Bioavailability of Phenytoin Sodium Capsules Available in Thailand Part II : *In vivo* Study

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Abstract

Four phenytoin brands, dilantin® and three local brands (brand A, B and C) were selected for the bioavailability study. The study was carried out in 16 healthy male Thai volunteers with the average age of 21 years old. A single oral dose of 300 mg (three capsules of 100-mg) phenytoin sodium was given to subjects following an 8 hour-overnight fast. The tested drugs were given in a single-blind randomized crossover with at least 2 weeks of washout period. Venous blood samples of approximately 5 ml were drawn before medication and at 1, 2, 4, 6, 8, 10, 12, 24, 48 and 72 hours post dosing. Plasma phenytoin concentrations were determined by HPLC assay.

The pharmacokinetic parameters were calculated from the plasma-concentration time curve of an innovator brand, dilantin® , by PCNONLIN program. Elimination rate constant and half-life were 0.2 h^{-1} and 19 h, respectively. The maximum concentration (Cmax) and time to peak (Tmax) were 1.98 μg/ml and 9.6 h, respectively. Bioavailability study was determined by comparing the area under the plasma concentration time curve (AUC), maximum plasma concentration (Cmax) and time to reach maximum plasma concentration (Tmax) by using ANOVA. The result indicated that two local brands (brand A and brand C) were not bioequivalent to the innovator in terms of Cmax and $AUC_{0-\alpha}$, whereas Tmax was not significantly different among these 4 brands. Cmax and AUC of brand A and C were significantly higher than the innovator brand. In addition, the plasma concentration time profile of brand C was also different from other brands with the steep peak which yielded a Cmax value double that of the Cmax of the innovator. However, brand B (from Research and Development Institute, Government Pharmaceutical Organization) was bioequivalent to dilantin® after 4 times of product formulation adjustment.

This present study demonstrated that the local products (brand A and brand C) were not bioequivalent with the innovator. Thus, the interchange from one brand to another must be performed cautiously or should be avoided, otherwise phenytoin blood levels should be monitored closely together with the clinical signs and symptoms of the patients.

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Phenytoin (diphenylhydantoin) is clinically effective in generalized tonic-clonic and partial seizures. The mechanism of action of phenytoin is to inhibit the repetitive firing of action potentials from the epileptic focus by blockade of use - dependent sodium channels[1-3].

The absorption of phenytoin after oral ingestion is slow and variable due to limited aqueous solubility. Peak plasma levels may reach as early as 3 hours or as late as 12 hours after oral single dose[4]. In therapeutic plasma concentrations phenytoin is highly bound (about 90%) to plasma protein, mainly albumin. The drug is eliminated almost entirely by hepatic metabolism. Less than 5 per cent of phenytoin is excreted unchanged in the urine. Phenytoin is extensively (60-70%) metabolized to inactive parahydroxylated phenytoin by hepatic enzyme system which is saturable at high plasma levels. At the plasma concentration below 10 μg/ml, the hydroxylation is first order, at higher concentrations the hydroxylation reactions approach saturation and the hydroxylation becomes zero order[5]. This means that at a higher concentration, plasma half-life increases with phenytoin concentration.

Optimum control without clinical signs of toxicity occur with phenytoin serum levels between 10 and 20 μg/ml. There may be wide interpatient variability in phenytoin serum levels with equivalent dosages. Based on the formulation of the innovator brand, dilantin® is available as an extended capsule and prompt capsule. The 100 mg extended capsule is used during maintenance therapy whereas prompt capsule is suitable for use in loading dose regimens. On top of this, these forms are not indicated on the lable as available in U.S.A.

There are at least 6 brands of phenytoin sodium available in Thailand and together with the special characteristics of phenytoin as mentioned above, bioequivalence studies are necessary to reveal the quality of these pharmaceutical formulations[6]. Therefore, this study was conducted to compare the bioavailability of local phenytoin sodium capsules with the innovator brand.

MATERIAL AND METHOD

Subjects

Sixteen healthy male volunteers between the age of 18-25 years were enrolled into the study. All subjects were determined to be healthy on the basis of medical history, physical examination and routine laboratory tests. Written informed consent was obtained from all subjects prior to the study.

Drugs

The 2 local products (brand A and C) and the innovator product (dilantin)® were purchased from a drug store in Bangkok as a whole bottle. Brand B was provided from the Research and Development Institute, Government Pharmaceutical Organization.

