Journal of Epilepsy and Clinical Neurophysiology

J Epilepsy Clin Neurophysiol 2010;16(2):69-73

Total, Unbound Plasma and Salivary Phenytoin Levels in Critically Ill Patients

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ABSTRACT

Objective: To assess the reliability of salivary phenytoin (PHT) concentrations and predicted free PHT levels by Sheiner-Tozer equation in order to substitute measured free PHT concentrations in critically ill patients. **Methodology:** Twenty-four neurocritically ill adult patients receiving intravenous PHT were included in the study. Analyses of total, free plasma and saliva PHT concentrations were performed by fluorescence polarization immunoassay. Plasma albumin levels were also determined. **Results:** Free PHT concentrations as well as salivary levels better correlate to clinical effect than total drug concentrations. Linear regression analysis showed a strong correlation between estimated free PHT concentrations by Sheiner-Tozer and measured free PHT levels ($r=0.835$; $p<0.001$) and salivary PHT concentrations and measured free PHT concentrations ($r=0.964$; $p<0.001$). Sheiner-Tozer equation could be misleading in the presence of displacing drugs. **Conclusions:** Saliva may serve as a feasible fluid to plasma in order to be used as a surrogate for free concentration monitoring of PHT in this population.

Keywords: Phenytoin, unbound concentration, saliva concentration; Sheiner-Tozer equation.

RESUMO

Níveis totais, livres em plasma, e salivares de fenitoína em doentes graves

Objetivo: Avaliar a confiabilidade de concentrações salivares de fenitoina (PHT) e níveis livres de PHT pronosticado por equação de Sheiner-Tozer, o efeito da substituição das concentrações medidas livres de PHT em doentes graves. **Método:** Vinte e quatro doentes adultos que recebem PHT intravenoso forem incluídos no estudo. Análises de PHT total, livre em plasma e saliva foram realizadas por uma técnica de imune fluorescência polarizassem. Os níveis de albumina em plasma foram também determinados. **Resultados**: Concentrações livres de PHT em plasma e saliva correlacionam melhor ao efeito clínico que concentrações de fármaco total. Análise de regressão lineal mostrou uma correlação forte entre concentrações livres de PHT estimadas por Sheiner-Tozer e os níveis livres de PHT medidos ($r=0.835$; $p<0.001$), e entre concentrações em saliva de PHT e concentrações livres medidas de PHT ($r=0.964$; $p<0.001$). A equação de Sheiner-Tozer poderia ser inadequada na presença de fármacos competidores da ligação às proteínas. **Conclusões**: Saliva pode servir como substituto do plasma para ser utilizado no controle de concentração livre de PHT em plasma nesta população.

Unitermos: Fenitoína, concentração livre, concentração em saliva; equação de Sheiner-Tozer.

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Received Mar. 26, 2010; accepted Apr. 30, 2010.

INTRODUCTION

As the measurement of total drug concentration (free drug plus protein-bound drug) is much easier and far cheaper than measurement of free drug concentration, therapeutic drug monitoring services generally determine only the total drug concentration. Although measurements of drug concentrations in plasma correlate far better with clinical effect than the drug dose does, the total concentration of a drug in blood does usually not represent the concentration of the drug at its receptor site. Only the free drug passes from the blood or plasma into the biophase and can interact with a receptor to produce a given pharmacological and therapeutic effect. Due to this fact, therapeutic levels of total drug depend directly on its plasma protein binding, so this factor plays an important role in monitoring drugs with high affinity to proteins (more than 80%).[1,2]

Routine measurement of free drug concentration may be desirable, but it is difficult to achieve in practice as equilibrium dialysis is time-consuming and requires large sample volumes and the numerous membranes commercially available that allow free drug to be measured in the ultrafiltrate are costly, such as the Amicon Centrifree micropartition system.

In critical care units, intravenous phenytoin (PHT) is the first-line drug in treatment of generalized tonic-clonic seizures.

PHT, a weak acid, is highly protein bound, primarily to albumin, which results in a free fraction of only 10%. Commonly, total serum PHT concentrations are measured and used as surrogates for free levels in adjustment of dosing regimes. Monitoring of total serum PHT, however, may be misleading because serum albumin may be reduced in critical disease states. This is due, in most cases to a systemic inflammatory response to surgery or sepsis with increased capillary permeability with large amounts of fluids and solutes leaking into the interstitial space. Others causes of hypoalbuminemia in this population are: reduced dietary protein intake, decrease in gene transcription mediated by interleukin-6 and tumour necrosis factor α, reduced hepatic synthesis or increased degradation and renal disease.[3,4] Hypoalbuminemia results in an increased fraction of unbound drug.

