Correlation between Serum and Salivary Phenytoin Concentrations in Thai Epileptic Children

Sahas Liamsuwan MD*,
Ulailuk Jaiweerawattana MSc*

** Division of Child Neurology, Queen Sirikit National Institute of Child Health, Department of Medical Services, Ministry of Public Health, College of Medicine, Rangsit University, Bangkok, Thailand*

***Objective**: To study the correlation between serum and salivary phenytoin concentration in Thai epileptic children.*
***Material and Method**: Children aged 5 to 12 years with diagnosed epilepsy who received phenytoin monotherapy seen in the neurological clinic at Queen Sirikit National Institute of Child Health were studied. The recruited patients were required to have good compliance, normal albumin level, and no evidence of cancer, HIV infection, hepatic, renal and salivary glands disease. Blood and saliva samples were collected and measured phenytoin level by fluorescence-polarization immunoassay technique.*
***Results**: Thirty patients, 19 males and 11 females, were studied. The average (mean $\pm$ SD) age and weight were 8.24 $\pm$ 2.09 years and 27.76 $\pm$ 9.86 Kilograms. Both serum and salivary phenytoin levels correlated with phenytoin doses as exponential type (R^2 = 0.4188, 0.3682, respectively). Equations for describing serum and salivary phenytoin levels by phenytoin dose were $y = 0.7403e^{0.3952x}$ and $y = 0.1431e^{0.3072x}$ respectively. Serum and salivary phenytoin levels were closely correlated as linear type (R = 0.880, R^2 = 0.967). The obtained equation of this relationship was $y = 10.165x$, where y = serum phenytoin level and x = salivary phenytoin level. Adverse drug reactions were found in 5 patients (6.6%), horizontal nystagmus 2 cases, hirsutism 2 cases and gingival hyperplasia 1 case.*
***Conclusion**: High correlation between serum and salivary phenytoin levels supported the use of saliva instead of blood for phenytoin monitoring in Thai children which were difficult in blood collection and had psychological trauma. The obtained equations in the present study could be applied for adjusting the dosage regimen and monitoring by using salivary phenytoin level in clinical practice.*

***Keywords**: Phenytoin, Salivary level, Thai children*

J Med Assoc Thai 2011; 94 (Suppl. 3): S172-S177
***Full text. e-Journal:** http://www.mat.or.th/journal*

Epilepsy is a common neurological disorder. The current definition of epilepsy is that an individual must experience at least two unprovoked seizures in separated by at least a 24-hour period[1]. The clinical manifestations of seizure involve sudden and transitory abnormal symptoms including alteration of consciousness, motor, sensory, autonomic or psychic function, Epilepsy can be found in anybody, but high incidence is reported in early childhood and the elderly[2,16].

Control of epilepsy is necessary for preventing acute physical harm and long-term morbidity associated with recurrent seizures. Treatment of epilepsy consists of avoidance of potential seizures precipitant, pharmacological treatment and non-pharmacological treatment, such as surgery. Treatment with antiepileptic drugs dose not cure, but only suppresses or prevents recurrence of seizure. However, patients whose epilepsy is completely controlled with medication can successfully stop therapy after a seizure-free period of at least two years[3,4].

Phenytoin was first introduced for clinical use in 1938, but it is still widely used as a standard medication on account of cheap price, high efficacy in partial and generalized tonic-clonic seizure[5,6]. The troubles in using phenytoin in a clinical setting result from its narrow therapeutic index, high protein binding and dose-dependent pharmacokinetics. The serum level of phenytoin and the degree of seizure control are directly related[7]. The elimination of phenytion occurs largely by hepatic microsomal mixed-function oxidase

