J. Pharm. Pharmacol. 1995, 47: 197–203
Received August 18, 1994
Accepted September 27, 1994

Anticonvulsant Activity of Phenytoin-lipid Conjugates, a New Class of Phenytoin Prodrugs

GERHARD K. E. SCRIBA, DIDIER M. LAMBERT* AND JACQUES H. POUPAERT*

*Department of Pharmaceutical Chemistry, School of Pharmacy, University of Münster, Hittorfstrasse 58–62, 48149 Münster, Germany, and *Laboratory of Medicinal Chemistry, School of Pharmacy, Catholic University of Louvain, CMFA 73.40, Avenue E. Mounier 73, 1200 Brussels, Belgium*

Abstract

The anticonvulsant activity of phenytoin-lipid conjugates obtained by covalent binding of 3-hydroxymethylphenytoin to dimyristoylglycerides via a succinidyl linkage, to 2-(1,3-dimyristoylglyceryl)butyric acid and to 3-myristoyl-2-myristoylmethylpropionic acid was evaluated in the maximal electroshock (MES) test and the seizure threshold test with subcutaneous pentetrazol.

The phenytoin-lipid conjugates were less active than the parent drug in the MES test after intraperitoneal administration as suspensions, but exhibited comparable activity when injected as a solution in dimethylsulphoxide. They also protected mice from MES-induced seizures following oral administration of aqueous suspensions of the compounds or when incorporated into emulsions. The anticonvulsant activity could be correlated to in-vitro pancreatic lipase-mediated hydrolysis. The *bis*-deacyl derivatives were at least as active but in some cases also more toxic than phenytoin. Oral administration of two of the lipid conjugates resulted in a faster onset of the anticonvulsant activity compared with the administration of an equimolar dose of phenytoin itself. All compounds were inactive in the subcutaneous pentetrazol test.

It is concluded that the lipids act as prodrugs of phenytoin, which is generated by lipolysis upon oral administration.

Phenytoin was introduced in 1938 as an antiepileptic agent (Merrit & Putnam 1938) and is still extensively used for the treatment of generalized tonic clonic seizures or elementary partial seizures (Rall & Schleifer 1990). However, due to its low water solubility and low lipid solubility the drug has shown erratic bioavailability when administered orally (Arnold et al 1970; Suzuki et al 1970). Superior availability was obtained by co-administration of lipids (Chakrabarti & Belpaire 1978) or by ester prodrugs of 3-hydroxymethylphenytoin (Yamaoka et al 1983; Varia et al 1984). Prodrugs are direct chemical precursors of drugs that can alter their overall delivery by modifying their lipophilicity, release or metabolism.

In an attempt to improve the oral bioavailability of phenytoin, three classes of phenytoin-lipid conjugates (Fig. 1) have recently been synthesized as potential prodrugs of phenytoin (Scriba 1993a, 1994). The first group of compounds included triglycerides with 3-hydroxymethylphenytoin, a prodrug of phenytoin, bound to position 2 (**2**) or position 1 (**4**) of a glyceride via succinic acid. In the second type of lipid derivatives, the drug has been linked to 2-(1,3-dimyristoylglyceryl)butyric acid. In these compounds the hydrolytically labile ester bond between the 2-hydroxy group of the glycerol backbone and succinic acid has been replaced by the metabolically stable ether functionality (**6**). In a third series of compounds, the glycerol moiety has been replaced by 3-hydroxy-2-hydroxymethylpropionic acid which allows the direct formation of an ester bond with the alcohol function of drugs without incorporating a spacer (**8**).

In-vitro, the phenytoin-lipid conjugates were degraded by pancreatic lipase similarly to natural triglyderides (Scriba 1993b, 1994). The enzyme cleaved with high positional specificity the ester bonds in positions 1 and 3 of the lipids. Thus, the *bis*-deacyl compounds **1**, **5** and **7**, which may be compared with 2-monoglycerides, were primarily formed after incubation of the lipids **2**, **6**, and **8**, respectively. When the drug was bound to position 1 of the glyceride via a succinoyl spacer (compound **4**) the succinic acid mono ester was primarily formed. All compounds eventually gave phenytoin.

Oral administration is generally considered the way of application of choice for a drug. According to the in-vitro studies the lipid conjugates can be expected to be hydrolysed by pancreatic lipase in the upper small intestine upon oral administration and, therefore, act as prodrugs of phenytoin. The present study was performed to evaluate this hypothesis and to determine the anticonvulsant activity of the phenytoin-lipid conjugates. Furthermore, a possible correlation between the in-vitro hydrolysis data and the pharmacological activity in-vivo was investigated. Special attention was directed towards the possibility of an improvement of the efficacy of the drug-lipid conjugates compared with the parent drug. The *bis*-deacyl derivatives **1**, **5** and **7**, which can be considered intermediates in the hydrolysis of the corresponding lipids, as well as the 1-monoglyceride, **3**, were also included in this study.

