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Original Article

Total Phenytoin concentration is not well correlated with active free drug in critically-ill head trauma patients

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ABSTRACT

Objective: Phenytoin is an antiepileptic drug used widely for prophylaxis and treatment of seizure after neurotrauma. Phenytoin has a complex pharmacokinetics and monitoring of its serum concentrations is recommended during treatment. Total phenytoin concentration is routinely measured for monitoring of therapy. In this study, we evaluated the correlation between phenytoin total and free concentrations in neurotrauma critically-ill patients to determine whether the phenytoin total concentration is a reliable predictor of free drug, which is responsible for the therapeutic effects.

Methods: A total of 40 adult head trauma patients evaluated for free (unbound) and total serum phenytoin concentrations. Patients were divided into two groups. Group A consists of 20 unconscious patients with severe head injury under mechanical ventilation and Group B consists of 20 conscious self-ventilated patients. Correlation and agreement between total and free phenytoin plasma concentrations were analyzed.

Findings: Pearson correlation analysis and Bland-Altman test showed weak to moderate correlation ($r = 0.528$) and poor agreement between free and total phenytoin concentrations in patients with severe trauma and higher Acute Physiology And Chronic Health Evaluation II (APACHE II) scores (Group A) and good correlation ($r = 0.817$) and moderate agreement in patients with mild to moderate trauma and lower APACHE II scores (Group B).

Conclusion: Our results indicated that total phenytoin serum concentration is not a reliable therapeutic goal for drug monitoring in severely-ill head trauma patients even in the absence of hypoalbuminemia, renal and hepatic failure. It seems justifiable to measure free phenytoin concentration in all severely ill neurotrauma patients.

Keywords: Critically-ill patients; free drug concentration; head trauma; hypoalbuminemia; Phenytoin

INTRODUCTION

One of the ominous consequences of traumatic brain injury (TBI) is seizure with overall incidence of 3.1% in head trauma patients; although, it largely depends on the severity of trauma and may occur in 17% of patients with severe TBI.[1] Phenytoin is an antiepileptic drug (AED) and because of its efficacy and some other characteristics such as having parenteral formulation and lower side-effects compared with other traditional AEDs, has extensive use in preventing early seizures in TBI patients.[2] About 90% of phenytoin is bound to serum albumin (Alb), hence only 10% of the drug is in unbound (free) form, which can cross physiologic barriers and is responsible for its clinical efficacy.[3] Phenytoin

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has a narrow therapeutic index and based on saturable metabolism and non-linear pharmacokinetic properties, many variables such as plasma protein binding, concomitant disease and other high-protein bound drugs can alter phenytoin kinetic.[4] This sensitive pharmacokinetic of phenytoin make a great challenge with its dose adjustment and highlight the need for therapeutic drug monitoring (TDM) in head trauma patients in order to obtain desirable drug levels.[5] It is best shown that in the presence of some known conditions, i.e., hypoalbuminemia, hepatic and renal failure and interaction with other high-protein-bound drugs, monitoring of total phenytoin cannot concisely predict free drug concentration and these are poorly correlated.[3,6] We hypothesized that in critically ill-patients, in addition to mentioned factors, there might be some other considerations, which make it necessary to monitor free fraction of phenytoin.

The objective of this study is to evaluate the correlation of free and total phenytoin concentrations in critically ill neurotrauma patients in the absence of hypoalbuminemia, renal and hepatic failure. We also intended to evaluate the influence of severity of illness in the monitoring of total and free phenytoin concentrations in TBI patients.

METHODS

This study was a prospective cohort conducted at three intensive care units (ICU) from October 2010 until July 2012. Forty adult head trauma patients who were admitted to neurosurgical ICU and required intravenous (IV) phenytoin for the prophylaxis of post injury seizures were enrolled in the study and divided into two groups. Group A consist of 20 comatic patients who had severe TBI with Glasgow Coma Scale (GCS) <8 and also required mechanical ventilation. Group B consist of 20 patients who were conscious and did not require mechanical ventilation (GCS ≥ 8), but have other risk factors for seizure including cortical contusion, depressed skull fractures, subdural or epidural hematoma, intracerebral hemorrhage, penetrating head injury or occurrence of seizure within 24 h after head injury. Initial Acute Physiology and Chronic Health Evaluation II (APACHE II) score and the sequential organ failure assessment (SOFA) score[7,8] calculated in time of recruitment for both groups. Patients were included if they were aged 18 or older, needed seizure prophylaxis as diagnosed by two intensivists and have baseline serum Alb > 3 g/dL. Patients were excluded if any of the following conditions present: Bradycardia (heart rate < 50 beets/min), second or third degree heart block, hypotension (mean atrial pressure < 65 mmHg), hepatic or renal insufficiency (i.e., total bilirubin > 2 mg/dL, alanine aminotransferase > 3 times of the upper limit of the normal range, serum creatinine > 1.5 mg/dL), patients with the history of phenytoin administration in the last 2 weeks or hypersensitivity to phenytoin. Patients with serum Alb < 3 and elevated serum creatinine excluded to eliminate the effects of hypoalbuminemia and uremia.