In addition, certain drugs such as valproic acid (VPA)[5] or endogenous compounds in patients with renal failure may further increase free PHT fraction by competitive displacement from protein-binding sites.

This increase in free drug fraction results in an increased distribution volume, increased clearance and therefore, a fall in total drug concentration.

Unfamiliarity with this pharmacological interaction in a critical care setting may lead to an increase in the dose of PHT in a patient with low total serum PHT level (less than 10 mg/L) who in fact has a normal free PHT level (between 1 and 2 mg/L).

PHT-related toxicities in patients with total drug levels in plasma within the population therapeutic range (10-20 mg/L) but with high free PHT levels have been reported in the literature [6]. Nystagmus, diplopia, vomiting, alterations of consciousness and paradoxically an increase in the number of seizures were reported in Lindow et. al.[7] This latter side effect could be misinterpreted and could lead to an increase in dose although in fact, levels in the biophase are elevated.

As the unbound fraction of PHT represents the active drug and is therefore of therapeutic interest, corrective algorithms, such as the Sheiner-Tozer equation [8,9] have been proposed to predict unbound PHT levels in adult patients with abnormal albumin levels. However, because the unbound level of PHT is highly dependent on a number of factors in addition to serum albumin level, the determination of free PHT levels using this equation might be misleading.

For a number of drugs, mainly those which are lipophylic and non significant ionized at salivary pH range, studies have demonstrated that the total concentration of a drug in saliva is equal to the concentration of free drug in plasma and therefore, more reflective of drug concentration in the biophase.[10,11,12] Saliva might be a natural plasma ultrafiltrate , making it a better surrogate for free PHT levels and, if this is the case, therapeutic drug monitoring in this fluid may be more advantageous than total plasma drug monitoring and much cheaper than free drug determination.

The purpose of this study was to assess the reliability of salivary PHT concentrations and to determine the validity of Sheiner-Tozer equation in this patient population by comparing both procedures with free PHT levels.

MATERIAL AND METHODS

Patients

The study population was composed of patients admitted to the intensive care unit of "Hospital de Clínicas" Dr. Manuel Quintela between January and December 2009.

Only patients needing routine monitoring of PHT were included in the study.

All were neurocritically ill patients and required phenytoin therapy for prophylaxis or suppression of seizures.

Blood and whole saliva samples were obtained simultaneously at steady state.

For saliva collection, citric acid crystals were placed on the tongue, and one millilitre saliva was collected with a syringe from the oral cavity.

Blood was collected by arm venipuncture and drawn into pre-heparinized tubes.

Blood samples obtained from the patients were used to measure unbound and total plasma concentrations.

The research protocol was approved by the Ethics Committee of the Hospital.

PHT and albumin assays

Blood samples were centrifuged and both plasma and saliva samples were stored at -20°C until analysis. Analysis was carried out in the clear supernatant of saliva (following centrifugation; 3000 g at room temperature for 10 min).

Analyses of total, free plasma and saliva PHT concentrations were performed by fluorescence polarization immunoassay using TDx analyser (Abbot Laboratories; Chicago, IL, USA). Free PHT drug was obtained by ultrafiltration using a SIGMA 3K18 refrigerated centrifuge and centrifree Micropartition System (Amicon-Centrifree), Approximately 1 mL of plasma was pipetted into the ultrafiltration device, then centrifuged at 2000 g at 25±2ºC for 20 minutes

Plasma albumin levels were determined by colorimetric analysis (Hitachi 911 Automatic Analyser, Roche Laboratories).

Corrective algorithms: Sheiner-Tozer equation

Free PHT concentration was predicted using the following equation:

$$C_{corr} = \frac{C_{exp}}{(1-\alpha)\,(C_{alb}/4{,}2)+\alpha} = \frac{C_{exp}}{0{,}21\,C_{alb}+0{,}1}$$

Being:

Cexp: experimental concentration of total plasma PHT (mg/L)

Calb: plasma albumin concentration (g/dL)

α: fraction of unbound PHT

Ccorr: total PHT concentration estimated by Sheiner-Tozer

This equation corrects total concentration in the presence of hypoalbuminemia considering a free PHT fraction of 10% and a plasma albumin level of 4.2 g/dL.