Study Design and Procedures

Comparative bioavailability of the 4 products was performed in a controlled, randomized single blind, cross-over study with washout intervals of 14 days. Each subject was given a single oral dose of three capsules of 100 mg phenytoin sodium with 180 ml of water in the morning following an 8-hour overnight fast. Food was abstained until four hours after drug administration. The venocath (G20) was inserted into a forearm vein for blood sampling in each subject, and the venocath was flushed with 100 IU/ml heparin in normal saline solution to prevent blood clotting. Serum blank was kept before drug administration. Five milliliters of blood samples were collected in heparinized tubes at the following intervals : 1, 2, 4, 6, 8, 10, 12, 24, 48 and 72 hours postingestion and then centrifuged at 3000 rpm for 10 minutes. The plasma was separated and kept at -80°C until analysis.

All meals and fluid were standardized both in regard to content, quantity and time of administration.

At several time points of blood sampling, volunteers were asked for the occurrence of adverse drug reactions, such as drowsiness, headache, nausea, vomiting, paresthesia, ataxia, and confusion.

Quantitative drug analysis

The quantitative analysis of phenytoin was determined by high pressure liquid chromatography. The HPLC model Consta Metric (Thermoseparation products) was used to detect phenytoin sodium. A reverse phase column Nucleosil® C_{18}, 10 μm (25 cm x 4.6 mm) was used as a separating column. Mixture of acetonitrile, methanol, and water 17, 25, and 58 per cent was used as a mobile phase. After thoroughly mixing, it was filtered through a 0.45 μm-membrane filter. The phenytoin

peak was detected at 254 nm using Spectro Monitor® 3200 (Thermoseparation).

Preparation of samples

Plasma samples were extracted in a 10-ml glass-stoppered centrifuge tube. Two hundred microlitres of plasma were mixed with 80 µl of 5 mol/L hydrochloric acid by using vortex mixer for 5 seconds. Then 2.5 milliliters of dicloromethane was added and mixed for 1 minute. Sufficient amount of solid ammonium sulfate was added to saturate the aqueous layer (added until solid ammonium sulfate precipitated). This mixture was mixed for 30 seconds and then centrifuged at 5,000 rpm for 5 minutes. The upper aqueous layer was discarded. Two millilitres of organic layer were transfered into a conical glass and evaporated in a water bath at 50°C. The residue was redissolved in 100 µl of a mixture of water (80 µl) and internal standard (phenobarbitone 20 µl). Twenty microlitres of sample were injected into the HPLC to quantitate phenytoin concentration.

Pharmacokinetic analysis

PCNONLIN (SCI software, Lexington, KY, U.S.A.) was used to estimate the following pharmacokinetic parameters : apparent volume of distribution (Vd/F), first-order absorption rate constant (K01), first-order elimination rate constant (K10), first-order elimination half-life (K10-HL), and area under the curve (AUC). The maximum plasma concentration (Cmax) and the time to reach the maximum concentration (Tmax) were obtained from raw data of the plasma-concentration time curve.

Statistical analysis

Bioequivalence parameter (AUC, Cmax, and Tmax) was compared by using ANOVA. The local product will be bioequivalent to the innovator brand if their AUC, Cmax and Tmax are in the range of 90 per cent confidence interval (CI) of the innovator product with the permission of ± 20 per cent deviation.

RESULTS

Sixteen healthy volunteers were enrolled in the study. Their aged ranged from 18-25 years (mean 20.9 ± 3.4 kg), weight from 51.0 to 62.1 kg (mean 56.3 ± 3.4 kg), and height from 160 to 175 cm (mean 167.1 ± 4.3 cm).

Table 1. Pharmacokinetics parameters of phenytoin.

Vd/F	175.18 L
first-order absorption rate constant	0.26 h^{-1}
first-order elimination half-life	19 h

Summarized pharmacokinetic data are presented in Table 1. The mean Vd/F was 175.2L. The absorption of phenytoin from the gastrointestinal tract was slow with the mean first-order absorption rate constant of 0.26 h^{-1} and the first-order absorption half-life of 5 h. Peak plasma concentration was reached at approximately 12 h. The elimination half life was 19 h, and the first-order elimination rate constant was 0.2 h^{-1}.

The mean plasma concentration-time curve of the four phenytoin sodium brands are shown in Fig. 1. After oral administration of the innovator product (dilantin®), absorption was slow with mean peak plasma phenytoin concentration of 1.74 µg/ml in 12 h. This value was in the same range of the mean Cmax of brand B which was 1.61 µg/ml with Tmax of 12 h. In contrast, after oral administration of brand A and C, the drugs reached Tmax within 10 h with the peak plasma concentration of 2.76 and 3.25 µg/ml, respectively.

