International Journal of Pharmaceutics, 81 (1992) 263–265

IJP 02717

Notes

New highly water-soluble phenytoin prodrugs

Alma Dal Pozzo and Maurizio Acquasaliente

Istituto di Chimica e Biochimica G. Ronzoni, via G. Colombo, 81, 20133 Milano (Italy)

(Received 17 June 1991)
(Modified version received 13 November 1991)
(Accepted 29 November 1991)

Key words: Phenytoin; Water-soluble prodrug; Polyethylene glycol

Summary

Six new prodrugs of phenytoin (DPH) were prepared, containing polyethylene glycols (PEGs) as the promoiety. All compounds, with one exception, proved to be freely water-soluble and showed good stability in water and in isotonic solution, pH 7.4, whereas they released the native drugs in the presence of human plasma. The behaviour observed in vitro indicates these new PEG esters of DPH to be potentially useful prodrugs in vivo.

Phenytoin (DPH), an anticonvulsant drug, is known to be erratically absorbed after both oral and parenteral administration, due to its very critical equilibrium of solubility (Bundgaard and Johansen, 1980).

Among several attempts at synthesizing DPH prodrugs with increased water solubility and the ability to release promptly DPH in vivo, the approach of Varia et al. (1984a,b) is particularly interesting. The authors obtained several *N*-hydroxymethyl phenytoin derivatives with good aqueous solubility which released the active principle in physiological systems.

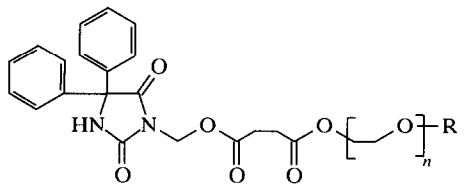
In order to improve further the water solubility and stability of the prodrugs, we prepared a new series of *N*-hydroxymethyl phenytoin esters, where the acyl moiety was represented by mono- and heterofunctional polyethylene glycols (PEGs), bearing ionizable or non-ionizable end substitutions. Among water-soluble oligomers for covalent modification of drugs, PEGs are considered to be very convenient due to their biocompatibility, and lack of antigenicity and toxicity (Abuchowski and Davis, 1979; Chen et al., 1981; Zalypsky et al., 1983; Ferruti and Dal Pozzo, 1987).

The structures of the six DPH derivatives prepared are represented in Scheme 1.

3-(Chloromethyl)-5,5-diphenylhydantoin was synthesized as described by Varia et al. (1984a). PEG monosuccinic half esters were synthesized as previously described (Dal Pozzo et al., 1989). Purification of the final products, when necessary, was achieved by column chromatography through silanized silica gel 60 (70–230 mesh, Merck) using acetone-water 4:6 as the mobile phase.

PEG succinic esters **1a–1d** were synthesized via activation of the carboxyl group of the corresponding PEG monosuccinic half-ester with te-

Correspondence: A. Dal Pozzo, Istituto di Chimica e Biochimica G. Ronzoni, via G. Colombo, 81, 20133 Milano, Italy.



1

Scheme 1.

Compound	n [a]	R
1a	3	CH_3
1b	17.3	CH_3
1c	22.3	H
1d	49.5	CH_3
1e	22.3	$CO-(CH_2)_2-COONa$
1f	22.3	$CO-CH_2-S-CH(CH_2-COONa)-COONa$

[a] Average values of n are given, since the PEGs used were polydisperse commercial samples.

trabutylammonium (TBA); the acid was dissolved in benzene together with an equivalent amount of anhydrous TBA-OH and taken to dryness. Excess 3-(chloromethyl)-5,5-diphenylhydantoin (up to 2 equiv.) was added to the residue and the reaction was carried out in dimethyl formamide at room temperature for 72 h. The reaction mixture, diluted 1:10 with water, was extracted with chloroform and the organic phase, after washing back with water and $NaHCO_3$, was dried over sodium sulphate and evaporated. After column chromatography, pure products were obtained in yields ranging from 60 to 80%. ^{1}H-NMR ($CDCl_3$), in ppm: 7.4 (s,arom.prot.); 5.62 (s,N-CH_2); 4.25 (t,CH_2O-CO); 3.68 (m,$(CH_2CH_2O)_n$); 3.38 (s,OCH_3); 2.65 (s,$COCH_2CH_2CO$). In compound **1c**, the signal at 3.38 ppm was absent.

3-(Hydroxymethyl)-5,5-diphenylhydantoin ester with PEG sodium succinate (**1e**) was synthesized from **1c** with succinic anhydride (2.5 equiv.) and pyridine (2.5 equiv.) in alcohol-free chloroform, at refluxing temperature for 24 h. The reaction mixture was evaporated to dryness and the residue dissolved in $NaHCO_3$-saturated solution; the solution, adjusted to pH 2, was extracted with $CHCl_3$. After evaporation of the solvent, the residue was purified by column chromatography (yield 60%). ^{1}H-NMR ($CDCl_3$) presented the same signals as described for compound **1c**.

Titration (0.1 M NaOH, with phenolphthalein) gave 98% of the calculated carboxyl groups.

The acid was dissolved in saturated $NaHCO_3$ solution at a concentration of 2%, and the solution was extracted with $CHCl_3$, which, after evaporation, gave **1e**, as the sodium salt, in quantitative yield.