Correspondence: G. K. E. Scriba, Department of Pharmaceutical Chemistry, School of Pharmacy, The University of Münster, Hittorfstrasse 58–62, 48149 Münster, Germany.

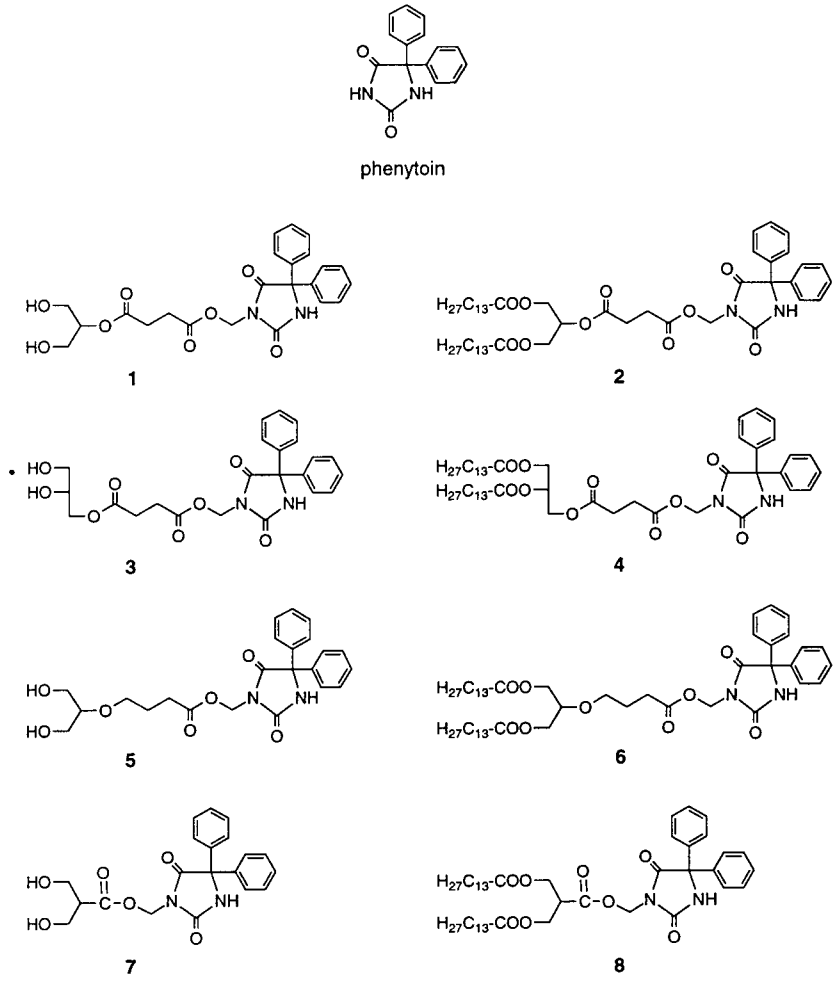

FIG. 1. Structures of the compounds.

Materials and Methods

Materials

The phenytoin-lipid conjugates and the corresponding *bis*-deacyl derivatives were synthesized as described previously (Scriba 1993a, 1994). Phenytoin and sodium oleate were purchased from Caelo (Hilden, Germany). Lecithin (60% from egg yolk) and methylcellulose (Tylose 1000) were obtained from Fluka (Neu-Ulm, Germany). Porcine pancreatic lipase (type II, crude preparation), triolein and sodium taurocholate were obtained from Sigma (Deisenhofen, Germany). Dimethylsulphoxide was distilled from sodium hydride. Buffers and solutions were made with double-distilled, de-ionized water.

In-vitro incubations

In-vitro incubations of the lipids with porcine pancreatic lipase were performed as described earlier (Scriba 1993b). Briefly, crude dispersions of the lipids obtained by sonication of a 1 : 4 mixture of a solution of the lipids in ethanol and a 25 mM solution of sodium taurocholate in water were incubated with pancreatic lipase (375 units mL^{-1}, assayed by Sigma (Deisenhofen, Germany) using triacetin as substrate at pH 7·4) in 0·1 M 1,4-piperazinediethanesulphonic acid (PIPES) buffer, pH 6·5, at 37°C. Samples of the incubations were quenched with ice-cold 0·5 M $HClO_4$, diluted with water/acetonitrile (80 : 20, v/v) and centrifuged. The clear supernatant was analysed by HPLC on a LiChrospher 100 RP-18 column (125 × 4·6 mm, 5 mm) (E. Merck, Darmstadt, Germany). For the analysis of the *bis*-deacyl derivatives, the mobile phase consisted of 0·05 M phosphate buffer, pH 5·8, containing 25% acetonitrile (v/v). The lipids were chromatographed using acetonitrile/water (95 : 5, v/v). The flow rate was 1·5 mL min^{-1}. The HPLC apparatus consisted of a Shimadzu LC 6A solvent delivery module, a Shimadzu SPD 6A UV detector operated at 254 nm and a Shimadzu C-R6A integrator (Shimadzu AG, Duisburg, Germany).

