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Bioavailability of Phenytoin Following Oral Administration of Phenytoin–lipid Conjugates to Rats

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Abstract

The bioavailability of phenytoin was evaluated in rats upon oral administration of phenytoin-lipid conjugates obtained by covalent binding of 3-hydroxymethylphenytoin to 1,3-dimyristoylglyceride via a succinidyl linkage, to 2-(1,3-dimyristoyl-2-glyceryl)butyric acid and to 3-myristoyloxy-2-myristoyloxymethylpropionic acid.

Despite differences of the phenytoin plasma concentrations all three compounds approximately doubled the AUC compared with the dosing of phenytoin itself. The early onset and the long duration of the anticonvulsant activity after administration of the triglyceride-derived conjugate could be correlated to the increased phenytoin plasma levels.

It is concluded that drug-lipid conjugates may be useful prodrugs for the oral delivery of poorly water-soluble drugs.

In an attempt to improve the oral absorption of poorly water-soluble drugs, lipid conjugates have been designed as lipase-driven drug delivery systems using phenytoin as a model compound (Scriba 1993a, 1994). In these derivatives (Fig. 1) 3-hydroxymethylphenytoin has been covalently bound to 1,3-dimyristoylglycerol via succinic acid (**1**), to 4-(1,3-dimyristoyl-2-glyceryl)butyric acid (**2**), and to 3-myristoyloxy-2-myristoyloxymethylpropionic acid (**3**). The glyceridomimetic prodrugs were effectively degraded in-vitro by pancreatic lipase yielding phenytoin (Scriba 1993b; Scriba et al 1995). Moreover, the lipase-mediated release of the drug correlated with the anticonvulsant activity of the compounds upon oral administration to mice (Scriba et al 1995). The conjugates **1** and **2** were more effective in antagonizing seizures than phenytoin itself 30 min postdose, suggesting efficient degradation of the lipids in-vivo. Therefore, the present study was conducted to compare the oral bioavailability of phenytoin upon administration of the glyceridomimetics with the availability of phenytoin itself.

Materials and Methods

Chemicals

Phenytoin was obtained from Caelo (Hilden, Germany), 5-(*p*-methylphenyl)-5-phenylhydantoin from Sigma (Deisenhofen, Germany), and heparin solution (25 000 int. units mL^{-1}) from Ratiopharm (Ulm, Germany). Phenytoin–lipid conjugates were synthesized as described by Scriba (1993a, 1994) and were at least 98% pure. All other chemicals were obtained from commercial sources at the highest purity available.

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Pharmacokinetics

Male Wistar rats (bred at the animal facilities at UCL), 220–260 g, were housed individually in a 12-h light–dark cycle with free access to commercial rodent chow and water. The animals were starved overnight and during the experiment but were allowed free access to water. Phenytoin (119 μmol kg^{-1}) and phenytoin–lipid conjugates (30 mg kg^{-1} equivalents of phenytoin) were administered as suspensions in 0·5% aqueous methylcellulose by oral intubation in a volume of 2 mL kg^{-1}. Approximately 300 μL blood were collected via the tail-clip method into Eppendorf tubes

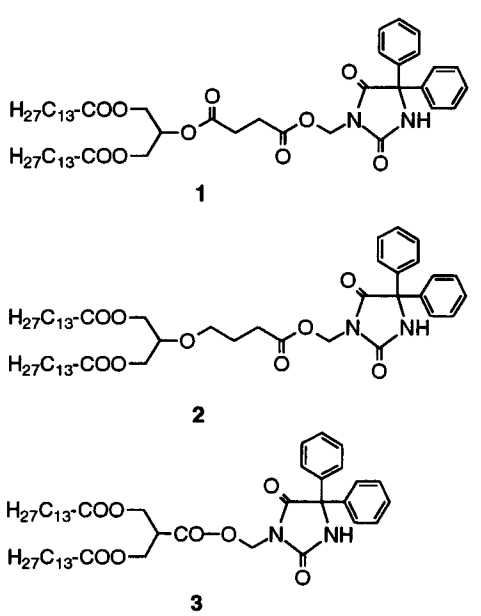

FIG. 1. Structures of phenytoin-lipid conjugates.