Dicarboxyethylthioacetyl-PEG 3-(hydroxymethyl)-5,5-diphenylhydantoin succinic ester (**1f**) was synthesized via an intermediate bromoacetate of **1c**, prepared according to a previously described method (Dal Pozzo et al., 1989). A solution of mercaptosuccinic acid (0.96 mmol) and triethylamine (2.88 mmol) in 5 ml $CHCl_3$ was added dropwise to a solution of the bromoacetate in 10 ml $CHCl_3$ and the mixture left at room temperature for 1 h. The reaction mixture was then diluted 1:3 with $CHCl_3$, washed with water and concentrated. The residue, purified by column chromatography, afforded the pure product (**1f**) in 60% yield. Titration gave 97.3% of the calculated carboxyl groups. The sodium salt was prepared as described above for **1e**. As compared with **1e**, ^{1}H-NMR presented a new signal at 2.75 ppm (d, $CH\underline{CH_2}COO^-$).

For determination of the DPH content (%) of the prodrugs, a suitable quantity of prodrug (equivalent to about 0.06 mmol of DPH) was dissolved in 2 ml of 1 N NaOH. After 20 min at room temperature the solution was diluted with methanol, neutralized with 1 N HCl, with the addition of 5-(4-hydroxyphenyl)-5-phenylhydantoin as internal standard, and injected onto the HPLC column (Lichrospher, Merck RP8, 5 μm; 35% CH_3CN in 0.01 M phosphate buffer, pH 7.4; $\lambda = 220$ nm). The results are listed in Table 1. The conversion of the prodrugs into DPH in human plasma was followed by HPLC, according to the same method as described above, after denaturation: an aqueous solution of each compound (equivalent to 10^{-5} M DPH) was injected into simple buffer or 10% buffer solution of human plasma (in undiluted plasma, hydrolysis was too rapid to be followed).

At given time intervals, 5 ml of CH_3CN-CH_3OH 4:1 was added to 1 ml of the plasma

TABLE 1

Physico-chemical characteristics of diphenylhydantoin pro-drugs – elemental analyses (C, H, N)

Compound	Formula	Mol. Wt	DPH content found in prodrug (%)
1a	$C_{27}H_{32}N_2O_9$	528	46.7
1b	$C_{55.6}H_{89.2}N_2O_{23.3}$	1157.2	22.03
1c	$C_{64.6}H_{107.2}N_2O_{28.3}$	1363.2	17.3
1d	$C_{120}H_{218}N_2O_{55.5}$	2574	9.37
1e	$C_{68.6}H_{110.2}N_2O_{31.3}Na$	1485.2	16.1
1f	$C_{70.6}H_{111.2}N_2O_{33.3}SNa_2$	1597.2	15.19

solution, and the mixture was stirred for 1 min followed by centrifugation at 3000 rpm for 20 min. The supernatant was evaporated under N_2, and the residue dissolved in methanol followed by injection.

The half-life for the conversion of **1a–1f** to DPH was determined to be around 3 h in 10% diluted plasma, exhibiting pseudo-first-order kinetics. On the other hand, the half-life in simple isotonic phosphate buffer was found to be 150 h. This indicates that the rapid cleavage is enzymatic in nature and that the prodrugs must undergo cleavage to DPH under in vivo conditions.

As expected, all derivatives, except **1a**, were freely and promptly soluble in water, in any proportions. Moreover, they showed very good stability in water on storage for 1 month at room temperature.

Based on these findings, the new derivatives **1b–1f** described in this paper appear to be very promising for use as prodrugs of DPH, this approach contributing to the overcoming of the drawbacks of low solubility and stability of the native drug.

Acknowledgement

This work was supported in part by the Italian CNR, Special Project on Fine Chemicals.

References

Abuchowski, A. and Davis, F.F., Preparation and properties of polyethyleneglycol-trypsin adducts. *Biochim. Biophys. Acta*, 578 (1979) 41–46.

Bundgaard, H. and Johansen, M., Pro-drugs and drug delivery systems. VIII: Bioreversible derivatization of hydantoins by *N*-hydroxymethylation. *Int. J. Pharm.*, 5 (1980) 67–77.

Chen, R.H.L., Abuchowsky, A., Van Es, T., Palczuk, N.C. and Davis, F.F., Properties of two urate oxidases modified by the covalent attachment of polyethyleneglycol. *Biochim. Biophys. Acta,* 660 (1981) 293–298.

Dal Pozzo, A., Vigo A. and Donzelli, G., New monofunctional derivatives of polyethyleneglycols via monotrityl intermediates. *Macromol. Chem.* 190 (1989) 2457–2461.

Ferruti, P. and Dal Pozzo, A., Oligomeric drug carriers. *Bioact. Compat. Polym.,* 2 (1987) 148–167.

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

Varia, S.A., Schuller, S. and Stella, V.J., Phenytoin prodrugs, IV: Hydrolysis of various 3-hydroxymethyl phenytoin esters. *J. Pharm. Sci.,* 73 (1984b) 1974–1980.

Zalipsky, S., Gilon, C. and Zilkha, A., Attachment of drugs to polyethyleneglycols. *Eur. Polym. J.,* 19 (1983) 1177–1183.