Correspondence to:
Liamsuwan S, Division of Child Neurology, Department of Pediatrics Queen Sirikit National Institute of Child Health, Bangkok 10400, Thailand.
Phone: 081-430-1133
E-mail: liamsuwan@hotmail.com

reaction, CYP 2C9 and CYP 2C19. The Michaelis-Menten equation is described as follows: D = (Vmax x Cp)/(Km + Cp) where D is the dose rate (mg/d), Vmax is the maximum rate of metabolism (mg/d), Km is the serum concentration at which the rate of metabolism is half-maximal (mg/L) and Cp is the serum or plasma concentration (mg/L). Moreover, Bauer and Dodson found that mean Vmax is significantly higher in children than in adults and declines during childhood[8,9]. Consequently, therapeutic monitoring of phenytoin is important and necessary for children who have age-dependent phenytoin metabolism. Therapeutic drug monitoring refers to the individualization of dosage by maintaining plasma or serum drug concentrations within the therapeutic range[10]. Blood sample has many disadvantages, such as invasive sampling, requiring the expertise of drawing blood and risk of complication from infection and thrombosis[11,12]. During the past 30 years, the use of saliva for therapeutic drug monitoring of anticonvulsant drugs was of interest. The published studies demonstrated that saliva concentration of phenytoin ranged from 0.04 to 5.12 mg/L and these concentrations are about one-tenth of the corresponding plasma levels. The correlation coefficients ranged from 0.85 to 0.99 and 0.96 to 0.99 for total and free phenytoin concentrations, respectively[11,27]. Kankiravatana studied 10 Thai epileptic children with an average age of 10 years and 5 months. All saliva and blood samples were measured by fluorescence-polarization immunoassay technique. It has been found that the average saliva phenytoin level was 1.23 $\pm$ 1.26 mg/L. Serum phenytoin concentration was highly related to saliva phenytoin concentration with a correlation coefficient of 0.989[13]. Monitoring drug concentration in blood is highly invasive; salivary drug concentration is more preferred by children and caregivers[14].

Accordingly, the purpose of the present study was to determine the correlation between determining the effect of dose-alteration on phenytoin concentration change in serum and saliva. The results may be used as a guide for adjusting the dosage regimen and monitoring therapy, by using salivary phenytoin concentration in clinical practice.

Material and Method

Thai epileptic children aged 5 to 12 years who received phenytoin monotherapy in the neurological clinic at Queen Sirikit National Institute of Child Health were recruited to the present study. The children were required to have good compliance, normal albumin level and no evidence of cancer, HIV infection, hepatic, renal and salivary glands disease. Each of the patients was followed-up for 4-6 visits (duration between each visit was 1 month). The patients were assessed for clinical outcome by interviewing and drug dosing/seizure record card and examined for adverse drug reaction at every visit. Saliva and blood samples were collected simultaneously. Phenytoin concentration in serum and saliva samples was measured by fluorescence-polarization immunoassay technique, TDX® analysis (Abbott Laboratories, USA).

Clinical and demographic data were described by descriptive statistics and tables. The serum and salivary phenytoin level correlation was assessed by simple linear regression and correlation analysis. If two variables normally distributed, correlation was tested by Pearson's correlation. Otherwise, one or two variables abnormally distributed, correlation was tested by Spearman's correlation. The difference was considered to be statistically significant at $p < 0.05$.

Results

Thirty epileptic children were recruited in the present study. Demographic characteristics summary of each recruited patient is presented in Table 1. The average age and weight of patient was 8.24 year-old and 27.76 kilograms. There were 19 (63.3%) boys and 11 (36.6%) girls. There were 14 (46.7 %) patients who had relatives with a history of epilepsy. From 90% (27) of patients investigated by electro-encephalography, 74% of these patients had abnormal electroencephalogram[15-17]. The average onset of first seizures was at 5.08 years old. Sixty percent (18 patients) had taken phenytoin for 6 months or more. Five patient (16.6%) experienced adverse drug reaction during the present study, (2 patients had nystagmus, hirsutism in 2 cases and gingival hyperplasia in one case). No serious side effect was found (Table 2). The average (mean $\pm$ SD) dose was 6.06 $\pm$ 1.18 mg/kg/day (ranging from 3.77 to 8.88 mg/kg/day). The median serum and the salivary phenytoin concentration were 8.2 mg/L (ranging from 1.18 to 43.34 mg/L) and 0.87 mg/L (ranging from 0.24 to 4.65 mg/L), respectively. By curve estimation analysis, serum phenytoin levels were correlated with phenytoin doses as exponential types. The appropriate equation for describing the correlation between serum phenytoin level and phenytoin dose was $Y = 0.7403e^{03952x}$, where Y = serum phenytoin levels and x = phenytoin dose. The coefficient of determination (R^2) was 0.4188. Serum and salivary phenytoin concentrations were closely associated as linear type

with Spearman's correlation coefficient of 0.880 (p-value < 0.05) from linear regression analysis; the equation for estimating serum phenytoin concentration from salivary phenytoin concentration was Y = 10.165X, where Y = serum phenytoin levels and X = salivary phenytoin level. The coefficient of determination (R^2) was 0.9670 (Fig. 1).