Comparative results of bioavailability in term of $AUC_{0-\alpha}$, Cmax, and Tmax between the local products (brand A, B, C) and the innovator are summarized in Table 2. Only brand B was bioequivalent with Dilantin® with a 90 per cent confidence interval within the 80-120 criteria for bioequivalency for AUC and Cmax. In contrast to brand A and C, their AUC and Cmax were significantly higher than the innovator brand. The plasma concentration time profile of brand C was different from other brands with the steep peak which yielded Cmax value as double that of the C max of the innovator. Two subjects complained of headache when they received phenytoin brand C. No other clinically important adverse events were observed.

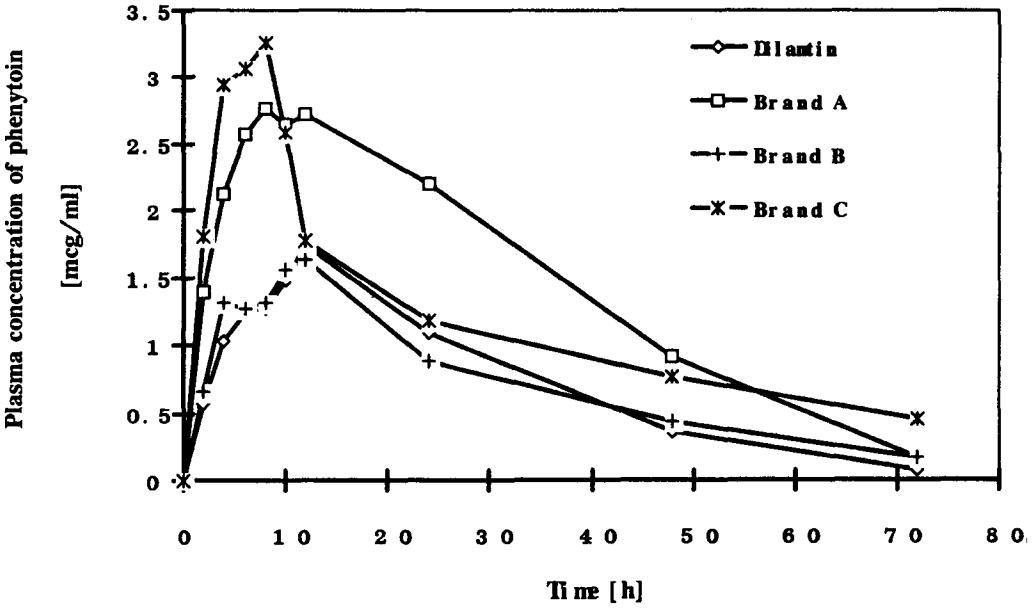

Fig. 1. Plasma concentration-time curve of 4 phenytoin brands.

Table 2. Summary of the results of a bioavailability study*.

Brand	Dilantin® Mean ± SEM	Brand A Mean ± SEM	Brand B Mean ± SEM	Brand C Mean ± SEM
$AUC_{0-\alpha}$ (μg./ml.min)	50.04±7.98	101.47±8.36	51.04±6.83	86.43±5.70
p-value		<0.001	NS	0.005
Cmax (μg/ml)	1.74±0.15	2.76±0.21	1.61±0.17	3.25±0.29
p-value		<0.001	NS	<0.001
Tmax(min)	11.5±0.77	9.50±0.46	11.67±0.51	10.40±0.43
p-value		NS	NS	NS
90% Confidence interval** for $AUC_{0-\alpha}$		180-225	84-120	157-188

* The bioavailability of a drug from 4 brands was studied in 16 healthy adult male volunteers using a randomized single blinded cross-over design.

** 90% Confidence interval were calculated by the equation of $x \pm t_{\alpha}(SEM)$ and then compared with the mean of the innovator. (x = mean value of generic phenytoin, t_{α} = percentage points of the *t* distribution, SEM = standard error of mean).

DISCUSSION

The result of pharmaceutical properties of phenytoin sodium capsules revealed the difference in various parameters, especially the dissolution profile (see Part I "*in vitro*" study). The present study was designed to evaluate the bioequivalence of these local products in comparison to the innovator, dilantin® . The secondary pharmacokinetic characteristics were analyzed analogously.

The results of our pharmacokinetic data were generally in the same range as the previous report[7,8]. We found that the elimination half life of phenytoin extended capsule was approximately 19 h. This parameter may have clinical implementations to predict the steady state (4-5 half-lives) serum level of phenytoin in first exposed epileptic patients. When plasma level determinations are necessary, they should be obtained at least 5-7

half-lives after treatment initiation. Dosage adjustment should also be performed after the steady-state.