Free drug levels at different albumin concentrations were estimated according to Sheiner-Tozer equation using 0.1 X C corr.

Statistical Analysis

Correlations between experimental and predicted free PHT levels, and salivary levels were performed by least-square regression analysis (SPSS, Inc., Chicago).

RESULTS

Twenty-four adult patients (9 female and 15 male) were included in this study. The mean age of patients was 51.5 years, with a range of 14 to 82 years. Thirteen were receiving PHT therapy for suppression of seizures and the remaining eleven as seizure prophylaxis.

Mean intravenous daily dose of PHT was 402 mg± 52.2 mg (range 375-500 mg).

A total of 44 PHT determinations were done.

Twenty-three patients were with hypoalbuminemia. The plasma albumin ranged from 1.5 g/dL to 3.4 g/dL with a mean of 2.58 g/dL±0.57 g/dL (normal: 3.5 g/dL-5.0 g/dL).

Five patients were also receiving a displacing drug (VPA).

Mean free PHT fraction in these patients was 0.169±0.080, significantly higher ($p<0.001$) than the one reported for patients with normal albumin (0.10).[9]

Only the patients with documented epilepsy were taken into account to correlate total and free levels with clinical status in order to know which concentration gives more reliable information regarding the pharmacological effect of PHT (Table 1).

From the seven epileptic patients with controlled seizures, six presented total PHT concentrations below therapeutic range (<10 mg/L). In five patients free concentrations were in the range of 1 mg/L to 2 mg/L.

Six patients presented symptoms of drug toxicity with total drug levels below 20 mg/L in four of them. However, their free PHT concentrations exceeded 2 mg/L. Toxic symptoms were nystagmus, lack of consciousness and exacerbation of seizures. In one patient, the administration of a loading dose because of a low total plasma concentration resulted in toxicity, whereas initially the free concentration in this patient was adequate with good seizure control.

Table 1. Mean salivary, total and unbound concentration of PHT and clinical status of the patient. Concentrations are expressed as mean ± SD. (A) stands for average.

Clinical status	Mean free plasma concentration (mg/L)	Mean total plasma concentration (mg/L)	Mean saliva concentration (mg/L)
Sub-therapeutic	0.82±0.51 (n=14)	6.0±4.1 (n=14)	0.78 ±0.58 (n=7)
Therapeutic	1.7±1.1 (n=7)	9.3±7.1 (n=7)	(A) 1.5 (n=2)
Toxic	3.9±1.7 (n=11)	23.8±9.6 (n=11)	(A) 5.4 (n=4)

Correlations

20 paired values of free plasma and salivary concentrations were obtained. Salivary samples with blood contamination were discarded.

In patients with hypoalbuminemia, estimation of free drug using Sheiner-Tozer equation was done using total plasma levels and plasma albumin.

Linear regression analysis showed a strong correlation between estimated PHT concentrations by Sheiner-Tozer and free levels (r^2=0.835; $p<0.001$) and salivary PHT concentrations and free concentrations (r^2=0.964; $p<0.001$) (Figures 1 and 2).

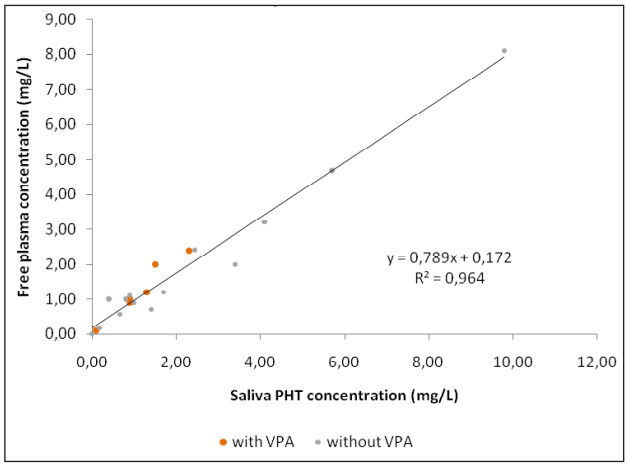

Figure 1. Saliva PHT concentrations versus free plasma concentrations in critically ill patients. Patients with VPA are highlighted.

