Bioavailability of Phenytoin and Anticonvulsant Activity after Oral Administration of Phenytoin-*bis*-hydroxyisobutyrate to Rats

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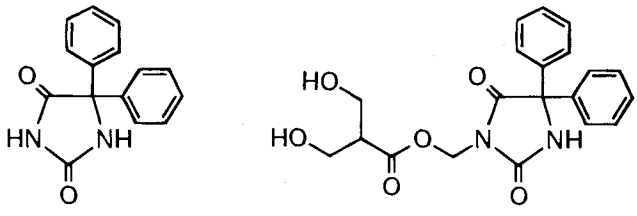
INTRODUCTION

Several prodrugs of phenytoin have been evaluated to overcome the drug's limitations due to its poor aqueous solubility and highly irritating properties (1). The phosphate ester of 3-hydroxymethylphenytoin is currently undergoing approval in the United States (2).

In the course of the investigation of lipid-derived prodrugs of phenytoin, 3-hydroxy-2-hydroxymethylpropionic acid (*bis*-hydroxyisobutyric acid) was designed as a glycerol mimic allowing direct esterification with 3-hydroxymethylphenytoin (3). Rapid release of phenytoin from the resulting phenytoin-*bis*-hydroxyisobutyrate (Fig. 1) by hydrolytic enzymes such as plasma esterases was observed *in vitro* (4). Moreover, the compound displayed anticonvulsant activity and a pharmacological profile similar to that of the parent drug after i.p. administration to mice (5). Therefore, it was concluded that the compound might act as a prodrug of phenytoin.

Oral administration is generally considered the route of choice for a drug. Therefore, the present study was conducted in order to evaluate the bioavailability and anticonvulsant activity of phenytoin-*bis*-hydroxyisobutyrate in comparison to phenytoin after oral administration to rats.



phenytoin phenytoin-*bis*-hydroxyisobutyrate

Fig. 1. Structures of the compounds.

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MATERIALS AND METHODS

Chemicals

Phenytoin was obtained from Caelo (Hilden, Germany) and heparin solution (25,000 I.E./ml) from Ratiopharm (Ulm, Germany). Phenytoin-*bis*-hydroxyisobutyrate was synthesized as described (3). All other chemicals were obtained from commercial sources at the highest purity available. Solutions were prepared in double-distilled, deionized water.

Pharmacokinetics

Male Wistar rats (bred at the animal facilities at UCL), weighing 210–240 g, were housed individually at a 12 hour light-dark cycle with free access to commercial rodent chow and water. The animals fasted overnight and during the experiment but were allowed water ad libitum. 119 μmol/kg of phenytoin and phenytoin-*bis*-hydroxyisobutyrate (30 mg/kg equivalents of phenytoin) were administered as suspensions in 0.5% methyl cellulose by oral intubation in a volume of 2 ml/kg. Approximately 300 μl of blood were collected via the tail clip method into Eppendorf tubes containing 20 μl of the heparin solution. The samples were centrifuged at 4°C at 4,000 g, the plasma was immediately separated, frozen at −80°C, and stored frozen until analyzed by gas chromatography as described earlier in detail (6). Phenytoin concentrations were calculated by the peak area ratio method using a calibration curve obtained from spiked plasma samples.

Pharmacology

Anticonvulsant testing was provided by the Antiepileptic Drug Development Program, Epilepsy Branch, Division of Convulsive, Developmental and Neuromuscular Disorders, National Institutes of Health, according to standard procedures (7) and included the maximal electroshock (MES) test and the seizure threshold test with subcutaneous pentetrazol (scMet test). The acute neurological toxicity was determined in the rotorod test.

Data Analysis

Noncompartmental analysis of the data was performed. The maximum plasma concentration (C_{max}) and the time to reach this concentration (t_{max}) were obtained directly from the plasma concentration *versus* time profiles. The area under the curve (AUC) was calculated by the log-linear trapezoidal method for the observed values and by extrapolation to infinity. The elimination half life ($t_{1/2}$) was estimated from the final segment of the plasma concentration curve. Statistical comparison was performed using the t-test for unpaired observations. $P < 0.05$ was considered statistically significant.