The present study demonstrates that 2 local products (brand A and C) were not bioequivalent with the innovator, dilantin® . The 90 per cent confidence intervals of the AUC, and Cmax were out of the required limits of ± 20 per cent. Brand C displayed the highest AUC and Cmax together with a different plasma-concentration time curve from the innovator brand. This was in accord with the unique pattern of its dissolution and the per cent labeled amount. (see Part I : *in vitro* study). High phenytoin serum levels from brand C also led to headache in two volunteers. As the Cmax and AUC of brand C was quite high and showed the characteristic of "prompt" release capsule, it should be avoided to administer as "300 mg once a day dosing". This regimen will result in toxic blood levels especially at the time when the steady state has already been established.

Concerning brand A, the dissolution profile of brand A was rather low when compared to the innovator brand, however, its bioavailability was much higher. This was probably due to different physicochemical characteristics of the excipient. This result also demonstrated that the *in vitro* dissolution test is not a reliable indicator of biological equivalence.

In general practice these products are frequently used interchangably, however, the regimen of drug administration usually follows the previous prescription. This definitely leads to the changes in phenytoin blood levels which can be subtherapeutic or toxic. Even this problem is not well-documented, physicians and pharmacists should be aware of the existence of the formulation forms of the products whether it is "extended" or "prompt" capsules.

Several studies of bioavailability of phenytoin from other countries[9-13] have been published and the results showed that phenytoin is the drug that has a bioavailability problem due to different manufacturers and batch to batch variation. This present study demonstrates that the local products (brand A and brand C) were not bioequivalent with the innovator, dilantin® . These results should alert physicians and pharmacists to be aware of selecting or interchanging the brand of phenytoin. Thus, the interchange from one brand to another must be performed cautiously or should be avoided, otherwise phenytoin blood levels should be monitored closely together with the clinical signs and symptoms of the patients. Not only biopharmaceutical problems and physicochemical characteristics[14] can affect the bioavailability, but also its narrow therapeutic range and dose-dependent kinetics will aggravate the chance of toxic effects. Careful monitoring of blood levels is essential after brand interchange in order to avoid subtherapeutic or toxic phenytoin blood levels and finally to increase the patients' quality of life. In terms of drug regulations, based on our study, *in vitro* specifications for prompt and extended phenytoin sodium capsules as well as the *in vivo* bioequivalence requirements for these two types of products are recommended.

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REFERENCES

1. Julien RM, Halpern LH. Effects of diphenylhydantoin and other antiepileptic drugs on epileptiform activity and Purkinje cell discharge rates on epileptiform activity and Purkinje cell discharge rates. Epilepsia 1972; 13: 387-400.
2. Mclean MJ, Macdonald RL. Limitation of high frequency repetitive firing of cultured mouse neurons by anticonvulsant drugs. Neurology 1984; 34 (suppl 1): 228.
3. Delorenzo RL. Phenytoin : mechanisms of action. In : Lery Rit, Mattson RH, Meldrum BS, eds. Autiepileptic drugs, 4 th ed. New York : Raven Press 1995; 271-82.
4. Triman DM, Woodbury DM. Phenytoin: absorption, distribution, and excretion. Ibid: 301-14.
5. Browne TR, LeDuc B. Phenytoin: chemistry and biotransformation. Ibid: 283-300.
6. Nation RL, Sansom LN. Bioequivalence requirements for generic products. Pharmacol Ther 1994; 62: 41-55.
7. Jusko WJ. Bioavailability and disposition kinetics of phenytoin in man. In: Kellaway P, Petersen I, eds. Quantitative analytic studies in epilepsy. New York: Raven Press, 1976: 115-36.
8. Martin E, Tozer TN, Scheiner LB, Riegelman S. The clinical pharmacokinetics of phenytoin. J Pharmacokinet Biopharm 1977; 5: 579-96.
9. Mikati M, Bassett N, Schachter S. Double-blind randomized study comparing brand-name and generic phenytoin monotherapy. Epilepsia 1992; 33: 359-65
10. Tsai J-J, Lai M-L, Yang Y-HK, Hung J-D. Comparison on bioequivalence of four phenytoin preparations in patients with multiple dose treatment. J Clin Pharmacol 1992; 32: 272-6.
11. Shah VP, Prasad Uk, Freeman C, Skelly JP, Cabana BE. Phenytoin II: in vitro-in vivo bioequivalence standard for 100-mg phenytoin sodium capsules. J Pharm Sci 1983; 72: 309-10.
12. Rosenbaum DH, Rowan AJ, Tuchman L, French JA. Comparative bioavailability of a generic phenytoin and Dilantin. Epilepsia 1994; 35: 655-60.
13. Soryal I, Richens A. Bioavailability and dissolution of proprietary and generic formulations of phenytoin. J Neurol Neurosurg Psychiatry 1992; 55: 688-91.
14. Meyer MC, Stranghn AB. Biopharmaceutical factors in seizure control and drug toxicity. Am J Hosp Pharm 1993; 50: S17-S22.

