Bioavailability of Phenytoin Sodium Capsules Available in Thailand Part I : *In vitro* Study

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Abstract

Phenytoin is commonly used as an antiepileptic drug worldwide. The unique properties of phenytoin such as poor water solubility and zero-order kinetics of its metabolism, together with difference in pharmaceutical formulations can result in dramatic changes in bioavailability of phenytoin capsule.

The innovator (Dilantin® , Parke Davis), three local brands (brand A, B and C) were investigated for pharmaceutical characteristics including drug content, content uniformity, and dissolution. All these tests were performed as described in the monograph of extended phenytoin sodium capsules in USP XXII with slight modification in HPLC analysis. It was found that all the products, except brand C, had drug content and content uniformity within the standard range. Brand A and brand C failed to meet the USP XXII specification for the dissolution test. Per cent dissolution of brand A was lower whereas per cent dissolution of brand C was much higher than the standard value. The qualities of innovator and brand B were within the pharmacopoeial specification.

This result revealed that phenytoin capsules available in Thailand did not have homogeneous pharmaceutical equivalence which may lead to difference in plasma phenytoin levels (see part II : *In vivo* study). Thus, changing the brand of phenytoin in stable epileptic patients should be performed with caution.

Phenytoin is commonly used as an antiepileptic drug worldwide. The unique properties of phenytoin such as poor water solubility and zero-order kinetics of its metabolism[1], together with difference in pharmaceutical formulations can result in dramatic changes in the dissolution and bioavailability of phenytoin capsules[2,3].

There are two forms of phenytoin i.e. phenytoin sodium and free phenytoin base, used in drug product formulations. Based on the formula-

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tion of the innovator brand, Dilantin® contains phenytoin sodium and is available as a prompt capsule, extended capsule, and injection form[4]. There are also chew tablets and the suspension form available as free phenytoin base[4]. The 100 mg extended capsule is the most frequently prescribed form for maintenance therapy. Phenytoin sodium extended capsule is characterized by a slow and extended rate of absorption with peak blood concentrations expected in 4 to 12 hours. The characteristics of both the prompt and extended forms must comply with the pharmacopeial specification, USP XXII[5].

In Thailand, there are at least 6 brands of 100 mg phenytoin sodium capsules commercially available. Since phenytoin sodium is listed as an "Essential Drug"[6], the purchasing of brand will be influenced by the medium price and the bidding system. Thus, the manufacturers who offer the lowest price will have a high opportunity of being selected. In this case, patients will have the chance to receive various brands of phenytoin throughout the long term treatment. In addition, phenytoin is not listed in the Public Health Ministerial Regulation for the requirement of dissolution test[7].

The purpose of this study was to assess the *in vitro* characteristics of the most commonly used phenytoin sodium capsules marketed in the country. The data will be useful for clinicians and pharmacists in selecting proper phenytoin sodium products.

MATERIAL AND METHOD

Test products

Four brands of 100 mg phenytoin sodium capsules were bought from one drug store in Bangkok as a whole bottle. The innovator brand, Dilantin® was used as the reference product. The local products were assigned as brand A, brand B (product of Research and Development Institute, Government Pharmaceutical Organization) and brand C.

Standard drug and chemical reagents

Phenytoin sodium and phenobarbitone sodium (internal standard) were obtained from Sigma (St. Louis, No, U.S.A.).

The other chemical reagents were of HPLC and analytical grade.

Chromatographic conditions for phenytoin sodium

The HPLC model Consta Metric® (Thermoseparation products) was used to detect the level of phenytoin sodium. A reverse phase column Nucleosil® C_{18}, 10 μm, (25 cm x 4.6 mm) was used as the separating column. The mobile phase composed of water: 0.83 per cent glacial acetic acid (50:50, v/v). It was filtered through 0.45 μm membrane filter and deaerated for 30 minutes by using a sonicator before use.