RESULTS

The structures of the compounds are shown in Fig. 1. Preliminary experiments verified that measurable quantities of the prodrug were not present in the samples. For these initial studies one aliquot of the samples was analyzed immediately, another aliquot was left at room temperature for 1 h to ensure complete hydrolysis of the prodrug. No differences in the

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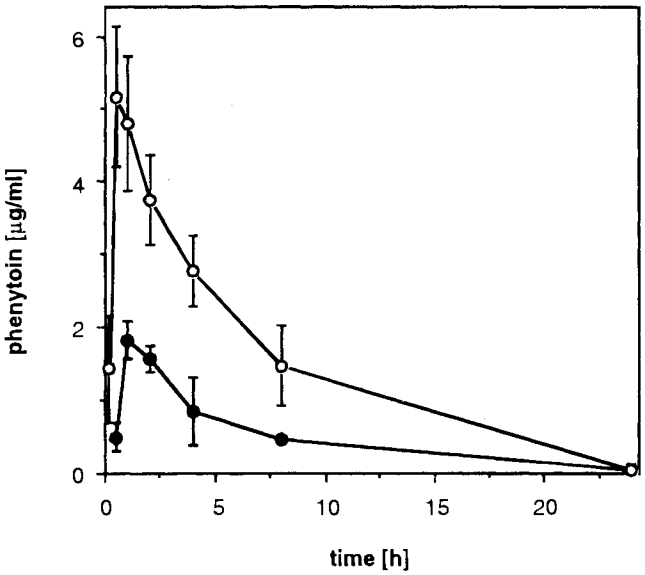

Fig. 2. Plasma concentration of phenytoin versus time profile following oral administration of equimolar doses of phenytoin (●) and phenytoin-*bis*-hydroxyisobutyrate (○) to rats (119 µmol/kg corresponding to 30 mg/kg phenytoin equivalents; n = 4 − 5 ± SD).

plasma concentrations of phenytoin between the two aliquots could be detected.

The plasma concentration *versus* time profile after oral administration of phenytoin and phenytoin-*bis*-hydroxyisobutyrate, respectively, is shown in Fig. 2. The pharmacokinetic parameters obtained by noncompartmental analysis of the plasma data are summarized in Table 1. Compared to the application of the parent drug, administration of the prodrug resulted in a higher bioavailability of phenytoin. An approximate 2.5-fold increase of C_{max} and a 3-fold increase of the AUC was observed. t_{max} was at 0.74 h. No significant differences regarding $t_{1/2}$ were found.

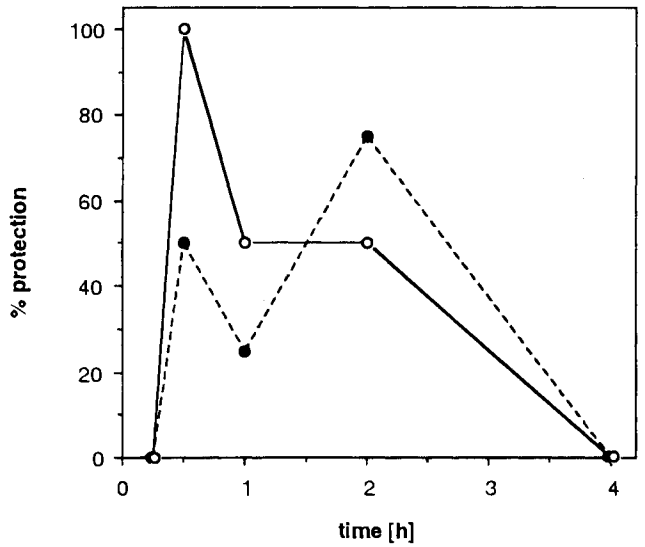

Fig. 3. Time-course of the MES activity after oral administration of 119 µmol/kg phenytoin (●) and 66 µmol/kg of phenytoin-*bis*-hydroxyisobutyrate (○) to rats. The values are expressed as percentage of the animals protected (n = 4).

The time course of the anticonvulsant activity determined in the MES test after oral administration of phenytoin-*bis*-hydroxyisobutyrate and phenytoin is shown in Fig. 3. The prodrug showed maximal protection against MES-induced seizures 30 min after oral application of a dose of 62 µmol/kg. Administration of almost twice the dose of phenytoin (119 µmol/kg) led to only incomplete protection. The maximal activity occurred at 2 h postdose. In contrast, an approximately equimolar dose of the prodrug fully antagonized seizures between 0.5 and 6 h and still protected 25% of the rats after 8 h (data not shown). The median effective doses (ED_{50}) of the compounds are summarized in Table 1.

