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A comparative study of the bioavailability of five different phenytoin preparations

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The concentration of phenytoin in saliva has been measured in 8 healthy volunteers at intervals after an intravenous dose and after single oral doses of five formulations commercially available in the United Kingdom. The six doses (all 300 mg) were given in random order and at least one week apart. There were no significant differences in the mean values of the peak saliva concentration, the time-to-peak and the area under the saliva concentration-time curve between the five oral formulations. The absolute bioavailability of phenytoin varied between 68 and 74%.

Phenytoin has been classified (Doluisio et al 1973) as a drug with 'high risk potential' with respect to bioavailability problems. The drug has a low therapeutic index and displays saturation kinetics at conventional doses (Richens 1979). Maintenance of a safe and effective steady-state plasma concentration requires that the absolute daily dose remains constant. Even minor alterations in the extent of absorption (bioavailability) can alter equilibrium substantially. It is therefore unfortunate that phenytoin has physicochemical properties which tend to render its absorption inconsistent and unreliable (Neuvonen 1979). The problems associated with differences in bioavailability between preparations of phenytoin have been documented in Europe, North America and Australasia (for references, see Neuvonen 1979). However, a comparison of the steady-state concentrations of phenytoin produced by five different preparations in a cross-over study in epileptic patients receiving regular treatment (Chen et al 1982) revealed only minor differences which, although statistically significant, were not considered to be clinically important. But claims of bioinequivalence continue to be made not only by certain manufacturers but also by epileptic patients who have experienced alteration in seizure frequency following product substitution.

We thought it reasonable to carry out a further comparison of different preparations marketed in Britain, and have examined saliva concentration-time profiles after single oral and intravenous doses in healthy volunteers.

Subjects and methods

Subjects were all healthy employees of the North Staffordshire Health Authority. All underwent physical examination and provided venous blood for determination of full blood count and biochemical indices of liver and kidney function prior to entry into the study. Written informed consent was obtained and the study received prior approval by the Ethical Committee of the Health Authority. The subjects were not permitted to take any medicines for 24 h before each study and fasted for 9 h before, and 3 h after, each dose. Each subject received 6 separate doses of phenytoin 300 mg (5 oral, 1 intravenous) in random order and at least one week apart. The oral doses were taken with 200 ml of water and the mouth was rinsed thoroughly both immediately and 15 min after the dose. The intravenous dose (Epanutin Ready-Mixed Parenteral, Parke-Davis; 300 mg in 6 ml) was given as a slow infusion in 50 ml of isotonic saline over 30 min. The oral preparations used are listed in Table 1.

Saliva samples (5 ml) for determination of phenytoin concentration were collected before and at hourly

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Table 1. Details of the five oral formulations.

Formulations	Supplier
Phenytoin tablets BP 100 mg soluble phenytoin tablets Lot: 1KK2	The Boots Co. Ltd, Nottingham, UK
Phenytoin tablets BP 100 mg sugar coated tablets Lot: 2AQ533	Evans Medical Ltd, Liverpool, UK
Phenytoin sodium tablets BP 100 mg Lot: BN CD22A	Thomas Kerfoot & Co. Ltd, Ashton-under-Lyne, UK
Phenytoin tablets BP 100 mg Lot: TH 671 BN1247	Cox, A. H. & Co. Ltd, Rustington, UK
Phenytoin capsules BP 100 mg Lot: BN 1M124	Parke-Davis & Co. Ltd, Pontypool, UK

intervals for 8 h after the dose. Further samples were collected after 12, 15, 24, 48, 72 and 96 h. Saliva flow was stimulated by chewing a piece of Parafilm (Gallenkamp). During the intravenous study, additional samples were collected at 15, 30 and 45 min after the commencement of the infusion. Samples were stored at −20 °C before analysis. The analytical methods of MacGee (1970) and Shihabi (1978) had to be modified in order to extract nanogram quantities of phenytoin from saliva and to ensure adequate selectivity. Saliva (1 ml) was made alkaline by adding 100 µl 1 M sodium hydroxide and any weakly basic ingredients extracted into 100 µl of organic extraction medium (ethyl acetate–dichloromethane–methanol–chloroform; 8:4:3:1 by volume). After vortex mixing (10 s) and centrifugation (4000 rev min^{-1}) for 3–4 min, the aqueous phase was transferred to a second tube containing 100 µl of organic extraction medium, 500 µl 0·1 M phosphate buffer (pH 4·5) and 20 µl of methanol, containing the internal standard, 5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH 100 mg $litre^{-1}$). After further vortex mixing (15 s) and centrifugation (4000 rev min^{-1} for 5 min), 4 µl of organic phase were taken up into a 10 µl glass syringe containing 3 µl of methylating agent (tetramethylammonium hydroxide 0·01% in methanol) and injected into the gas chromatograph.

A standard curve was described using phenytoin sodium BP (Evans Medical Ltd) and MPPH (Aldrich Chemical Co.). All reagents were of analytical grade (Analar). Chromatography (Pye Unicam GCD) was carried out using a flame ionization detector and a glass column (1 m × 6 mm o.d. 4 mm i.d.) packed with 1·6% SP1000 on 2CLQ, 100/120 mesh (Magnus Scientific). The packed column was conditioned for 48 h at 350 °C under nitrogen at a flow rate of 90 ml min^{-1}. The operating temperatures were 220 °C (column), 310 °C (injector) and 350 °C (detector), with carrier gas (nitrogen) flow 30 ml min^{-1}. Retention times for phenytoin and internal standard were 3·8 and 4·8 min, respectively. The limit of sensitivity was 0·15 mg $litre^{-1}$. Coefficients of variation range from 6% at 0·25 mg $litre^{-1}$ to 5·5% at 2 mg $litre^{-1}$.