Twenty microlitres of sample were injected into the HPLC system. The phenytoin peak was detected at 254 nm using Spectro Monitor® 3200 (Thermoseparation). Phenytoin concentration in the samples was determined against phenytoin sodium standard curve obtained by linear regression of its concentration *versus* peak area ratio between phenytoin sodium and the internal standard (phenobarbitone sodium).

Dissolution test

The dissolution test of phenytoin sodium capsule was assessed by using dissolution apparatus (Soltex Model AT7). The capsule was dissolved in a dissolution basket which was immersed in water. The dissolution basket was rotated at the rate of 50 rpm and the temperature of the medium was 37± 0.5°C. Two millilitres of the medium were aliquoted at 30, 60 and 120 minutes to quantitate the amount of dissolved phenytoin by using the HPLC method.

Content uniformity

The content uniformity is a parameter to determine the uniformity of each dosage unit.

Ten capsules of each brand were individually analysed. Each capsule was dissolved in 100 ml of methanol in volumetric flask, filtered and collected the filtrate, then 3 ml of the filtrate was pipetted and transfered to a 10 ml-volumetric flask. 0.5 ml of internal standard (phenobarbitone 1 mg/ml) was added and adjusted to 10 ml with water. Twenty microlitres of the solution was injected to the HPLC for phenytoin concentration. The acceptable range of pheyntoin sodium in each capsule should not be less than 93.0 per cent and not more than 107.0 per cent of the labeled amount (100 mg).

Assay

The drug powder from 20 capsules of each brand was transfered as completely as possible into a beaker and mixed thoroughly. 100 mg of this mixed powder was transfered to 100 ml volumetric flask, then dissolved with methanol and adjusted to 100 ml with methanol. This solution was filtered, then 3 ml of the filtrate was pipetted and transfered to a 10 ml volumetric flask. 0.5 ml internal standard (phenobarbitone 1 mg/ml) was added and adjusted to 10 ml with water. Then phenytoin concentration was analysed by HPLC.

Data analysis

The dissolution, content uniformity, and assay content of phenytoin sodium capsule were analysed according to the specification of phenytoin extended-release capsules in USP XXII(6). The result was reported as "met" or "failed" the pharmacopoeial specification.

RESULTS

The phenytoin peak was clearly separated from the phenobarbitone peak (internal standard) with the retention time of 2.7 and 4.4 minutes, respectively. (Fig. 1) The standard curve of phenytoin was linear over the range of 0.5-30 μg/ml with the r^2 of 0.9998.

The pharmaceutical characteristics of all four tested products are shown in Table 1.

The dissolution profile of each brand is also graphically presented in Fig. 2. Of the four brands, only dilantin® and brand B were found to meet the pharmacopoeial standard for the extended phenytoin capsule in all aspects. For brand A, its dissolution profile was much lower than the standard requirement, however, the other properties such as content uniformity and per cent assay were in the acceptable range. On the other hand, the dissolution profile of brand C did not show the pattern of extended capsule. Per cent dissolution of brand C was 77.1, 78.7 and 76.7 at 30, 60 and 120 minutes, respectively. These values were much higher than the innovator brand, Dilantin® . Moreover, the content uniformity (122.2±6.4) and the drug content (120.1) of brand C were also higher than the upper range of the acceptable limit (93-107%).

These results revealed that brand A and brand C were pharmaceutical inequivalent to the innovator brand.

DISCUSSION

This study showed that all these products were not pharmaceutically equivalent. Two local products, brand A and C, failed to meet the USP standard for the dissolution profile. For any drug to be absorbed, it must be dissolved first. Thus, the rate of dissolution from either tablet or capsule can markedly affect the rate of absorption and the bioavailability of the drug in the body.

