypercholesterolemia and mixed hyperlipidemia, the blood level of free phenytoin was elevated, with the degree of change being dependent on the type of hyperlipidemia. The effect was shown to be related to displacement of the drug from albumin by fatty acids.[14] Doucet et al.[15] found differences in the protein binding of phenytoin in diabetics and concluded that the difference in protein binding between diabetic and control sera was related to the glucose-independent modification of albumin. In patients with renal disease and in some with hepatic disease, there may be not only a low albumin concentration but also a decrease in protein binding.[16,17]

It is generally recognized that only free phenytoin molecules are pharmacologically active. The development of side effects has been reported to correlate better with free phenytoin concentrations rather than with total phenytoin concentrations.[18,19] Banh et al. showed that most patients who developed phenytoin toxicity had a normal or low total phenytoin concentration but an elevated free serum phenytoin concentration.[18]

Our study also showed that free phenytoin levels calculated using total phenytoin concentrations were unsuitable for directing therapy in hypoalbuminemic patients, and that free concentrations should be routinely monitored.

Fig. 2. The mean difference (solid line) between actual and calculated free phenytoin. One tenth of the Sheiner-Tozer corrected value was 0.65 ± 0.88 µg/mL; 95% confidence interval, -1.11 to 2.41, and considerable scatter is observed in this relationship.

Fig. 3. Phenytoin binding ratio and serum albumin concentration in patients. The binding ratio of free to total phenytoin is greater (25.3% free phenytoin) in patients with hypoalbuminemia (less than 3.5 g/dL) ($p = 0.002$).

Table 1. Cross Tabulation of Percent Difference and Albumin Level, Demonstrating a Much Greater Percent Difference in Hypoalbuminemic Patients ($p = 0.029$)

	Percent difference ≥ 20%	Percent difference < 20%	Total
Albumin < 3.5	29	5	34
Albumin ≥ 3.5	8	7	15
Total	37	12	49

Serum albumin concentrations < 3.5 g/dL have previously been shown to affect phenytoin binding ratios and to contribute to phenytoin intoxication.[20] Decreased protein binding leads to an increase in the free fraction of the drug. The free fraction of the drug is the portion that is available for metabolism and clearance by the liver. In essence, increzasing the free fraction of the drug increases the substrate present to the liver for metabolism, thereby potentially increasing the metabolism of the drug. This may result in a decrease in the total concentration of the drug while the free concentration remains constant. In this setting, patients are at particular risk for toxicity if clinicians increase doses in order to achieve therapeutic total concentrations.[21] In our patients, we found that 15 patients (31%) had free concentrations above the therapeutic level (> 2 µg/mL) despite total concentrations within the therapeutic range. Among these 15 patients, 10 patients had severe hypoalbuminemia.

The Sheiner-Tozer equation was recently used in a study to calculate free concentration in critically ill neurosurgical patients.[12] The authors found that, in this uniform patient population, the equation provided an unbiased and precise means of estimating free concentration if direct measurement was unavailable or impractical. The authors stated that the reason for the precision in their patient population, and hence the reason why their results differed from those of previous reports, was that their patients did not have clinical conditions that alter protein binding.[22]

Considering our results, methods of calculating the free concentration of phenytoin in patients with hypoalbuminemia should be used with caution.

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