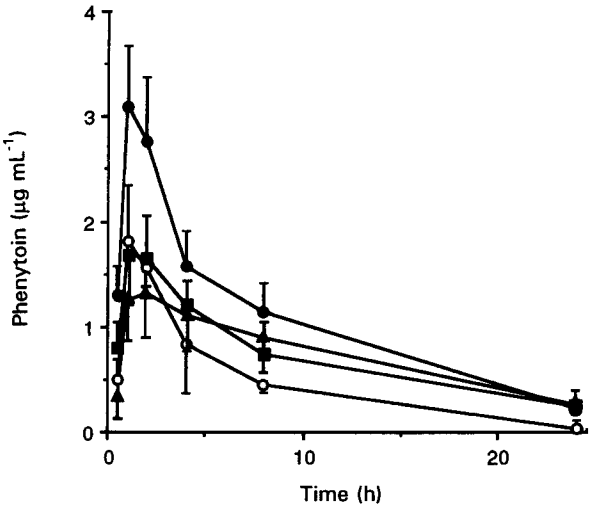

FIG. 2. Plasma concentration of phenytoin following oral administration of phenytoin (○) and phenytoin–lipid conjugates **1** (●), **2** (■), and **3** (▲) to rats. A dose of 119 μmol kg^{-1} (30 mg kg^{-1} equivalents of phenytoin) was applied by oral intubation (n = 4–5 ± s.d.).

containing 20 μL heparin solution. Following centrifugation at 4°C and 4000 *g*, the plasma was immediately separated and stored at −80°C until analysed by gas liquid chromatography (GLC) as described by Stella (1977) with minor modifications. The modification was the use of a 25 m HP-1 column (Hewlett Packard, Düsseldorf, Germany) for separation of phenytoin and the internal standard, 5-(*p*-methylphenyl)-5-phenylhydantoin, using helium as the carrier gas at a flow rate of 0·75 mL min^{-1}. The column temperature was maintained at 225°C. The detector temperature was set to 300°C, the injector temperature to 270°C. The retention times of phenytoin and 5-(*p*-methylphenyl)-5-phenylhydantoin were 9·0 and 11·7 min, respectively. The assay was linear in the range 0·5–25 μg mL^{-1} with a detection limit of approximately 0·2 μg mL^{-1}. The precision was 7·1% at 0·5 μg mL^{-1} and better than 2·6% at higher concentrations. Phenytoin concentrations were calculated by the peak area ratio method using a calibration curve obtained from plasma samples containing added phenytoin.

Data analysis

Noncompartmental analysis of the data was performed (TOPFIT 2.0). The maximum plasma concentration (C_{max}) and the time to reach this concentration (t_{max}) were obtained from the plasma concentration–time profiles. The area under the curve (AUC) was calculated by the logarithmic trapezoidal method for the observed values and by extrapolation to infinity. The elimination half-life ($t_{\frac{1}{2}}$) 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.

Pharmacology

Anticonvulsant testing in the maximal electroshock (MES) test and the determination of the acute neurological toxicity in the rotorod test after oral administration to CF1 mice was provided by the National Institute of Neurological Disorders and Stroke Epilepsy Branch, National Institutes of Health, Bethesda, MD, USA, according to standard procedures (Krall et al 1978). For these evaluations the compounds were suspended in 0·5% aqueous methylcellulose.

Results

The structures of the phenytoin–lipid conjugates are shown in Fig. 1. The plasma concentration of phenytoin upon oral administration of equimolar doses of phenytoin and the lipid conjugates **1**, **2**, and **3** to rats are summarized in Fig. 2. Application of triglyceride **1** resulted in higher plasma concentrations of phenytoin than administration of the parent drug, while lipid conjugates **2** and **3** gave similar or lower plasma concentrations of phenytoin after the first 4 h. At 8 h all conjugates produced higher plasma levels of phenytoin than the drug itself.

The pharmacokinetic parameters obtained by noncompartmental analysis of the plasma data are summarized in Table 1. While differences in C_{max} were observed upon administration of the glyceridomimetics **1** to **3**, all derivatives approximately doubled the AUC compared with phenytoin. Lipid **1** was the most effective of the compounds tested.