Pharmacology

Male OF1 mice, 20–30 g, were obtained from Iffa Credo (Les Oncins, France) and housed in colony cages in a 12-h light–dark cycle with free access to commercial rodent chow and water. During the experiment the animals were only allowed free access to water. Maximal electroshock seizures (MES) were induced by delivering an electrical stimulus of 50 mA to mice for 0·2 s via corneal electrodes. Blockade of the tonic extension of the hind limbs was considered as protection against seizures (Swinyard et al 1952). The compounds were dissolved in dimethylsulphoxide or suspended in 0·5% methylcellulose solution (w/v) by sonication. The

microscopically determined particle size of the suspensions was 5 to 30 μm. Emulsions were prepared as follows: the appropriate amounts of the lipids were dissolved in 400 μL triolein containing 30 mg mL^{-1} lecithin. While vortexing, 1·6 mL of a 5% aqueous solution of glycerol containing 5 mM sodium oleate adjusted to pH 7 was added. The resulting crude emulsion was sonicated five times for 30 s while cooling with ice. The particle size of the emulsions ranged between 1 and 5 μm with sparse droplets up to 25 μm. Regardless of the route of administration, a constant volume of 2 mL kg^{-1} of the freshly made preparations was applied throughout the experiments. ED50 values were calculated according to Litchfield & Wilcoxon (1949).

Additional MES tests of the compounds **1**, **3**, **5** and **7**, the seizure threshold tests with subcutaneous pentetrazol and the rotorod tests were provided by the Antiepileptic Drug Development Program, Epilepsy Branch, Division of Convulsive, Developmental and Neuromuscular Disorders, National Institutes of Health, according to standard testing procedures and included phases I, II, and VIa (Krall et al 1978; Porter et al 1984). Phase I identifies the anticonvulsant activity (MES and pentetrazol tests) and the neurotoxicity (rotorod test) in male Crossworth Farm #1 (CF1) mice while Phase II quantifies the anticonvulsant activity and neurotoxicity. In phase VIa, the potency of the compounds is determined following oral administration to rats. For all these evaluations the compounds were dissolved or suspended in 0·5% aqueous methylcellulose.

Results

The half-lives of the pancreatic lipase-catalysed hydrolysis of the lipid conjugates **2**, **4**, **6** and **8** are summarized in Table 1. Although the degradation of the lipids proceeded effectively for all derivatives, the compounds differ significantly in their rate of release of the drug.

The phenytoin-lipid conjugates exhibited only a low activity at doses of up to 340 μmol kg^{-1} in the MES test after intraperitoneal administration to mice as a suspension in 0·5% aqueous methylcellulose except for lipid **2**. The median effective dose (ED50) was determined to be 80·8 (48·1–129·4) μmol kg^{-1}. At a dose of 250 mg kg^{-1} (equivalent to 285 μmol kg^{-1}), lipid **2** showed no neurological deficit in the rotorod test, at a dose of 500 mg kg^{-1} (equivalent to 570 μmol kg^{-1}); **2** was toxic for five out of eight animals. All compounds showed good activity in the MES test when administered as dimethylsulphoxide solutions. Table 2 summarizes the initial screening of the compounds 30 min and 3 h after administration of 40 μmol kg^{-1} of the drugs dissolved in dimethylsulphoxide. Generally, the lipids were less active after 30 min than after 3 h. Compounds which did not contain phenytoin, such as 1,3-dimyristoylglycerol, 2-(1,3-dimyristoylglyceryl)butyric acid and 3-myristoyloxy-2-myristoyloxymethylpropionic acid, did not exhibit any anticonvulsant activity, nor did dimethylsulphoxide at the dose of 2 mL kg^{-1} used throughout the experiments.

Table 1. Half-lives of the pancreatic lipase-mediated degradation of phenytoin-lipid conjugates.

Lipid	Degradation of lipid $t_{\frac{1}{2}}$ (min)	Release of phenytoin $t_{\frac{1}{2}}$ (min)
2	1·5 ± 0·4	17·5 ± 6·4
4	7·8 ± 2·0	193 ± 24
6	8·4 ± 1·6	27·3 ± 3·8
8	10·8 ± 2·4	145 ± 31

The compounds were incubated in 0·1 M PIPES buffer, pH 6·5, at 37°C with 375 units mL^{-1} porcine pancreatic lipase in the presence of 25 mM Ca^{2+} and analysed as described in the experimental section. Each value is the mean ± s.d. (n = 3).

Table 2. MES activity of the compounds, after intraperitoneal administration of a dose of 40 μmol kg^{-1} in dimethylsulphoxide expressed as percent of the animals protected against seizures.