Patients were closely observed to restrict medications, which are known to affect phenytoin metabolism (cimetidine, calcium channel blockers, azole anti fungals, ciprofloxacin, erythromycin, chloramph enicol and phenobarbital) or alter its protein binding (warfarin, aspirin, heparin, tolbutamide, valproic acid or sulfonamides) throughout the study. This study was approved by Institutional Investigational Review Board for human and animal studies in Tehran University of Medical Sciences. Consent forms obtained from all patients or their legally authorized representatives.

Patients received phenytoin sodium as an IV loading dose of 15 mg/kg (50 mg/mL in 40% propylene glycol and 10% ethanol) followed by 375 mg/day maintenance dose (4-7 mg/kg/day divided in three doses), administered by IV infusion at 8 h intervals. Each dose was diluted in 100 mL of 0.9% saline and administered over 30 min. Blood samples for phenytoin trough level (30 min before the next dose) were collected in 4th day of therapy, the time that is expected for phenytoin to reach the steady state. Blood samples were obtained from central venous catheter. Venous blood samples (5 mL) were centrifuged at room temperature for 10 min at 3000 rpm. The serum samples were transferred and stored at −70°C temperature until final assay. Serum samples were filtered through suitable ultrafilter (amicon, cut-off = 5000 Da) for 30 min at 1000 rpm for the preparation of free fraction.

A high-performance liquid chromatography (HPLC) method was developed for the analysis of total and free phenytoin serum concentrations. Water: acetonitril (60:40) was used as mobile phase with flow rate of 1.4 mL/min and the eluent was monitored at 220 nm. For determination of phenytoin concentrations, serum samples were deproteinized with the same volume of acetonitrile. About 100 µL ultrafiltrate and 50 µL of deproteinized supernatant were injected into the HPLC column for determination of free and total phenytoin concentrations, respectively. Samples were introduced to HPLC column (Eurospher-Germany, C18 [5 µ, 150 mm × 4.6 mm with precolumn]) through a rheodyne injector fitted by a 100 µL loop. Column temperature was maintained at 30°C during the assays.

Continuous variables were presented as mean ± standard deviation and categorical variables as frequency (%). To compare patient characteristics between Group A and B, the Chi-square and the independent Student's *t*-test were used for categorical and continuous data, respectively. Pearson correlation coefficient and Bland-Altman (difference) plot were used to compare the correlation and agreement respectively between the total and free phenytoin concentration measurement. Data were analyzed with SPSS (version 11.5) software [Statistical Package for the Social Sciences; Chicago, Illinois, USA] and $P < 0.05$ was considered to be significant.

RESULTS

Forty 18-80 years old patients with head trauma (88.9% men and 11.1% women) who were receiving phenytoin and were eligible to participate in the study were enrolled and blood samples as well as their demographic and clinical parameters were collected. In Group A, there were 20 head trauma patients with GCS ≤ 8 who were under mechanical ventilation and in Group B there were 20 head trauma patients with GCS = 8-15 who did not need mechanical ventilation. In Group B, one patient due to false early diagnosis and three patients due to receiving phenobarbital during study period, excluded from the study and finally, data from 16 patients were analyzed. There were no significant differences in initial collected parameters between two groups except for GCS, SOFA and APACHE II scores [Table 1]. The values of mean free and total serum phenytoin concentrations, also the ratio of free/total phenytoin in both Groups A and B are presented in Table 1.

In Group A, 8 patients (40%) finally died in ICU and 12 patients discharged. In Group B, all patients finally discharged from ICU. In both groups, phenytoin total serum concentration only in 50-55% of patients (11 patients in Group A and 8 patients in Group B) was in the therapeutic range (10-20 mg/L). Five (25%) patients in Group A and 2 (12.5%) patients in Group B had sub-therapeutic phenytoin levels. Four (20%) patients in Group A and 6 (37.5%) of patients in Group B had toxic levels (above 20 mg/L). Of four patients in Group A, which had toxic phenytoin levels, two patients had levels above 40 mg/L, which considered highly toxic. In Group B, none of the patients had levels above 40 mg/L.

