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PHARMACOKINETICS OF A SINGLE DOSE OF PHENYTOIN IN MAN MEASURED BY RADIOIMMUNOASSAY

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1 Serum concentrations of phenytoin were studied by radioimmunoassay in five normal volunteers following a single oral dose of phenytoin sodium (100 mg).

2 Two distinct peaks were found at 2.5-3.5 and 10-12 h after ingestion. Maximum serum concentrations ranged from 1.56 μg/ml to 2.76 μg/ml. The mean plasma half-life of the drug under these conditions was 9.81 ± 0.66 (s.e. mean) hours.

Introduction

The clinical value of measuring plasma anticonvulsant concentrations in the control of therapy is now well recognized (Kutt, 1972; Marks, Lindup & Baylis, 1973; Marks, Morris & Teale, 1974). Phenytoin (diphenylhydantoin, DPH) has long been the drug of choice for the treatment of many types of epilepsy. The minimum effective serum concentration of phenytoin is generally in the region of 10 μg/ml (Buchthal, Svensmark & Schiller, 1960) and the optimum therapeutic range 10-20 μg/ml. Low levels of the drug may not control seizures, while high concentrations may result in side effects such as nystagmus, ataxia, somnolence and even convulsions. Inter-personal variations, including differences in the rates of absorption, genetically determined differences in drug metabolism, drug interactions and the effects of disease, create difficulties in predicting the blood concentration from a given dose and suggest that monitoring of the serum phenytoin level may be helpful in difficult cases.

Several analytical techniques, such as gas-liquid chromatography (g.l.c.) and thin-layer chromatography (t.l.c.) are available but involve complex extraction procedures and require relatively large volumes of blood. Radioimmunoassays for phenytoin have already been described (Tigelaar, Rapport, Inman & Kupferberg, 1973; Cook, Kepler & Dix Christensen, 1973) and are capable of being carried out on a micro sample of serum (1 μl) without prior extraction and with high sensitivity and precision. They can also be applied to the analysis of large numbers of samples.

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Methods

Drug administration and blood sampling

Each of five adult volunteers ingested tablets of phenytoin sodium (2 x 50 mg) after an overnight fast. No food was permitted for a further 2 hours. Blood (1 ml) was collected by venepuncture before ingestion of phenytoin and further 1 ml samples collected at intervals over the next 102 h (Table 5). Serum was separated from the clot and stored at 4°C until analysed.

Phenytoin radioimmunoassay

All serum samples from an individual were analysed in the same batch using a radioimmunoassay for plasma phenytoin developed in this laboratory (Robinson, 1975).

Production of immunogen The phenytoin antiserum was raised in sheep against a conjugate of phenytoin to bovine serum albumin (BSA) prepared as follows:

Phenytoin sodium (149 mg) was dissolved in distilled water (3.0 ml) together with BSA (201 mg). The water-soluble carbodiimide, 1-ethyl-3-diisopropylamino-carbodiimide HCl, was then added and the solution stirred overnight at room temperature. The conjugate solution was purified by dialysis against distilled water and freeze-dried. The concentration of unconjugated phenytoin in the dialysates was determined by u.v. analysis and the amount of phenytoin coupled to the BSA calculated. The phenytoin-BSA conjugate contained 32 moles phenytoin/mole BSA.

Immunization techniques Phenytoin-BSA (5.0 mg) was dissolved in distilled water (1.0 ml)

and emulsified with Freund's complete adjuvant (2.0 ml). The emulsion was administered intramuscularly to two sheep (Suffolk-Cob hybrid) over six sites on the legs. Three booster injections, containing 2.5 mg conjugate in the same volume of emulsion, were given at monthly intervals.

After a 3-month rest period the sheep were given a fourth booster containing 2.5 mg conjugate in 1.0 ml distilled water, emulsified with 2.0 ml adjuvant consisting of Marcol 52 with 10% Arlacel A (Robinson, Morris & Marks, 1975). Similar boosters were given five, seven and eight months later.

Venous blood samples were collected from the external jugular vein 10 days after each booster and the antiserum stored at 4°C.