Compound	MES activity (%)	
	30 min	3 h
Phenytoin	100	100
1	100	100
2	63	100
4	33	100
5	100	100
6	33	100
7	88	100
8	25	78

n = 7–9 animals.

The phenytoin-lipids also protected mice from MES-induced seizures following oral administration of a suspension in aqueous methylcellulose or when incorporated into a triolein/lecithin emulsion. Table 3 summarizes the results obtained with a dose of 35 μmol kg^{-1} of each compound. Regardless of the nature of the preparation, the compounds were always more active after 1 h and 4 h, respectively, than 30 min after the administration. The lipids **2** and **6** showed a higher activity in the MES test than the compounds **4** and **8**. Application of the conjugates **2** or **6** provided a better protection against MES-induced seizures 30 min after the administration than the parent drug phenytoin. The ED50 of phenytoin and the most active derivates **2** and **6** are shown in Table 4.

The time course of the anticonvulsant activity of the phenytoin-lipid conjugate **2** after intraperitoneal and oral administration to mice is summarized in Fig. 2. Following intraperitoneal administration the activity peaked around 6–8 h and the compound was still active after 24 h. No antagonism of the seizures was noted for the first 2–3 h. In contrast, after oral administration, the maximum of the MES activity was reached after 2–4 h.

The *bis*-deacyl derivatives were active in the MES test after intraperitoneal administration in dimethylsulphoxide solution or as a suspension in aqueous methylcellulose. The data of the compounds **1**, **5** and **7** after administration in dimethylsulphoxide are shown in Table 2. In phase I of the Antiepileptic Drug Development Program all derivatives showed activity at a dose of 30 mg kg^{-1} or less and were classified as active compounds (class 1). Except for ester 3, the compounds also protected rats against MES-induced seizures after oral administration (phase VIa, data not shown). The median effective dose (ED50) and median toxic dose (TD50) of **1**, **5** and **7** in comparison with phenytoin are summarized in Table 5. Following intraper-

Table 3. MES activity of the phenytoin-lipid conjugates in mice after oral administration of a dose of 35 μmol kg^{-1} of the compounds as a suspension and incorporated into a triolein/lecithin emulsion.

Compound	Suspension			Emulsion		
	30 min	1 h	4 h	30 min	1 h	4 h
Phenytoin	33	100	100	n.d.	n.d.	n.d.
2	67	90	90	57	100	100
4	17	n.d.	67	14	66	71
6	70	n.d.	100	33	100	100
8	17	33	60	14	50	80

The values are expressed as percent of the animals (n = 3–7) protected against seizures. n.d.: not determined.

itoneal injection, the compounds proved to be at least as active as phenytoin. A protective index (PI = TD50/ED50) of about 5·4–5·5 was calculated for these derivatives. Compound **1** was about three times as active as phenytoin after oral administration to rats with no apparent toxicity up to 660 μmol kg^{-1}, the highest dose tested (Table 5).

All compounds including the parent drug phenytoin were inactive in the seizure threshold test with subcutaneous pentetrazol.

Discussion

It has been observed that mixed triglycerides formed by substitution of a fatty acid moiety by a drug are rapidly cleaved in-vivo after intravenous application (Mergen et al 1991). Thus, when dissolved in a water-miscible organic solvent the conjugates **2**, **4**, **6** and **8** should be hydrolysed in-vivo resulting in pharmacologically active concentrations of phenytoin. The lower anticonvulsant activity after intraperitoneal administration of a suspension of the conjugates can be attributed to the extremely low aqueous solubility of the lipids (Scriba 1993b). The slow release or dissolution of the prodrugs from the suspension at the injection site resulted in lower phenytoin levels. While the maximum of the MES activity of phenytoin was found at about 2 h (Krall et al 1978), the peak of the anticonvulsant activity of lipid **2** was found at about 6 h. Glyceride derivatives without phenytoin i.e. 1,3-dimyristoylglycerol, 2-(1,3-dimyristoylglyceryl)butyric acid and 3-myristoyloxy-2-myristoyloxymethylpropionic acid did not antagonize seizures in the MES test. Thus, phenytoin can be considered as the pharmacologically active species; the delay of the onset of the activity suggests that the lipids have to be hydrolysed in order to exert any anticonvulsant activity.

The solvent dimethylsulphoxide did not antagonize seizures in the MES test at the dose used throughout the experiments. This is in agreement with the results obtained by Stankova & Mares (1992). However, dimethylsulphoxide is a well-known penetration enhancer (Reynolds 1993), and has also been reported to facilitate the entry of proteins (Broadwell et al 1982) and a variety of drugs (De La Torre 1970; Iwen & Miller 1986; Mori et al 1992) into the brain. Thus, its enhancing effect has to be taken into consideration and might contribute to the efficacy of the phenytoin derivatives when they are administered in this solvent. On the other hand, the increased activity of the lipids in dimethylsulphoxide might result from a fairly rapid release of the prodrugs from the injection site with minimal precipitation of the compounds.