The anticonvulsant activities of glyceridomimetic **1** and phenytoin following oral administration to mice were compared in the MES test, the acute neurological toxicity was determined in the rotorod test (Fig. 3). Lipid **1** effectively antagonized MES-induced seizures between 2 and 6 h. The anticonvulsant effect peaked around 2 h (determined with a lower dose than shown in Fig. 3A) and lasted for at least 8 h. The maximum of the seizure antagonizing activity of

Table 1. Pharmacokinetic parameters obtained from phenytoin plasma levels after oral administration of phenytoin and phenytoin–lipid conjugates **1**, **2**, and **3** to rats (n = 4–5 ± s.d.).

Compound	C_{max} (μg mL^{-1})	t_{max} (h)	AUC (μg h mL^{-1})	$t_{\frac{1}{2}}$ (h)
Phenytoin	1·90 ± 0·11	1·59 ± 0·40	10·0 ± 1·1	3·48 ± 0·46
1	3·08 ± 0·58**	1·40 ± 0·10	25·1 ± 3·9***	6·68 ± 1·09***
2	1·96 ± 0·26	1·67 ± 0·67	19·9 ± 7·4*	8·93 ± 4·74**
3	1·51 ± 0·34	2·30 ± 0·56	19·3 ± 5·7*	9·14 ± 1·69***

*$P < 0·05$; **$P < 0·01$; ***$P < 0·001$ when compared with phenytoin data using the *t*-test for unpaired observations.

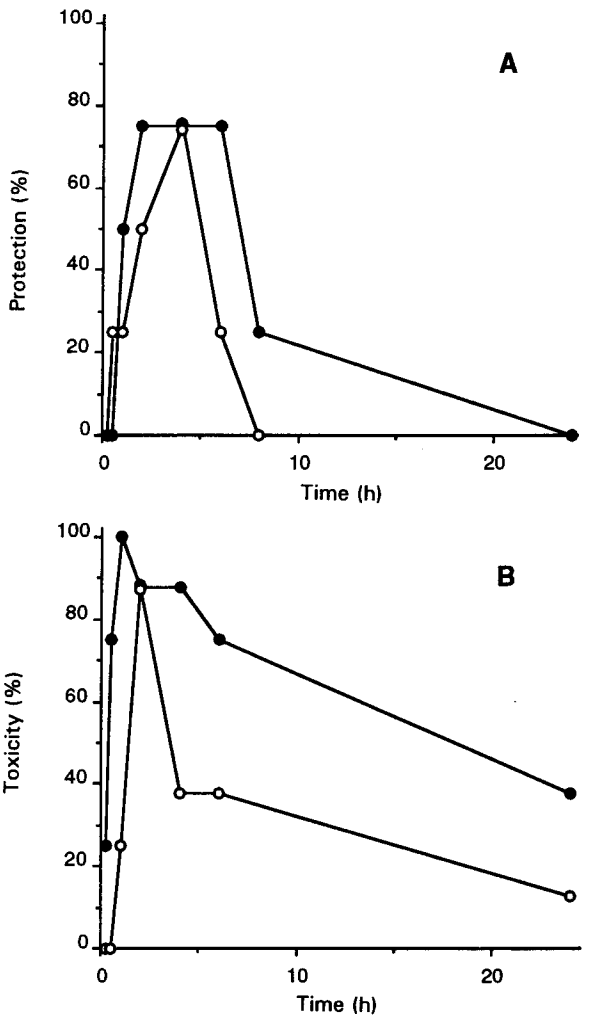

FIG. 3. Time course of the anticonvulsant activity (A; MES test) and acute neurological toxicity (B; rotorod test) of phenytoin (○) and phenytoin–lipid conjugate **1** (●) after oral administration to mice. The values are expressed as percentage of animals protected in the MES test (n = 4) or unable to grasp the rotorod (n = 8). The animals were dosed with phenytoin, 40 μmol kg^{-1} (MES) and 395 μmol kg^{-1} (rotorod), or with lipid **1**, 40 μmol kg^{-1} (MES) and 570 μmol kg^{-1} (rotorod).

phenytoin was observed after 4 h. The drug was inactive 8 h after the administration. The acute toxicity of the conjugate **1** peaked after 1 h, the toxicity of phenytoin after 2 h (Fig. 3B).