Table 1. Demographic data

Male No. (%)	19 (63.33)
Age (year)	8.24 ± 2.09
Weight (kg)	27.76 ± 9.86
Family history of seizure No. (%)	14 (46.67)
Onset of disease (year)	5.08 ± 3.11
Type of seizure min. (%)	
complex partial seizure	5 (16.67)
partial to secondary generalized seizure	2 (6.67)
generalized tonic seizure	10 (33.33)
generalized clonic seizure	1 (3.33)
generalized tonic-clonic seizure	10 (33.33)
atonic seizure	2 (6.67)
Duration of seizure min. (range)	3 (1-15)
Abnormal EEG No. (%) (total 27)	20 (74.07)

Table 2. Adverse drug reaction

Case No.	Type of ADR	Serum PHT level (mg/L)
1.	Horizontal nystagmus	27.68
11.	Hirsutism	35.22
13.	Gingival hyperplasia	21.15
18.	Horizontal nystagmus	22.20
26.	Hirsutism	3.48

ADR = adverse drug reaction, mg/L = milligram per liter, No. = number of subject, PHT = phenytoin

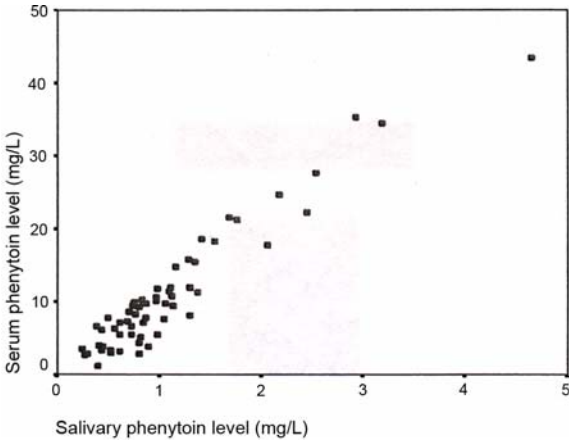

Fig. 1 Relationship between serum and salivary phenytoin concentrations

Discussion

Age-dependent pharmacokinetic and a narrow therapeutic range are important factors that promoting the necessary phenytoin concentration monitoring in children[18-23]. Non-invasive sampling and the development of new analytical technique, more sensitive and specific detector, are promising factors of salivary drug monitoring[20,24-26]. Determining phenytoin levels in serum specimen with TDX® analyzer was recommended by manufacture. Accordingly, some validation procedures, specificity, sensitivity, precision and accuracy, were tested and recorded by manufacture. Level of 19.03 mg/L all phenytoin in serum samples were stable until analysis. Many investigators determined salivary phenytoin level using FPIA technique since 1988[13,30-31]; however, the reliability of this technique for salivary phenytoin assays had not been reported. Furthemore, determining phenytoin level in saliva specimen with TDX® system had not been recommended by manufacture. For these reasons, validation procedures of salivary phenytoin assay were performed in the present study. However, after 8 weeks, salivary phenytoin level changed less than 10%. Therefore, all saliva samples were stable until analysis.

Nonlinear pharmacokinetic of phenytoin is described by the Michaelis-Menten equation. Km and Vmax are important parameters of elimination process. As previously reported, Km is not influenced by age, but mean Vmax being significantly higher in children than in adults and progressively declining during childhood. Bauer and Blouin revealed that the mean Vmax value of less than the 3 year-old group was statistically higher than the older group ($p < 0.01$)[8]. Accordingly, patients aged between 5 to 12 years were screened into the present study. The lowest age in the present study started at 5 years based on a suggestion by Kankirawatana[14]. The average compliance rates were more than 98% in both techniques. Citric acid was used in the present study because it required less cooperation[30]. Fifty-nine pairs of serum and salivary phenytoin level were tested for correlation. Salivary phenytoin level was highly correlated with serum phenytoin levels ($R = 0.880$, $p < 0.05$). The finding agreed with other studies. The correlation coefficient between serum and salivary phenytoin levels ranged