Assay procedure The protocol for the phenytoin radioimmunoassay is illustrated in Table 1.

Tritiated-phenytoin (^{3}H-DPH; specific activity 56 ci/mmol; 5.0 pmol/ml) was used as the label in the radioimmunoassay. All the dilutions of the antisera, label, standards and samples were made with 0.1 M phosphate buffered saline, pH 6.75, with 1% gelatin (PBSG).

The amount of antiserum binding 50% of the added label, in the absence of unlabelled phenytoin, was determined from antiserum dilution curves and was the amount used in the assay.

The standard solutions for the assay were prepared by dissolving phenytoin sodium (2.5 mg) in PBSG (25 ml) and diluting a 10 µl aliquot of this solution to 10 ml with PBSG giving a concentration of 100 ng/ml. This second solution was double-diluted to give the range of standards used in the assay.

The reagents were added to the assay tubes in the order indicated in the protocol. Following the addition of the antiserum the contents of the tubes were mixed and incubated at room temperature for 2.5 hours. The tubes were then placed in iced-water for 15 min before the addition of dextran-coated charcoal (DCC) for phase separation. The DCC suspension contained 0.63% w/v Norit A charcoal and 0.063% w/v dextran T-70 in PBSG. Five min after the addition of the DCC the tubes were centrifuged at 2000 rev/min and 4°C for 5 min, and aliquots of the supernatants taken for liquid scintillation counting.

Immediately before analysis all the sera for the experiment were diluted 1:50, 1:100, 1:200, 1:400, 1:800, 1:1600 and 1:2000 with PBSG.

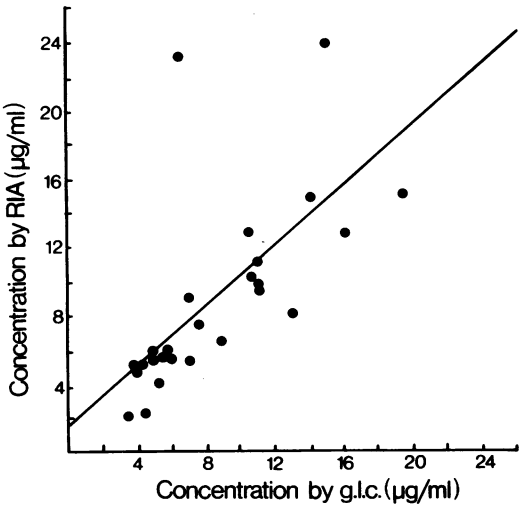

Figure 1 Comparison of radioimmunoassay (RIA) and g.l.c. methods for measuring serum phenytoin concentrations. ($y = 0.8801x + 1.4937$, $r = 0.7825$, $n = 30$).

Table 1 Assay protocol for phenytoin radioimmunoassay

				Standard curve		
	Total (µl)	*Non-specific binding* (µl)	*Antiserum dilution curve* (µl)	*Maximum bound* (µl)	*(Zero)* (µl)	*STD/Sample* (µl)
Buffer	300	300	200	100	200	100
STD/Sample	–	–	–	–	–	100
Label	100	100	100	100	100	100
Antiserum	–	–	100	200	100	100
Dextran coated charcoal	–	200	200	200	200	200
Buffer	200	–	–	–	–	–
Aliquot for counting	200	200	200	200	200	200

Duplicate measurements were made at each dilution.

None of the twelve structurally related anticonvulsants (Table 3) tested interfere with the assay. The major metabolites of phenytoin, i.e. 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (HPPH), shows less than 0.4×10^{-5}% cross-reactivity in the radioimmunoassay, and it is likely that its conjugate with glucuronic acid cross-reacts even less.

A comparison of the radioimmunoassay with a gas-liquid chromatography method (Papadopoulos, Baylis, Fry & Marks, 1973) in current use at West Park Hospital, Epsom, showed no significant difference between the two assays (Figure 1).