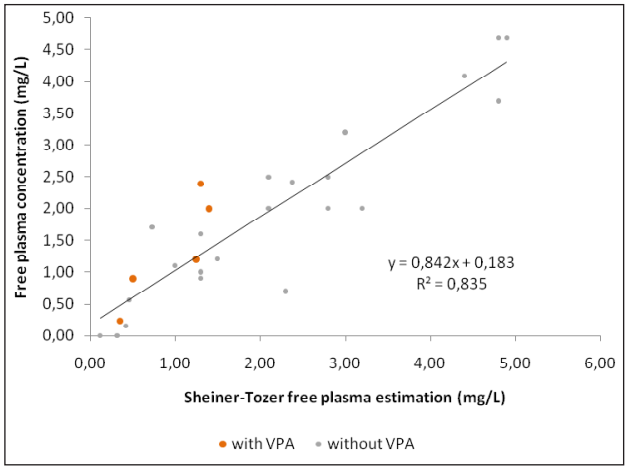

Figure 2. Free PHT estimation by Sheiner-Tozer equation versus free plasma concentrations in critically ill patients. Patients with VPA are highlighted.

DISCUSSION

Total and free drug concentrations and clinical status

From the results obtained, we could state that the mean free fraction of PHT is significantly higher in critically ill patients compared to other patient population. The main cause of this elevated free fraction is the high incidence of hypoalbuminemia (96%) in the population studied. Only one patient had plasma albumin within the acceptable range, however VPA (1800 mg/day) was part of his therapy. In this patient, the variation of the free fraction of PHT due to competition for binding to albumin between PHT and VPA was observed. This patient showed a markedly different free fraction: 10.5 % compared to 15.6 %, with different blood VPA concentrations: 13.3 mg/L and 42.3 mg/L respectively. Therefore, competitive displacement from albumin by VPA presumably affects significantly free fraction depending on the VPA concentrations.

As it is shown in Table 1, free PHT concentrations better correlate to clinical effect of epileptic patients than total drug concentrations. The latter did not distinguish between subtherapeutic and therapeutic effect.

Alternative to plasma ultrafiltration

The present work studied which alternative: Sheiner-Tozer estimation or saliva better acts as a surrogate for plasma ultrafiltrate measurement.

Figures 1 and 2 show the relationship between free drug in plasma versus saliva and versus free drug estimated by Sheiner-Tozer equation. Those pairs of values with VPA are highlighted. As it can be observed, these values are randomly distributed, not affecting the correlation with the free drug. However, most of these patients had very low VPA levels, so the data suggest that the increase in free fraction could be attributed mainly to low plasma albumin in these cases. On the other hand, when VPA concentration gets near the therapeutic range, the displacement from protein binding is not longer despicable and Sheiner-Tozer estimation yields unreliable values. This fact could be evidenced in one patient with plasma VPA concentration of 54.5 mg/L and plasma albumin of 3.0 g/dL. PHT monitoring revealed a total PHT level of 9.5 mg/L, free PHT level of 2.4 mg/L and a salivary PHT level of 2.3 mg/L. Nevertheless, the resulting Sheiner-Tozer equation gave a prediction of free PHT concentration of 1.3 mg/L.

Concerning the use of saliva as monitoring fluid, our results showed not only a good correlation between this fluid and the clinical status of the patient (Table 1) but also a highly significant correlation between salivary and free plasma concentrations, not affected by displacing drugs.

Moreover, saliva presents advantages with respect to Sheiner-Tozer estimation, as it does not require previous knowledge of albumin or total drug concentration.

CONCLUSIONS

To conclude, we can state that total plasma concentrations do not provide a reliable estimate of pharmacological effects. Routing monitoring of free PHT plasma concentrations would be preferable for more accurate dosing of medication and for the improvement of seizure control and the reduction of adverse effects. One major disadvantage is the associate cost of this procedure for routine monitoring.

Sheiner-Tozer estimation could be a solution if no displacing drugs are included in the treatment.

In view of this, salivary therapeutic drug monitoring may serve as a viable alternative to plasma free concentration monitoring of PHT in this population due to its low cost and its good correlation with the effect.

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