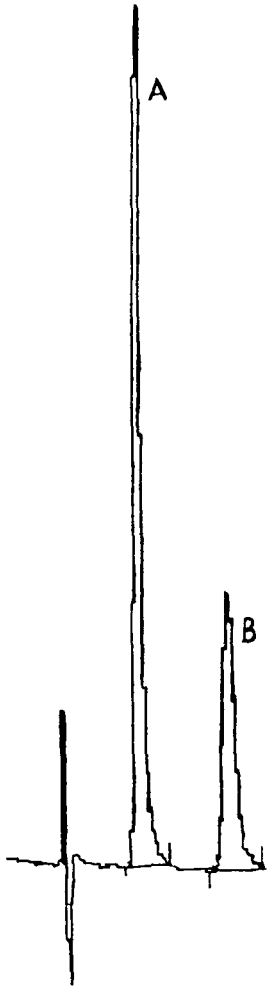

Fig. 1. HPLC chromatogram of phenytoin sodium in *in vitro* study.
A = phenobarbitone peak (internal standard)
B = phenytoin peak

Table 1. Dissolution, content uniformity, and drug content of 4 brands of phenytoin capsules.

Properties	Brand				Standard USP XXII specification
	Dilantin®	A	B	C	
Dissolution*					
% dissolution at (mean±SD)					
30 min	31.6 ± 3.5	17.7 ± 2.3	33.8 ± 1.6	77.1 ± 3.9	<40%
60 min	57.1 ± 5.3	25.0 ± 1.9	55.0 ± 3.8	78.1 ± 4.8	>55%
120 min	78.6 ± 6.6	29.4 ± 4.6	75.2 ± 2.9	76.7 ± 3.4	>70%
Content Uniformity**					
mean±SD	106.9 ± 2.1	107.9 ± 1.6	101.2 ± 2.53	122.2 ± 6.4	93.0 - 107.0%
%CV	1.92	1.47	2.58	5.22	
Drug Content					
% labeled amount	103.5	107	102.7	120.4	93.0-107.0%

* n = 12 capsules of each brand
** n = 10 capsules of each brand

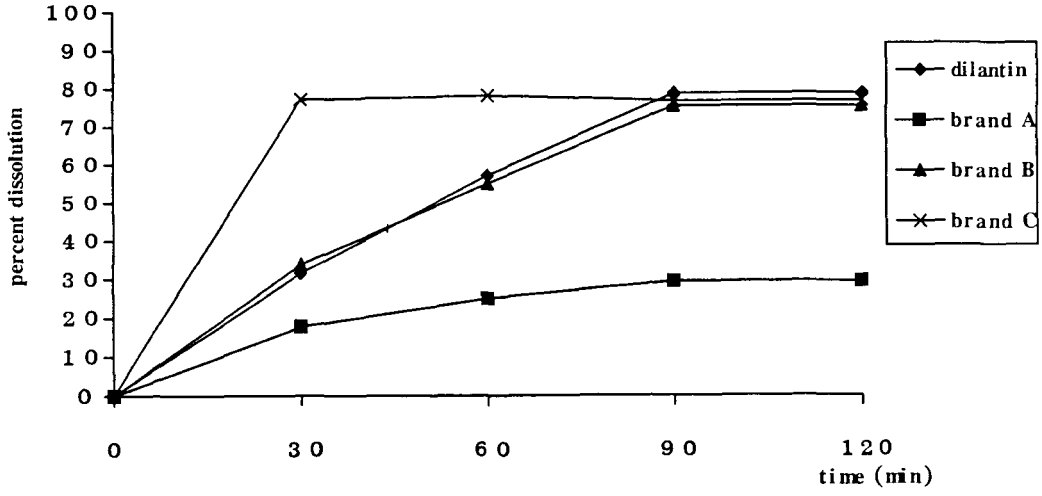

Fig. 2. Dissolution profile of four phenytoin brands.

Brand A differed significantly from the others in its deaggregation and the dissolution characteristics. A capsule of brand A dispersed within 15 minutes after starting the dissolution test and the powder gradually precipitated, whereas, Dilantin and brand B remained as a capsule-shaped powder aggregate without precipitation after the dissolution of the gelatin shell. This may be due to the difference in the excipients, other components in the formulation and the process of manufacturing.