Results

Table 2 shows the final dilutions of the antisera collected after each booster injection. The avidity of the antiserum for phenytoin was 4.49×10^{8} $lmol^{-1}$ in the serum collected after the fourth booster and increased to 1.25×10^{10} $lmol^{-1}$ following the sixth booster. The sensitivity of the radioimmunoassay, calculated by the method of Albano & Ekins (1970), was 321 ± 41 pg phenytoin/ml. The mean standard curve obtained during this experiment is shown in Figure 2.

All five volunteers showed two plasma phenytoin peaks. The first 2.5-3.5 h and the second 10-12 h after phenytoin ingestion (Table 4). Statistical analysis of the results showed that in three of the five subjects (W.A., D.T., S.W.) the second peak was significant and could not be due to experimental error.

Plots of serum phenytoin concentrations against time in the two subjects at either extreme are illustrated in Figure 3. Linear plots after the second peak showed an exponential fall in all of the subjects. The half-life of phenytoin calculated from this portion of the curve in each individual is shown in Table 5.

Table 2 Final dilutions of antisera collected after each booster immunization

Booster	*Final dilution*
1	–
2	1:4
3	1:6
4	1:640
5	1:100
6	1:1280
7	1:1000

Table 3 Anticonvulsants structurally related to phenytoin that do not interfere in the radioimmunoassay

Ethotoin	Ethosuximide
Mephenytoin	Phenobarbital
Trimethadione	Methylphenobarbital
Paramethadione	Primidone
Phensuximide	Amino-glutethimide
Methsuximide	Pheneturide

Table 4 Plasma phenytoin (DPH) levels in five volunteers after a single dose of phenytoin (100 mg) by mouth

	Phenytoin concentration (ng/ml)				
Time (h)	*B.M.*	*W.A.*	*D.T.*	*S.W.*	*J.R.*
0	0.0	0.0	0.0	0.0	0.0
1.0	1523.6	420.0	2707.1	1322.1	929.3
2.0	1814.6	1284.3	2762.1	1620.0	1204.6
3.0	1850.0	2184.3	2479.6	2130.7	1564.3
3.5	1414.3	2060.7	2491.4	2255.0	1292.1
4.0	1577.9	1911.4	2190.0	2725.7	1137.9
4.5	1427.9	1921.4	2161.4	2172.1	1111.4
5.0	1121.1	1595.7	1690.0	1922.9	1052.9
7.0	963.7	1161.4	2377.1	2728.6	1055.0
10.0	1027.1	2218.6	–	2175.7	1188.6
12.0	868.2	1605.4	2072.9	–	1269.2
25.0	407.1	690.7	1313.6	657.1	331.5
33.0	172.0	298.0	687.9	556.5	228.3
49.0	70.0	61.7	233.1	167.5	32.5
56.5	27.2	45.0	56.0	82.5	22.5
78.5	4.5	10.0	20.0	40.0	20.0
102.5	12.3	<0.3	–	–	–

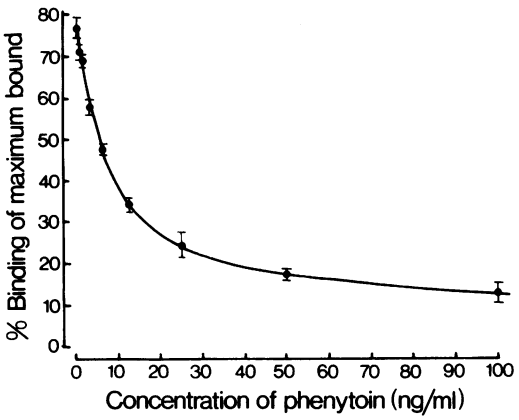

Figure 2 Mean ± s.e. mean (n = 10) phenytoin standard curve obtained in the pharmacokinetic studies. Maximum binding: 69.08 ± 1.64% T; non-specific binding: 4.32 ± 1.20% T.

Table 5 Serum half-lives of phenytoin following a single oral dose of phenytoin sodium (100 mg).