The area under the saliva concentration-time curve (AUC) for each preparation of phenytoin was determined using the trapezoidal rule. Bioavailability (F) was calculated from the equation:

$$F = \frac{AUC\,(oral)}{AUC\,(i.v.)} \times \frac{Dose\,(i.v.)}{Dose\,(oral)}$$

Doses of phenytoin sodium formulations were corrected for molecular weight. The mean values derived from the concentration-time profiles for each preparation were compared using Student's paired *t*-test.

Results

Eight volunteers (6 M, 2 F) aged between 22 and 47 (mean 31·1 years) took part. There were no clinically important abnormalities of full blood count or of liver and kidney function. The first four volunteers to be studied all experienced discomfort during the intravenous infusion of phenytoin with pain in the arm coming on a few minutes after commencement of the infusion and lasting for 5–10 min. Two of these individuals experienced prolonged discomfort for 24–48 h after the infusion and one developed superficial thrombophlebitis in the infusion arm. No further volunteers received the intravenous infusion.

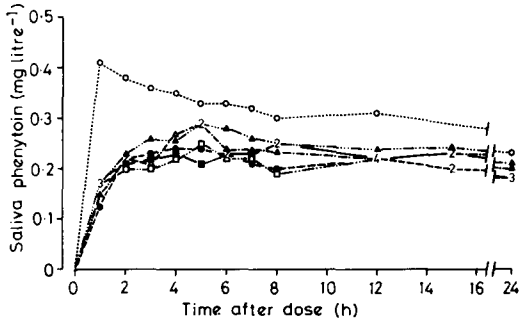

FIG. 1. Mean concentration-time curves for saliva phenytoin after each of the intravenous and five oral preparations. Symbols ○ = intravenous dose (n = 4), ● = Boots, □ = Evans, ■ = Kerfoot, △ = Cox, ▲ = Parke-Davis. Numerals replace symbols where more than one symbol occupies a single intercept.

The mean saliva phenytoin concentration-time curves during the first 24 h after the six doses are shown in Fig. 1. By 36 h after the dose, phenytoin concentration had usually fallen to a level which was too low to measure with confidence. The mean time required to reach the maximal concentration after an oral dose (t_{max}) ranged between 3·5 and 4·4 h. There were no significant differences between formulations. The observed mean peak saliva phenytoin concentrations (C_{max}) after oral doses were in the range of 0·23–0·31 mg $litre^{-1}$ and the five preparations did not differ significantly. Mean AUC values for the first 24 h after each dose lay between 4·37 and 4·79 mg $litre^{-1}$ for the five preparations. Mean bioavailability (calculated in the four subjects who received an intravenous dose) varied

between 68 and 74%. Neither AUC_{0-24} nor F differed significantly between the five preparations. The expanded results for these derived indices are shown in Table 2.

Table 2. Mean (±s.d.), C_{max}, AUC_{0-24} and F values for the five oral formulations.

Supplier	C_{max} mg litre^{-1}	t_{max} h	AUC_{0-24} mg h^{-1} litre	F* %
Evans	0·23 ± 0·07	4·38 ± 1·51	4·37 ± 0·83	67·5 ± 12·9
Boots	0·25 ± 0·06	3·50 ± 1·07	4·37 ± 0·77	67·5 ± 11·4
Kerfoot	0·25 ± 0·06	4·00 ± 1·77	4·77 ± 0·78	73·6 ± 12·0
Cox	0·31 ± 0·09	4·25 ± 0·71	4·79 ± 0·73	74·0 ± 11·3
Parke-Davis	0·25 ± 0·04	4·38 ± 1·06	4·62 ± 0·88	71·3 ± 13·6

* The calculated F values are based on volunteers who tolerated i.v. phenytoin.

Discussion

This study was designed following anecdotal reports from local epileptic patients, which questioned the bioequivalence of certain commonly available formulations of phenytoin. Recognizing the inconvenience and difficulty which would be incurred in a comparison of five phenytoin preparations at steady-state in non-resident epileptic patients, as well as the need to ensure perfect patient compliance, we elected to compare single doses in healthy volunteers. The use of saliva was justified by the numerous samples required, some at unsocial hours, and by the recommendation of Paxton & Wilcox (1980) who had used the same technique.

It has been argued (Neuvonen 1979) that first-order kinetics may not be uniformly applicable when single intravenous and oral doses of phenytoin are compared, because of the different peak concentrations produced, and that an under-estimate of absolute bioavailability may, therefore, result. Mean peak concentrations achieved in this study after intravenous and oral doses did not differ by much and we do not believe that the resulting range of absolute bioavailability is an under-estimate; earlier studies have produced similar values (Lund et al 1974; Gugler et al 1976).

Although a single dose study is the recommended way to assess absolute bioavailability (Koch-Weser 1974), it invites the criticism, in the case of phenytoin, that the findings would be different at steady-state because of dose-dependent kinetics (Lund et al 1974). The transition from first-order to zero-order kinetics would mean that even minor (i.e. not statistically significant) differences between formulations could result in clinically important changes in phenytoin concentration. The similarity between our results and those of the earlier steady-state comparison (Chen et al 1982) seem to refute this criticism, and support the assumption that there are no important differences in bioavailability between the oral preparations of phenytoin currently marketed in Britain.

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