from 0.846 to 0.996 were reported in children[3,28-31]. Only in a pediatric group, the correlation coefficient of serum and salivary phenytoin levels ranged from 0.920 to 0.996[3,30]. The correlation coefficient of serum and salivary phenytoin levels in the present study was slightly less than Lifshitz's result, despite using similar analytical procedure (citric acid-stimulated saliva and FPIA method)[28,30]. The correlation of the determination of obtained equation, Y = 10.165X where Y = serum phenytoin level and X = salivary phenytoin level, was very high. Therefore, this equation could be applied in clinical practice. Only in children, the average of serum-saliva ratios ranged from 10.52 to 10.75[13,30]. When comparing a mean $\pm$ SD of serum-saliva ratios with results of Lifshitz et al (10.52 $\pm$ 1.11), Kankirawatana (10.62 $\pm$ 5.50) and the present study (9.75 $\pm$ 3.02), Kankirawatana's study had the highest deviation of mean of serum-saliva ratios. Anavekar et al revealed the special interest of phenytoin-secreting mechanism[28]. They reported that whole salivary phenytoin levels of patients who had serum phenytoin levels in supratherapeutic levels were more underestimated of mean serum free phenytoin levels. Further work in a large number of patients with high phenytoin levels is needed to clarify this point.

Conclusion

The result of the present study, a high correlation between serum and salivary phenytoin levels, supported the use of saliva instead of blood for phenytoin of altered serum and altered salivary phenytoin levels was very useful for monitoring phenytoin therapy by using salivary phenytoin levels in clinical practice. However, the application of these equations in clinical practice needs further study in a large number of patients to test the validity. Since, the usefulness of phenytoin monotherapy, having a good compliance and no drug interaction that means limited influencing factors then good correlation was seen. In addition, the value of salivary phenytoin monitoring in patients with supratherapeutic level should be proved. Furthermore, before a salivary phenytoin monitoring program is performed as a routine work in the future, the variation of correlation between serum and salivary phenytoin levels within 24 hours should be clarified because it will imply the appropriate time of collecting saliva from the patient's home before delivering to analyze at the hospital.

Potential conflicts of interest

None.

References

1. Berg AT, Testa FM, Levy SR, Shinnar S. The epidemiology of epilepsy. Past, present, and future. Neurol Clin 1996; 14: 383-98.
2. Hauser WA, Annegers JF, Kurland LT. Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1935-1984. Epilepsia 1993; 34: 453-68.
3. Lott RS. Seizure disorder. In: Young LY, Koda-Kimble MA, editors. Applied therapeutic: the clinical use of drugs. 6th ed. Vancouver, WA: Applied therapeutic; 1997: 52.1-52.7.
4. Leppik IE. Issues in the treatment of epilepsy. Epilepsia 2001; 42 (Suppl 4): 1-6.
5. Garnett WR. Antiepileptics. In: Shumacher GE, editor. Therapeutic drug monitoring. East Norwalk, CT: Appleton & Lange; 1995: 367-74.
6. McNamara JO. Drug effective in the therapy of the epilepsies. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, editors. Goodman & Gilman's the pharmacological basis of therapeutic. 9th ed. New York: McGraw-Hill; 1996: 468-70.
7. Kutt H. Phenytoin: relation of plasma concentration to seizure control. In: Woodbury DM, Penry JK, Pippenger G, editors. Antiepileptic drugs. 2nd ed. New York: Raven Press; 1982: 241-6.
8. Bauer LA, Blouin RA. Phenytoin Michaelis-Menten pharmacokinetics in Caucasian paediatric patients. Clin Pharmacokinet 1983; 8: 545-9.
9. Birkett DJ. Therapeutic drug monitoring. Aust Presc 1997; 20: 9-11.
10. Liu H, Delgado MR. Therapeutic drug concentration monitoring using saliva samples. Focus on anticonvulsants. Clin Pharmacokinet 1999; 36: 453-70.
11. Cone EJ, Jenkins AJ. Saliva drug analysis. In: Wong SHY, Sunshine I, editors. Handbook of analytical therapeutic drug monitoring and toxicology. Boca Raton, FL: CRC press; 1997: 303-4.
12. Ferguson DB. The physiology and biology of saliva. In: deBurgh Norman JE, McGurk M, editors. Color atlas and text of the salivary glands: diseases, disorders and surgery. London: Mosby-Wolfe; 1995: 40-4.
13. Kankirawatana P. Salivary antiepileptic drug levels in Thai children. J Med Assoc Thai 1999; 82: 80-8.
14. Siegel IA. Use of saliva to monitoring drug concentrations. In: Sreebny LM, editor. The salivary system. Boca Raton, FL: CRC press; 1987: 158-68.
15. Camfield CS, Camfield PR, Gordon K, Wirrell E,