Subject	$T\frac{1}{2}$ *(h)*
B.M.	9.50
W.A.	8.75
D.T.	12.00
S.W.	10.50
J.R.	8.30

Mean $T\frac{1}{2}$ = 9.81 ± 0.66 (s.e. mean)

Discussion

The radioimmunoassay used in this experiment is sufficiently sensitive to detect 321 ± 41 pg DPH/ml which is up to 1000 times more sensitive than other techniques in current use. It is virtually specific for phenytoin, showing less than 0.4×10^{-5}% cross-reactivity with its major metabolite ((HPPH) and no cross-reactivity with any of 12 chemically related drugs.

Many reports indicate that the peak plasma phenytoin concentrations occur 4-8 h after ingestion of a single oral dose (Glazko, Chang, Baukema, Dill, Goulet & Buchanan, 1969; Woodbury & Swinyard, 1972; Kutt & Louis, 1972). We were surprised, therefore, to observe a second peak 10-12 h after a single oral dose of phenytoin (100 mg). Similar results have previously been reported by Lund, Alvan, Berlin & Alexanderson (1974), using a g.l.c. method, though they themselves did not comment upon

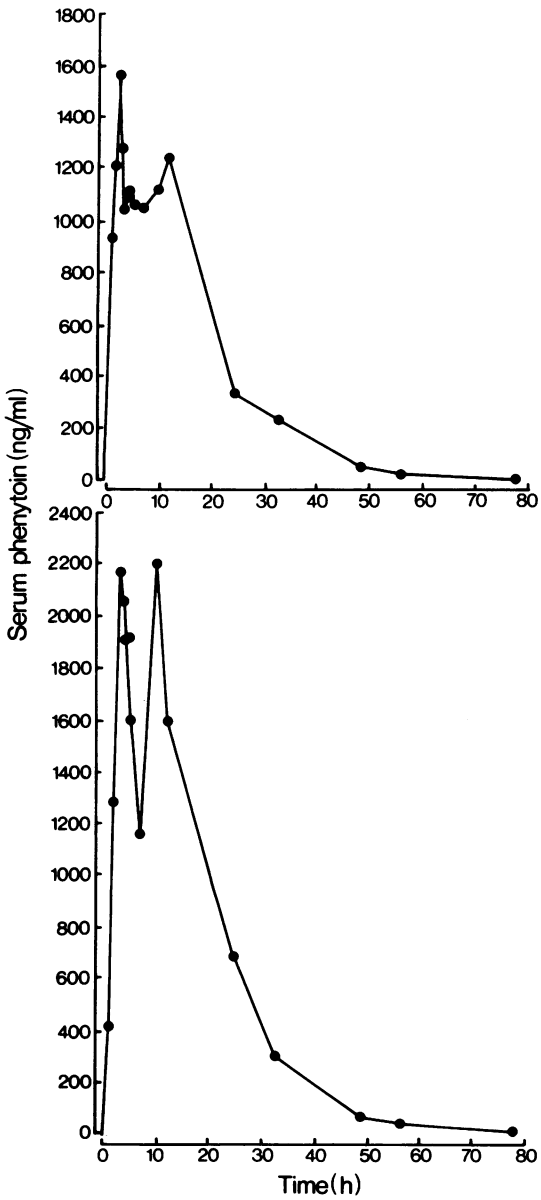

Figure 3 Serum phenytoin concentration following a single oral dose of phenytoin sodium (100 mg) in two subjects.

them. A possible reason for this, and the failure of other investigators to observe the second peak, may be the relative insensitivity of their analytical techniques.

The disappearance of phenytoin from the blood after the 12 h sample and the plasma half-life determined by radioimmunoassay agree with the

findings of others (Glazko *et al.*, 1969; Woodbury & Swinyard, 1972; Kutt & Louis, 1972). There is some evidence that at doses above 100 mg the plasma half-life of phenytoin in man is dose-dependent (Arnold & Gerber, 1970), but information with regard to smaller doses is not currently available.

The high sensitivity of the radioimmunoassay enables blood level determinations to be made on samples obtained by finger-prick. This is extremely important for the monitoring of therapy in infant epileptics. The method also provides a valuable research tool and experiments to elucidate the findings described here are currently being investigated.

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