As shown in Table 1, there was no significant difference in free ($P = 0.08$) and total ($P = 0.68$) serum phenytoin concentrations between two groups. By contrast, there was a significant difference in mean free/total serum concentrations between Groups A and B ($P = 0.004$). Pearson correlation analysis showed a significant but poor to moderate correlation between free and total phenytoin concentrations in Group A ($r = 0.528$, $P = 0.017$), but a good correlation between free and total phenytoin concentrations in Group B ($r = 0.817$, $P \leq 0.001$). Agreement analysis with Bland-Altman test showed a poor agreement between free and total phenytoin concentrations in Group A and moderate agreement in Group B. Plots of Bland-Altman analysis for Groups A and B are presented in Figures 1 and 2 respectively.

Table 1: Demographic and clinical parameters and phenytoin concentration analysis in patients of both study groups

Variable	Group A (*N*=20) (%)	Group B (*N*=16) (%)	*P* value
Age	38.7±14.97	34.13±16.16	0.37
Gender			
Male	17 (85)	15 (93.8)	-
Female	3 (15)	1 (6.2)	-
Mechanism of injury			
Car accident	14 (70)	11 (68.8)	-
Motor vehicle accident	4 (20)	3 (18.8)	-
Falling	2 (10)	2 (12.5)	-
Initial GCS	5.95±1.96	14.19±1.38	<0.001
Initial APACHE II	15.25±3.7	7.38±2.25	<0.001
Baseline serum albumin concentration	3.79±0.31	3.67±0.23	0.083
Baseline serum creatinine concentration	0.91±0.41	1.05±0.25	0.172
Free concentration of phenytoin (mg/L)	1.10±0.56	0.82±0.29	0.08
Total concentration of phenytoin (mg/L)	16.17±10.96	17.56±8.35	0.68
Free/total phenytoin concentration	0.08±0.04	0.05±0.01	0.004

Data are presented as mean±SD or *N* (%) where applicable, Group A: Patients with GCS<8 and under mechanical ventilation, Group B: Patients with GCS ≥8 who do not need mechanical ventilation, APACHE II=Acute physiology and chronic health evaluation II, GCS=Glasgow coma scale, SD=Standard deviation

DISCUSSION

The results of this study indicate that by increasing the severity of trauma and illness in critically ill TBI patients and with higher APACHE II and SOFA scores, the correlation and agreement between free and total serum concentrations decrease substantially. In most patients, total serum phenytoin concentration is measured for dose adjustment in routine practice. Measurement of free phenytoin is more expensive, require a longer time to analysis and is not available everywhere. Friel *et al.*, reported there is better correlation between brain phenytoin concentrations and free serum levels relative to total phenytoin levels in serum, both in epileptic and non-epileptic patients.[9]

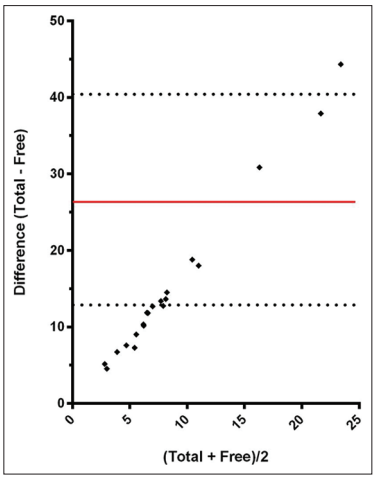

Figure 1: Plot of agreement analysis with Bland-Altman test for Group A. Total: Total concentration of phenytoin, Free: Free concentration of phenytoin

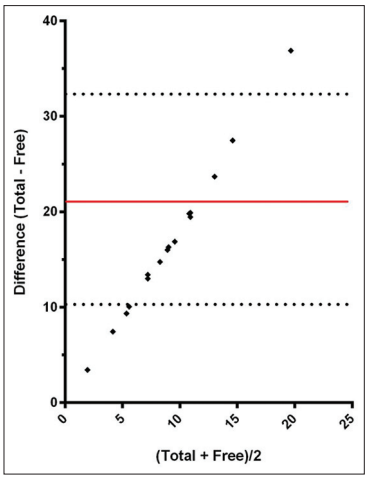

Figure 2: Plot of agreement analysis with Bland-Altman test for Group B. Total: Total concentration of phenytoin, Free: Free concentration of phenytoin