Dooley JM. Incidence of epilepsy in childhood and adolescence: a population-based study in Nova Scotia from 1977 to 1985. Epilepsia 1996; 37: 19-23.
16. Hopkins A, Shorvon S, Cascino G. Epilepsy. 2nd ed. Cambridge: Chapman & Hall Medical; 1995: 10.
17. Eriksson KJ, Koivikko MJ. Prevalence, classification, and severity of epilepsy and epileptic syndromes in children. Epilepsia 1997; 38: 1275-82.
18. Pellock JM. The classification of childhood seizures and epilepsy syndromes. Neurol Clin 1990; 8: 619-32.
19. Philip J, Holcomb IJ, Fusari SA. Phenytoin. In: Florey K. Analytical profiles of drug substances. Vol.13. London: Academic Press; 1984: 418.
20. Browne TR, LeDuc B. Phenytoin: Chemistry and biotransformation. In: Levy RH, Mattson RH, Meldrum BS, editors. Antiepileptic drug. 4th ed. New York: Raven Press; 1995: 283-300.
21. Payakachat N. Pharmacokinetics and bioavailability of phenytoin in healthy Thai 1995 [thesis]. Bangkok: Chulalongkorn University; 1994.
22. Treiman DM, Woodbury DM. Phenytoin: absorption, distribution, and excretion. In: Levy RH, Mattson RH, Meldrum BS, editors. Antiepileptic drugs. 4th ed. New York: Raven Press; 1995: 301-14.
23. Battino D, Estienne M, Avanzini G. Clinical pharmacokinetics of antiepileptic drugs in paediatric patients. Part II. Phenytoin, carbamazepine, sulthiame, lamotrigine, vigabatrin, oxcarbazepine and felbamate. Clin Pharmacokinet 1995; 29: 341-69.
24. Wagner JG. Time to reach steady state and prediction of steady-state concentrations for drugs obeying Michaelis-Menten elimination kinetics. J Pharmacokinet Biopharm 1978; 6: 209-25.
25. Bruni J. Phenytoin toxicity. In: Levy RH, Mattson RH, Meldrum BS, editors. Antiepileptic drugs. 4th ed. New York: Raven Press; 1995: 345-9.
26. Seymour RA. Connective tissue disorder. In: Davies DM, Ferner RE, de Glanvile H, editors. Davies's textbook of adverse drug reaction. 5th ed. London: Chapman & Hall Medical; 1998: 240-1.
27. Haeckel R, Hanecke P. Application of saliva for drug monitoring. An in vivo model for transmembrane transport. Eur J Clin Chem Clin Biochem 1996; 34: 171-91.
28. Anavekar SN, Saunders RH, Wardell WM, Shoulson I, Emmings FG, Cook CE, et al. Parotid and whole saliva in the prediction of serum total and free phenytoin concentrations. Clin Pharmacol Ther 1978; 24: 629-37.
29. Horning MG, Brown L, Nowlin J, Lertratanangkoon K, Kellaway P, Zion TE. Use of saliva in therapeutic drug monitoring. Clin Chem 1977; 23: 157-64.
30. Lifshitz M, Ben Zvi Z, Gorodischer R. Monitoring phenytoin therapy using citric acid-stimulated saliva in infants and children. Ther Drug Monit 1990; 12: 334-8.
31. Kanjanasilp J. Phenytoin therapeutic drug monitoring for patients at Prasat Neurological Institute [thesis]. Bangkok: Chulalongkorn University; 1994.