Discussion

The plasma levels of phenytoin were evaluated after oral administration of the phenytoin-lipid conjugates **1**, **2** and **3**. Application of compound **1** resulted in the highest plasma levels while dosing of the glyceridomimetic **3** led to only low drug concentrations. This observation may be correlated to the pancreatic lipase-mediated release of phenytoin from the conjugates in-vitro, which decreased in the order **1** > **2** ≫ **3** (Scriba 1993b; Scriba et al 1995). The slow liberation of the drug from **3** is supported by the fact that t_{max} occurred 2·3 h after the administration, while a t_{max} of about 1·5 h was found for lipids **1** and **2**. A shift of t_{max} has also been observed upon the co-administration of lipids and phenytoin (Chakrabarti & Belpaire 1978) and after the oral dosing of phenytoin prodrugs in tributyrin (Yamaoka et al 1983).

In-vitro, pancreatic lipase cleaved with high positional specificity the fatty acid ester bonds in position 1 and 3 of the glycerol moiety of conjugate **1** yielding phenytoin-2-monoglyceride, which was subsequently hydrolysed to give phenytoin (Scriba 1993b). In the present study, an early peaking of the phenytoin plasma concentrations as well as the anticonvulsant activity in the MES test and the toxic effect in the rotorod test were observed. These results suggest that conjugate **1** is also efficiently degraded by lipase in-vivo followed by absorption of free phenytoin rather than the intact lipid conjugates. The intestinal degradation of triglyceride prodrugs of acetylsalicylic acid and *p*-chlorophenoxyisobutyric acid has been described (Owen & Billimoria 1977; Kumar & Billimoria 1978).

Despite differences in C_{max}, application of the phenytoin–lipid conjugates improved the bioavailability of the drug by a factor of 2–2·5 compared with administration of phenytoin itself. 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. The rapid release of phenytoin from **1** may be expected to yield finely dispersed phenytoin which can be readily absorbed. The concomitant resulting fatty acids may also contribute to the absorption process (van Hoogdalem et al 1989). Interestingly, administration of conjugate **3**, which was only a poor substrate for pancreatic lipase, resulted in almost the same bioavailability as the rapidly degraded lipid **1**.

The pharmacokinetic data of phenytoin and phenytoin-lipid conjugate **1** correlated with the anticonvulsant activity and toxicity in mice. Oral administration of **1** resulted in an earlier peaking of both the activity in the MES test and the toxic effect in the rotorod test. In contrast to the parent drug, the lipid prodrug was still active after 8 h. Both observations can be attributed to the increased plasma levels and bioavailability of phenytoin obtained upon administration of the glyceridomimetic **1**.

An increase of the elimination half-life was found upon administration of the lipid conjugates. It is known that phenytoin displays nonlinear, dose-dependent elimination kinetics (Ashley & Levy 1973; Vincuna et al 1980). However, except for lipid **1**, only plasma levels comparable with or lower than those obtained after an equimolar dose of phenytoin were observed during the first 4 h. Thus, this apparent increase of $t_{\frac{1}{2}}$ might be attributed to the constant release of phenytoin rather than to a slower metabolism or elimination of the drug. An increase of $t_{\frac{1}{2}}$ has also been observed after the co-administration of phenytoin with lipids (Chakrabarti & Belpaire 1978) or after the dosing of phenytoin prodrugs in triglyceride preparations (Yamaoka et al 1983).

In summary, the results obtained suggest that lipid conjugate formation increases the bioavailability of phenytoin. The drug is released by pancreatic lipase in the intestines upon oral administration of the glyceridomimetic prodrugs. The rate of release of phenytoin determines the drug plasma levels and is dependent on the structure of the lipid conjugates. We conclude that drug–lipid conjugates might

represent useful prodrugs for the oral delivery of poorly water-soluble compounds.

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