The phenytoin-lipid conjugates protected mice against MES-induced seizures after oral administration as suspensions or when incorporated into triolein/lecithin emulsions. No comparison with the parent drug could be made in the case of the emulsions, because a reasonable triolein emulsion containing phenytoin could not be formulated due to the low water solubility and low lipid solubility of phenytoin (Schwartz et al 1977; Varia et al 1984). Regardless of the preparation, the lipids **2** and **6** were more active than **4** and **8**. This observation can be correlated with the in-vitro release of phenytoin from the lipid conjugates by pancreatic lipase which occurred 5–11 times faster from **2** and **6** than from **4** and **8** and can be explained by the degradation

Table 4. Median effective dose (ED50) of phenytoin and the lipids **2** and **6** after intraperitoneal and oral administration to mice.

Compound	Intraperitoneal solution in dimethylsulphoxide		Oral suspension in methylcellulose		Oral triolein emulsion	
	30 min	3 h	30 min	4 h	30 min	4 h
Phenytoin	13·8 (10·2–18·6)	12·0 (7·0–20·8)	58·5 (31·2–109·8)	10·7 (7·4–15·5)	n.d.	n.d.
2	24·8 (13·8–44·6)	9·3 (4·2–12·5)	23·9 (18·1–31·6)	13·6 (9·3–19·8)	26·0 (14·0–41·4)	15·8 (10·8–23·4)
6	n.d.	n.d.	23·4 (14·2–38·6)	14·5 (9·8–21·2)	n.d.	n.d.

n.d.: not determined. OF1 mice were treated with the compounds 30 min, 3 and 4 h, respectively, before the MES test. ED50 values were calculated from 5–7 doses (n = 5–9 animals per dose) according to Litchfield & Wilcoxon (1949). The values are expressed in μmol kg^{-1}; 95% confidence intervals are given in parentheses.

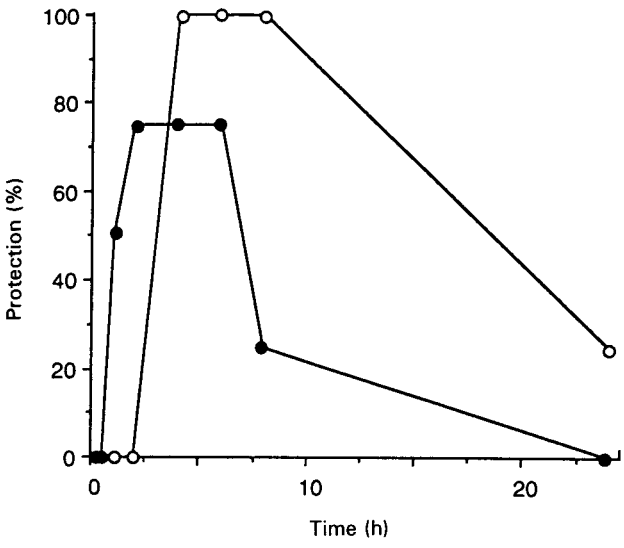

FIG. 2. Time course of the anticonvulsant activity after intraperitoneal and oral administration of a suspension of the phenytoin-lipid conjugate **2** to mice. ○, Intraperitoneal administration of a dose of 100 mg kg^{-1} (equivalent to 114 μmol kg^{-1}); ●, oral administration of a dose of 30 mg kg^{-1} (equivalent to 34 μmol kg^{-1}). The data are expressed as percent of animals protected (n = 4). The data were determined by the Anticonvulsant Drug Development Program of the Epilepsy Branch, National Institutes of Health.

mechanism determined in-vitro (Scriba 1993b). The lipase-mediated hydrolysis of the derivatives **2** and **6** yielded primarily the monoglycerides **1** and **5**, respectively, which were rapidly hydrolysed to eventually give phenytoin. In contrast, the intermediates generated from **4** and **8** upon lipolysis only slowly released phenytoin. Due to the positional specificity of lipase degradation, the glyceride **4** furnished phenytoin succinic acid mono ester. Succinic acid mono esters are generally known as poor substrates for hydrolytic enzymes (Larsen et al 1988; Seki et al 1988). Lipolysis of **8** furnished **7** which was also relatively stable, probably due to steric factors. Because the same degradation mechanism can be assumed after oral administration of the lipid conjugates, it can be concluded that the anticonvulsant activity is due to phenytoin rather than to the intact drug-lipid conjugates. Therefore, the lipids act as prodrugs of phenytoin. The rate of release of the drug determines the pharmacological activity. The efficient lipase-mediated release of phenytoin from lipid **2** is further demonstrated by the fact that the maximum of the anticonvulsant activity was found at 2–4 h after oral administration compared with 6–8 h after intraperitoneal administration of the compound.