การศึกษาความสัมพันธ์ของระดับยาเฟนิโทอินในซีรัม และในน้ำลายในเด็กไทยที่เป็นโรคลมชัก

สหัส เหลี่ยมสุวรรณ, อุไลลักษณ์ ใจวีระวัฒนา

วัตถุประสงค์: *เพื่อหาความสัมพันธ์ของระดับยาเฟนิโทอินในซีรัมและในน้ำลาย ในเด็กไทยที่เป็นโรคลมชัก*

วัสดุและวิธีการ: *ศึกษาในแผนกกุมารเวชกรรมกรรมผู้ป่วยนอกคลินิกประสาท สถาบันสุขภาพเด็กแห่งชาติมหาราชินี โดยคัดเลือกผู้ป่วยอายุ 5 ถึง 12 ปี ที่ได้รับยาเฟนิโทอินเป็นยาควบคุมอาการชักเพียงตัวเดียว โดยเลือกผู้ป่วยที่ให้ความร่วมมือเป็นอย่างดี มีระดับอัลบูมินปกติ นอกจากนั้นผู้ป่วยไม่มีประวัติป่วยด้วยโรคมะเร็ง ติดเชื้อเอชไอวี โรคตับ โรคไต หรือโรคของต่อมน้ำลาย ตัวอย่างเลือด และน้ำลายของผู้ป่วยแต่ละรายถูกเก็บ และนำไปตรวจระดับยาเฟนิโทอินด้วยวิธีการฟลูโอไรเซชั่นอิมมูโนแอสเส*

ผลการศึกษา: *ผู้ป่วยที่ให้ความร่วมมือจนสิ้นสุดการวิจัยมี 30 คน เป็นผู้ป่วยชาย 19 คน และหญิง 11 คน อายุเฉลี่ย (ค่าเฉลี่ย $\pm$ ค่าเบี่ยงเบนมาตรฐาน) 8.24 $\pm$ 2.09 ปี และมีน้ำหนักเฉลี่ย (ค่าเฉลี่ย $\pm$ ค่าเบี่ยงเบนมาตรฐาน) 27.76 $\pm$ 9.86 กิโลกรัม ผลการศึกษาพบว่าระดับยาเฟนิโทอินในซีรัมและในน้ำลายมีความสัมพันธ์กับขนาดยาในรูปแบบเชิงพหุ โดยมีค่า R^2 = 0.4188 และ 0.3682 ตามลำดับ สมการที่อธิบายการเปลี่ยนแปลงของระดับยาเฟนิโทอินในซีรัม และในน้ำลายที่เกิดจากการเปลี่ยนแปลงของขนาดยาได้แก่ $Y = 0.7403e^{0.3952x}$ และ $Y = 0.1431e^{0.3072x}$ ตามลำดับ ระดับยาเฟนิโทอินในซีรัม และในน้ำลายสัมพันธ์กันอย่างมากในรูปเชิงเส้นตรงโดยมีค่า R = 0.880 และ R^2 = 0.967 สมการของความสัมพันธ์นี้ คือ Y = 10.165X ซึ่ง Y = ระดับยาเฟนิโทอินในซีรัม และ X = ระดับยาเฟนิโทอินในน้ำลาย อาการไม่พึงประสงค์จากยาพบได้ 5 ราย (6.6%) ได้แก่ nystagmus 2 ราย, hirsutism 1 ราย, เหงือกบวม 1 ราย ไม่พบภาวะแทรกซ้อนที่รุนแรง*

สรุป: *ระดับยาเฟนิโทอินในซีรัมมีความสัมพันธ์กันอย่างมากกับระดับยาเฟนิโทอินในน้ำลาย ซึ่งข้อมูลนี้ช่วยสนับสนุนการติดตามการใช้ยาเฟนิโทอินโดยการวัดระดับยาในน้ำลาย ซึ่งการเจาะเลือดในเด็กมีความยุ่งยาก และมีผลกระทบต่อจิตใจของเด็ก และสมการต่างๆ ที่ได้สามารถนำไปปรับใช้ในการปรับขนาดยา และการติดตามการใช้ยาเฟนิโทอินโดยการวัดระดับยาเฟนิโทอินในน้ำลายได้ในอนาคต*