ชีวประสิทธิผลของยาฟีไนโตอินโซเดียมชนิดแคปซูลที่มีจำหน่ายในประเทศไทย ตอนที่ 2 : การศึกษาในคน

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ทำการศึกษา bioavailability ของ phenytoin 4 ชนิดคือ dilantin® และชนิดที่ผลิตในประเทศไทยอีก 3 บริษัท (บริษัท A, B และ C) ในอาสาสมัครชายสุขภาพดีจำนวน 16 คน มีอายุเฉลี่ย 21 ปี ให้อาสาสมัครรับประทานยา phenytoin sodium ขนาด 300 มิลลิกรัม (3 แคปซูลของ 100 มิลลิกรัม) หลังจากอดอาหารในตอนกลางคืนมา 8 ชั่วโมง โดยอาสาสมัครทุกคนถูกสุ่มให้ได้รับยาทั้ง 4 ชนิดสลับกันแบบ single-blind โดยมีระยะห่างอย่างน้อย 2 สัปดาห์ เก็บตัวอย่างเลือดประมาณ 5 มิลลิลิตร ก่อนได้รับยาและที่ 1, 2, 4, 6, 8, 10, 12, 24, 48 และ 72 ชั่วโมงภายหลังได้รับยา หาความเข้มข้นของ phenytoin ในพลาสมาโดยใช้วิธี HPLC

ค่าพารามิเตอร์ทางเภสัชจลนศาสตร์คำนวณจาก plasma-concentration time curve ของยาต้นแบบ dilantin® โดยใช้ PCNONLIN program ค่า elimination rate constant และค่าครึ่งชีวิตมีค่า 0.2 ชั่วโมง$^{-1}$ และ 19 ชั่วโมงตามลำดับ ระดับยาสูงสุดในเลือด (Cmax) และระยะเวลาที่ได้ระดับยาสูงสุด (Tmax) มีค่า 1.98 ไมโครกรัม/มิลลิลิตร และ 9.6 ชั่วโมงตามลำดับ การศึกษา bioavailability ทำได้โดยการเปรียบเทียบค่าพื้นที่ใต้เส้น plasma concentration time curve (AUC), ค่าระดับยาสูงสุด (Cmax) และระยะเวลาที่ให้ระดับยาสูงสุด (Tmax) โดยใช้สถิติ ANOVA ผลการศึกษาพบว่ายา 2 บริษัทที่ผลิตในประเทศไทย (brand A และ brand C) ไม่มี bioequivalent กับยาของบริษัทต้นแบบในแง่ของ Cmax และ $AUC_{0-\alpha}$ ในขณะที่ค่า Tmax ไม่มีความแตกต่างอย่างมีนัยสำคัญทั้ง 4 บริษัท ค่า Cmax และ AUC ของบริษัท A และ C มีค่าสูงกว่าของบริษัทต้นแบบอย่างมีนัยสำคัญทางสถิติ นอกจากนี้รูปร่างของ plasma concentration time curve ของ brand C ยังแตกต่างจากบริษัทอื่นๆ โดยมี peak ที่ชัน ซึ่งให้ค่า Cmax เป็น 2 เท่าของยาจากบริษัทต้นแบบ อย่างไรก็ตามยาบริษัท B (ผลิตภัณฑ์ของสถาบันวิจัยและพัฒนา องค์การเภสัชกรรม) มี bioequivalent กับ dilantin ภายหลังจากการปรับสูตรถึง 4 ครั้ง

จากการศึกษานี้พบว่า ยาบริษัท A และ C ไม่มี bioequivalent กับยาต้นแบบ ดังนั้นควรเปลี่ยนบริษัทยาด้วยความระมัดระวังหรือควรหลีกเลี่ยงการเปลี่ยนบริษัท ในกรณีที่หลีกเลี่ยงไม่ได้ควรติดตามระดับยา phenytoin อย่างใกล้ชิด รวมทั้งดูอาการแสดงทางคลินิกของผู้ป่วยร่วมด้วย

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