There may be some excipients in brand A which can bind to phenytoin and form the undissolved complex of the drug, however, in the GI tract these excipients may enhance phenytoin absorption (see part II result).

The *in vitro* dissolution pattern of brand C was not an extended form. In USP XXII, prompt phenytoin sodium capsule must dissolve more than 85 per cent within 30 minutes. Brand C dissolved 77 per cent at 30 minutes and seemed to be for-

mulated as the prompt capsule. Following the USP XXII, prompt phenytoin sodium capsule must be labelled as "Not for once-a-day dosing". If the manufacturer intended to formulate brand C as a prompt form, it should be labelled as such and adjust per cent dissolution at 30 minutes to be more than 85 per cent. Brand C may have a benefit for use in a loading dose in epileptic patients.

For brand B, the Research and Development Institute, Government Pharmaceutical Organization formulated this product which met the pharmacopoeial requirement after 4 times of formulation adjustment.

Although phenytoin has been used for a long time in Thailand, the dissolution standards of the various formulations are not specified in Thai Pharmacopoeia 1993[8]. Since phenytoin is still the standard drug for the treatment of epilepsy, combined with its narrow therapeutic range and the dose-dependent kinetics, a little change in the formulation and other pharmaceutical properties such as dissolution profile and content uniformity might lead to a drastic fluctuation in blood level and may cause clinical problems. Thus, the standard of the phenytoin capsules either prompt or extended forms should be reconsidered by the Thai FDA and it should be kept in mind that changes of the formulation, even the excipient, can cause dramatic changes in blood levels of the drug.

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ชีวประสิทธิผลของยาฟีไนโตอินโซเดียมชนิดแคปซูลที่มีจำหน่ายในประเทศไทย ตอนที่ 1 : การศึกษาในหลอดทดลอง

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Phenytoin เป็นยากันชักที่นิยมใช้ทั่วไป คุณสมบัติพิเศษของ phenytoin เช่น การละลายน้ำที่ต่ำและการทำลายยาที่เป็น zero-order kinetics รวมทั้งความแตกต่างของการตั้งสูตรตำรับมีผลอย่างมากต่อการเปลี่ยนแปลง bioavailability ของ phenytoin

ได้ทำการตรวจคุณสมบัติของยา เช่น ปริมาณยา, ความสม่ำเสมอของตัวยาสำคัญ และการละลายของยาต้นแบบ (Dilantin®, Parke Davis) และยาที่ผลิตในประเทศ 3 บริษัท (brand A, B และ C) การทดสอบทั้งหมดทำตามที่กำหนดไว้ใน monograph ของ extended phenytoin sodium capsules ใน USP XXII โดยมีการปรับวิธีการวิเคราะห์โดย HPLC เล็กน้อย พบว่ายาทุกตัวยกเว้น brand C มีปริมาณยาและความสม่ำเสมอของตัวยาสำคัญอยู่ในค่ามาตรฐาน brand A และ brand C ไม่เข้ามาตรฐานของ USP XXII สำหรับการทดสอบการละลาย เปอร์เซ็นต์การละลายของ brand A ต่ำกว่ามาตรฐานในขณะที่ของ brand C มีค่าสูงกว่าค่ามาตรฐานมาก คุณสมบัติของยาต้นแบบและ brand B เข้าตามมาตรฐานของตำรายา

ผลการศึกษานี้แสดงให้เห็นว่ายาแคปซูล phenytoin ที่มีจำหน่ายในประเทศไทยมีคุณสมบัติของสูตรตำรับแตกต่างกัน ซึ่งอาจส่งผลให้เกิดความแตกต่างของระดับยา phenytoin ในพลาสมาได้ (ดูส่วนที่ 2 : การศึกษาในคน) ดังนั้นการเปลี่ยนยี่ห้อของ phenytoin ในผู้ป่วยที่ควบคุมการชักได้แล้วควรทำด้วยความระมัดระวัง

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