As a poorly water-soluble drug, the absorption of phenytoin from the intestinal tract is known to depend on the rate of dissolution of the crystalline material and, therefore, on the size and form of the phenytoin crystals (Glazko 1972; Sanson et al 1975). The major absorption site of the drug is the upper small intestine (Dill et al 1956), which is also the site of lipolysis (Mead et al 1986). Based on their ED50 values, the conjugates **2** and **6** were approximately twice as effective as phenytoin in antagonizing MES-induced seizures 30 min after oral administration. Thus, lipase-catalysed degradation of the lipid conjugates can be expected to yield very fine dispersed phenytoin, which results in superior absorption compared with the administration of phenytoin itself.

The activity of pancreatic lipase is known to depend on the interfacial area of the lipid droplets (Mead et al 1986). However, the onset of the anticonvulsant activity appeared to be slower when the compounds were administered as emulsions compared with suspensions (Table 3), although the emulsions generally had a smaller particle size than the suspensions. This may be explained by the fact that lipase possesses a higher affinity or a higher V_{max} for natural triglycerides such as triolein than for phenytoin-lipid conjugates. Thus, when an emulsion is applied, lipase hydrolyses triolein preferentially whereas upon administration of an emulsion the entire activity of the enzyme can be used for the degradation of the drug-lipid conjugates. Detailed mechanistic studies will be necessary to confirm this mechanism. However, this observation is also a further indication that hydrolysis of the lipid conjugates is required in order to exert the pharmacological activity. In fact, it has been recently demonstrated by Alvarez & Stella (1989) that suspensions of esters of 3-hydroxymethylphenytoin can be substrates for pancreatic lipase.

The *bis*-deacyl derivatives **1**, **5** and **7** which can be compared with 2-monoglycerides as intermediates of the lipase-catalysed hydrolysis as well as the isomeric

Table 5. Median effective dose (ED50), median toxic dose (TD50) and protective index (PI) of phenytoin and the *bis*-deacyl derivatives **1**, **5** and **7** after intraperitoneal and oral administration.

Compound	Mice (intraperitoneal)			Rats (oral)		
	ED50 (MES)	TD50 (rotorod)	PI (TD50/ED50)	ED50 (MES)	TD50 (rotorod)	PI (TD50/ED50)
Phenytoin[a]	37·7 (32·2–41·2) [2 h]	260 (208–286) [2 h]	6·9	118 (79·9–178) [2 h]	>11900 [0·5 h]	>100
1	32·6 (24·5–42·4) [4 h]	179 (149–197) [1 h]	5·5	34·5 (30·0–42·2) [1 h]	>660 [0·25–24 h][b]	>20
5	29·4 (25·1–36·0) [4 h]	159 (148–175) [1 h]	5·4	n.d.	n.d.	–
7	30·2 (27·0–34·6) [1 h]	167 (132–201) [1 h]	5·5	n.d.	n.d.	–

[a] Data reported by Krall et al (1978) and Porter et al (1984). [b] Tested between 0·25 and 24 h. n.d.: not determined.
The data were determined by the Anticonvulsant Drug Development Program of the Epilepsy Branch, National Institutes of Health. ED50 values were calculated from 4–5 doses (n = 8–16 animals per dose). The values are expressed in μmol kg^{-1}; 95% confidence intervals are given in parentheses. The time of testing is listed in square brackets.

1-monoglyceride **3** were included in the study. All compounds exhibited good anticonvulsant activity in the MES test upon intraperitoneal administration as solutions in dimethylsulphoxide or as suspensions in methylcellulose to mice and, except for compound 3, also after oral administration to rats. The ED50 and TD50 of the compounds **1**, **5** and **7** following intraperitoneal application of a suspension indicated the derivatives were as active, but appeared to be also more toxic than phenytoin. The protective indices (PI = TD50/ED50) of 5·4–5·5 were lower than the index of 6·9 determined for phenytoin, although this difference is not statistically significant. The observations can be attributed to the approximate 50-fold increase of the aqueous solubility of the *bis*-hydroxy prodrugs compared with the parent drug (Scriba 1993b). Rat plasma rapidly released phenytoin from the compounds **1**, **5** and **7** in-vitro with half-lives of less than 0·5 min (Scriba 1993b, 1994). Thus, the derivatives would also be rapidly cleaved by plasma esterases in-vivo. It seems, therefore, more likely that phenytoin is the pharmacologically active species and that both the efficacy and the toxicity are due to phenytoin itself. However, it cannot be totally excluded that the increased toxicity is due to the prodrug.