Studies conducted in non-critically ill epileptic patients, found a strong correlation (r = 0.98) between free and total serum concentrations and suggest that routine monitoring of free phenytoin levels is unnecessary in patients with Alb levels ≥ 3 g/dL.[10,11] But in the area of critical care, there are other considerations; Zielmann *et al*., conclude that in critically ill trauma patients, there are various factors that have influence on phenytoin concentration; therefore, it seems justifiable to measure free phenytoin concentration in critically ills to prevent toxicities.[5] In a retrospective study in critically ill pediatric patients, there was only a moderate correlation (r = 0.795) between free and total serum phenytoin concentrations and authors concluded that total phenytoin concentration is not a well-qualified measure for monitoring treatment in critically ill pediatric patients.[12] The result of explained study in pediatric patients is in agreement with this study by the explanation that in our adult critically ill-patients, correlation was even weaker in patients with more severe illness (r = 0.528).

Several studies have reported that unbound phenytoin concentrations increase in critically ill-patients. Major causes described are hypoalbuminemia, renal or hepatic failure and co-administration with highly protein-bound drugs.[5,12,13] Fedler and Stewart found that in patients with hypoalbuminemia, monitoring of free phenytoin concentration has better correlation with desired clinical outcome.[14] In one study conducted in severe neurotrauma patients, it is shown that there was a strong inverse correlation between Alb levels and free phenytoin concentrations (r = 0.85). Furthermore, they showed that there is a week correlation between free and total phenytoin concentrations in these patients (r = 0.60), which is highly in agreement with our findings in severe head injury patients. In the mentioned study, this correlation did not compare between patients with low and high initial APAHE II scores and hypoalbuminemia accounted as the major cause for such findings.[15]

Krasowski and Penrod evaluated the free and total phenytoin levels correlation and difference with adjusted phenytoin levels (estimated by Sheiner-Tozer equation) as an alternative method in hypoalbuminemic conditions. The authors stated that free phenytoin concentration should be measured as possible and if this is not feasible, estimation of phenytoin levels by formula, could be used as a supplement tool for total phenytoin measurement.[16]

One major difference between our study and others, is that in our study none of the patients had renal or liver impairment and none of them was hypoalbuminemic (Alb < 3.5) at the time of drug's level measurement. The mean free/total value was lower than expected (<10%) that is in contradiction with some previous results in critically ill settings.[5,17] One reason might be the normal baseline Alb levels in our patients, since those studies assume that decreasing in protein binding related to hypoalbuminemia is mainly responsible for increasing in free phenytoin levels. Shohrati *et al*., evaluated phenytoin pharmacokinetics in 20 severe neurotrauma patients and found that phenytoin actual maximum metabolic rate (Vmax) in these patients is significantly higher than expected and concluded that this could be the reason of low phenytoin levels in their patients.[18] Critical illness, trauma and acute inflammation can cause elevation in acute-phase proteins in serum such as α_1-acid glycoprotein (AAG).[19-21] In addition to Alb, phenytoin also is able to bind to AAG in human serum and it

is shown that increasing in AAG levels can increase phenytoin protein binding therefore leads to decrease in free fraction of phenytoin.[22] Thus, decreased free/total phenytoin levels in our patients especially those with more severe injuries, might be due to probable increasing in drug's Vmax, acute inflammatory response after severe neurotrauma and critical illness in these patients.

In conclusion, the results of our study along with other studies emphasize the importance of TDM of phenytoin, especially in critically ill-patients. Phenytoin dosage should be individualized and patients should be monitored continuously to prevent both toxicity and under-treatment. Monitoring of free (unbound) serum phenytoin is more reliable measure compared with total drug levels in critically ill-patients especially those with more severe head trauma and high APACHE II score.

AUTHORS' CONTRIBUTION

All of the authors have contributed in data gathering. Sadeghi K, made substantial contributions in reviewing the literature and preparing the manuscript. Mojtahedzadeh M, Najafi A, Ahmadi A, and Rouini MR, had substantial contributions to conception and design of the study, selection of the patients and editing the manuscript. Beigmohammadi MT, Hadi F, Mahmoodpoor A, Farhudi Sh, and Hendoui N, participated in the selection of eligible patients and collecting samples and patients' data in three intensive care units. Hamishehkar H, carried out data analyzing and edited the manuscript. All of the authors gave approval for the final edition of this article.

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