Upon oral administration to rats, phenytoin-*bis*-hydroxyisobutyrate did not exhibit any toxicity in the rotorod test at doses of up to 1300 µmol/kg and was inactive in the seizure threshold test with subcutaneous pentetrazol (scMet) at doses of up to 650 µmol/kg. 3-Hydroxy-2-hydroxymethylpropionic acid was inactive in either test at concentrations up to 2500 µmol/kg.

DISCUSSION

Compared to the parent drug, administration of the ester resulted in substantially increased C_{max} and AUC. The relatively low bioavailability obtained after dosing of phenytoin might be attributed to the slow and incomplete dissolution of the compound due to its poor aqueous solubility. Prodrug formation resulted in an approximate 40-fold increase of the water solubility (4). However, this increase is only moderate compared to the about 4,000- to 5,000-fold increase of the aqueous solubility obtained with other prodrugs such as fosphenytoin (8). Efficient enzymatic hydrolysis of the bis-hydroxyisobutyrate prodrug as indicated by the early t_{max} might contribute to the rapid absorption process.

The slight increase of the elimination half life ($t_{1/2}$) was not statistically significant. It is known that phenytoin displays nonlinear, dose-dependent pharmacokinetics in the rat (9,10). Thus, the higher plasma levels obtained after administration of the prodrug might be responsible for a slower metabolism and/or elimination of phenytoin.

The pharmacokinetic data correlated with the anticonvulsant activity in the MES test. Administration of phenytoin-*bis*-hydroxyisobutyrate resulted in a faster peaking of the MES activity and a lower ED_{50} than the administration of phenytoin itself. These observations can be attributed to the increased bioavailability of phenytoin after administration of the derivative.

No measurable levels of the prodrug could be detected in the plasma samples, only phenytoin. The exact site of the hydrolysis of the *bis*-hydroxyisobutyrate prodrug is unknown. However, the high plasma levels of phenytoin after 15 min as well as the early peaking of t_{max} and of the anticonvulsant activity after oral administration (0.5 h) compared to a maximal activity at 1 h after intraperitoneal administration (5) suggest that the prodrug is hydrolyzed during the absorption process.

Upon oral administration, phenytoin-*bis*-hydroxyisobutyrate did not exhibit any significant activity in the scMet test or any neurological toxicity in the rotorod test at the doses tested. No activity in the scMet test nor acute toxicity have been demonstrated for phenytoin (7). Thus, coupling to the *bis*-hydroxyisobutyrate did not alter the pharmacological profile of

Table 1. Pharmacokinetic Parameters Obtained from Phenytoin Plasma Levels and Median Effective Dose Determined in the MES Test After Oral Administration of Phenytoin and Phenytoin-*bis*-hydroxyisobutyrate to Rats

	Pharmacokinetic parameters[a]				MES test	
Compound	AUC [μg·h/ml]	C_{max} [μg/ml]	t_{max} [h]	$t_{1/2}$ [h]	Time of test[b] [h]	ED_{50}[c] [μmol/kg]
Phenytoin	10.0 ± 1.1	1.90 ± 0.11	1.25 ± 0.5	3.48 ± 0.46	2	91.9 (84.8–100.7)
Phenytoin-*bis*-hydroxy-isobutyrate	28.5 ± 4.2	5.25 ± 0.99	0.74 ± 0.1	4.25 ± 1.01	0.5	43.2 (29.4–59.1)
	$P < 0.001$[d]	$P < 0.001$	$P < 0.05$	n.s.[e]	—	$P < 0.001$

[a] Mean ± SD, n = 4 − 5 animals.
[b] Time of maximal activity.
[c] ED_{50} calculated from 5 doses, 8 animals per dose.
[d] t-test for unpaired observations.
[e] n.s., statistically not significant.

the drug as it has been described for dihydropyridine esters of 3-hydroxymethylphenytoin (11).

Phenytoin-*bis*-hydroxyisobutyrate displayed an anticonvulsant activity comparable to that of the drug itself upon intraperitoneal administration to mice (4) but was more active than phenytoin after oral dosing to rats. Thus, the present results suggest that glyceride-derived prodrugs might be useful for the oral delivery of phenytoin.

In conclusion, the oral administration of phenytoin-*bis*-hydroxyisobutyrate resulted in a higher bioavailability and anticonvulsant efficacy than the application of the phenytoin itself while not altering the pharmacological profile of the drug. 3-Hydroxy-2-hydroxymethylpropionic acid-derived prodrugs might represent useful prodrugs for the oral delivery of poorly water-soluble compounds.

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