Following oral administration to rats, compound **1** was more active than phenytoin and displayed about 3·4 times more activity in the MES test. Moreover, the maximum of the MES activity peaked earlier than for the parent drug. Pancreatic lipase has been found to hydrolyse the *bis*-deacyl derivatives in-vitro, and this hydrolysis can be expected to occur in-vivo. Of the four compounds, **1** was most effectively degraded with a half-life of approximately 6·5 min (Scriba 1993b). Combined with the increased aqueous solubility the rapid enzymatic hydrolysis of **1** resulted in an increased availability of the drug and, thus, a faster onset of the activity compared with phenytoin itself. A reasonable comparison between the toxicity data of **1** and the parent drug could not be made because **1** was only studied up to a dose of 660 μmol kg^{-1}, where no neurotoxicity was noted in the rotorod test. It has generally been observed that rats are much less susceptible to the neurological toxicity of phenytoin than mice (Porter et al 1984).

Compared with the ED50 reported by the Anticonvulsant Drug Development Program for the MES test (Krall et al 1978; Porter et al 1984) lower values were determined in the present study. According to the supplier, the OF1 mouse used in our experiments is equivalent to the CF1 strain used by NIH. Although we are not aware of major variations of the testing procedures between the two laboratories, the differences may be due to some differences between the two testing protocols; variations of the ED50 depending on the strain and age of the mice or the testing procedure have been reported (Meldrum 1986).

None of the compounds tested was active in the pentetrazol test. This has been reported for phenytoin (Krall et al 1978; Porter et al 1984). Thus, coupling to lipids or the corresponding *bis*-deacyl structures did not alter the pharmacological profile of the drug as described for dihydropyridine esters of 3-hydroxymethylphenytoin (Shek et al 1989). Structure-activity relationship studies (Nakamura et al 1965; Poupaert et al 1984) have shown that *N*-3 alkylated phenytoin derivatives were either inactive or presented only a low activity in the MES test. Moreover, those derivatives which were active also showed activity in the pentetrazol test. Therefore, the data further indicate that phenytoin itself is the pharmacologically active species. The lipid conjugates can be considered prodrugs of phenytoin.

In conclusion, the results obtained suggest that the phenytoin-lipid conjugates are prodrugs of phenytoin which do not alter the pharmacological profile of the parent drug. Following oral administration, the conjugates are hydrolysed by lipase resulting in some cases in an increased availability of phenytoin compared with that observed after application of phenytoin itself. Our observations suggest that drug-lipid conjugates can be useful as a lipase-driven drug-delivery system for poorly-soluble drugs.

Acknowledgements

This work was supported by the Fonds der Chemischen Industrie. The authors are indepted to Dr James P. Stables and his staff at the Epilepsy Branch, Division of Convulsive, Developmental and Neuromuscular Disorders, National Institutes of Health, Bethesda, MD, USA.

References

Alvarez, F. J., Stella, V. J. (1989) Pancreatic lipase-catalyzed hydrolysis of esters of hydroxymethyl phenytoin dissolved in various metabolizable vehicles, dispersed in micellar systems, and in aqueous suspensions. Pharm. Res. 6: 555–563

Arnold, K., Gerber, N., Levy, G. (1970) Absorption and dissolution studies on diphenylhydantoin capsules. Can. J. Pharm. Sci. 5: 89–92

Broadwell, R. D., Salcman, M., Kaplan, R. S. (1982) Morphologic effect of dimethyl sulfoxide on the blood-brain barrier. Science 217: 164–166

Chakrabarti, S., Belpaire, F. M. (1978) Bioavailability of phenytoin in lipid containing dosage forms in rats. J. Pharm. Pharmacol. 30: 330–331

De La Torre, J. C. (1970) Relative penetration of L-DOPA and 5-HTE through the blood-brain barrier using dimethyl sulfoxide. Experientia 26: 1117–1118

Dill, W. A., Kazenko, A., Wolf, L. M., Glazko, A. J. (1956) Studies of 5,5-diphenylhydantoin (Dilantin) in animals and man. J. Pharmacol. Exp. Ther. 118: 270–279

Glazko, A. J. (1972) Panel discussion case histories on bioavailability of drugs—diphenylhydantoin. Pharmacology 8: 163–177

Iwen, P. C., Miller, N. G. (1986) Enhancement of ketoconazole penetration across the blood-brain barrier of mice by dimethyl sulfoxide. Antimicrob. Agents Chemother. 30: 617–618

Krall, R. L., Penry, J. K., White, B. G., Kupferberg, H. J., Swinyard, E. A. (1978) Antiepileptic drug development: II. Anticonvulsant drug screening. Epilepsia 19: 409–428

Larsen, C., Kurtzhals, P., Johansen, M. (1988) Kinetics of regeneration of metronidazole from hemiesters of maleic acid, succinic acid and glutaric acid in aqueous buffer, human plasma and pig liver homogenate. Int. J. Pharm. 41: 121–129

Litchfield, J. T., Wilcoxon, F. J. (1949) A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99–113

Mead, J. F., Alfin-Slater, R. B., Howton, D. R., Popjak, G. (1986) Lipids, Chemistry, Biochemistry and Nutrition. Plenum Press, New York, pp 255–283

Meldrum, B. S. (1986) Pharmacological approaches to the treatment of epilepsy. In: Meldrum, B. S., Porter, R. J. (eds) Current Problems in Epilepsy, Vol. 4. New Anticonvulsant Drugs. John Libbey, London, pp 17–30

Mergen, F., Lambert, D. M., Goncalves Saraiva, J. C., Poupaert, J. H., Dumont, P. (1991) Antiepileptic activity of 1,3-dihexadecanoylamino-2-valproyl-propan-2-ol, a prodrug of

valproic acid endowed with a tropism for the central nervous system. J. Pharm. Pharmacol. 43: 815–816

Merrit, M. H., Putnam, J. T. (1938) Sodium diphenylhydantoinate in treatment of convulsive disorders. J. Am. Med. Assoc. 111: 1068–1073

Mori, N., Wada, J. A., Sato, T., Saito, H., Kumashiro, H. (1992) Important roles of *N*-methyl-D-aspartate receptors in expression of amygdaloid-kindled seizure demonstrated by intraperitoneal administration of L-aspartate in dimethyl sulfoxide. Epilepsia 33: 583–588

Nakamura, K., O'Hashi, K., Nakatsuji, K., Hirooka, T., Fujimoto, F., Ose, S. (1965) The anticonvulsant activity of 3-ethoxycarbonyl-5,5-diphenylhydantoin (P-6127) in animals. Arch. Int. Pharmacodyn. 2: 261–270

Porter, R. J., Cereghino, J. J., Gladding, G. D., Hessie, B. J., Kupferberg, H. J., Scoville, B., White, B. G. (1984) Antiepileptic drug development program. Cleveland Clin. Q. 51: 293–305

Poupaert, J. H., Vandervorst, D., Guiot, P., Moustafa, M. M. M., Dumont, P. (1984) Structure-activity relationships of phenytoin-like anticonvulsant drugs. J. Med. Chem. 27: 76–78

Rall, T. W., Schleifer, L. S. (1990) Drugs effective in the therapy of the epilepsies. In: Goodman-Gilman, A., Rall, T. W., Nies, A. S., Taylor, P. (eds) The Pharmacological Basis of Therapeutics. 8th edn, Pergamon Press, New York, pp 439–443

Reynolds, J. E. F. (ed.) (1993) Martindale. 30th edn, The Pharmaceutical Press, London, p. 1101

Sanson, L. W., O'Reilly, W. J., Wiseman, C. W., Stern, L. M., Derhan, J. (1975) Plasma phenytoin levels produced by various phenytoin preparations. Med. J. Aust. 62: 593–595

Schwartz, P. A., Rhodes, C. T., Cooper, J. W. (1977) Solubility and ionization characteristics of phenytoin. J. Pharm. Sci. 66: 994–997

Scriba, G. K. E. (1993a) Phenytoin-lipid conjugates as potential prodrugs of phenytoin. Arch. Pharm. 326: 477–481

Scriba, G. K. E. (1993b) Phenytoin-lipid conjugates: chemical, plasma esterase-mediated, and pancreatic lipase-mediated hydrolysis in-vitro. Pharm. Res. 10: 1181–1186

Scriba, G. K. E. (1994) Synthesis and in vitro evaluation of 4-(2-glyceryl)butyric acid: a glyceride mimic for drug delivery via drug-lipid conjugates. Arch. Pharm. 327: 347–348

Seki, H., Kawaguchi, T., Higuchi, T. (1988) Specificity of esterases and structure of prodrug esters: reactivity of various acylated acetaminophen compounds and acetylaminobenzoated compounds. J. Pharm. Sci. 77: 855–860

Shek, E., Murakami, T., Nath, C., Pop, E., Bodor, N. S. (1989) Improved anticonvulsant activity of phenytoin by a redox brain delivery system III: brain uptake and pharmacological effects. J. Pharm. Sci. 78: 837–843

Stankova, L., Mares, P. (1992) Antimetrazol action of two potential anticonvulsants, CM 40907 and SR 41378, in immature rats. Physiol. Res. 41: 451–457

Suzuki, T., Saitoh, Y., Nishihara, K. (1970) Kinetics of diphenylhydantoin disposition in man. Chem. Pharm. Bull. 18: 405–411

Swinyard, E. A., Brown, W. C., Goodman, L. S. (1952) Comparative assays of antiepileptic drugs in mice and rats. J. Pharmacol. Exp. Ther. 106: 319–330

Varia, S. A., Schuller, S., Sloan, K. B., Stella, V. J. (1984) Phenytoin prodrugs III: water-soluble prodrugs for oral and/or parenteral use. J. Pharm. Sci. 73: 1068–1073

Yamaoka, Y., Roberts, R. D., Stella, V. J. (1983) Low-melting phenytoin prodrugs as alternative oral delivery modes for phenytoin: a model for other high-melting sparingly water-soluble drugs. J. Pharm. Sci. 72: 400–405