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Evaluation of the prodrug potential of the sulfate esters of acetaminophen and 3-hydroxymethyl-phenytoin

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Summary

Potassium acetaminophen sulfate (Ia) and potassium 3-sulfonyloxymethyl-5,5-diphenylhydantoin (IIa) were evaluated as potential prodrugs of acetaminophen (Ib) and phenytoin (IIc), respectively. Both sulfate esters were stable in neutral solutions and Ia had a hydrolysis half-life of 24 h in 0.2 M hydrochloric acid at 37°C. Compound Ia exhibited no anti-writhing activity after oral or intraperitoneal administration to mice when compared to Ib. Compound IIa was rapidly eliminated from plasma with a half-life of ~ 34 min after intravenous administration to rats with no detectable IIc in the plasma up to 4 h post-administration. It was concluded that neither Ia nor IIa would be a potentially useful prodrug.

Introduction

The literature is inconclusive as to whether sulfate esters of alcohols and phenols can serve as prodrugs (Conolly et al., 1972; Conway et al., 1968; Dolly et al., 1971; Kawamura et al., 1971; Levy and Matsuzawa, 1966; Miyabo et al., 1981; Powell and Olavesen, 1981; Sinkula and Yalkowsky, 1975) by being cleaved in vivo by sulfatases (Roy, 1960). To help clarify this point, two sulfate esters, Ia and IIa, were synthesized and evaluated as potential prodrugs of acetaminophen, Ib, and phenytoin, IIc, respectively.

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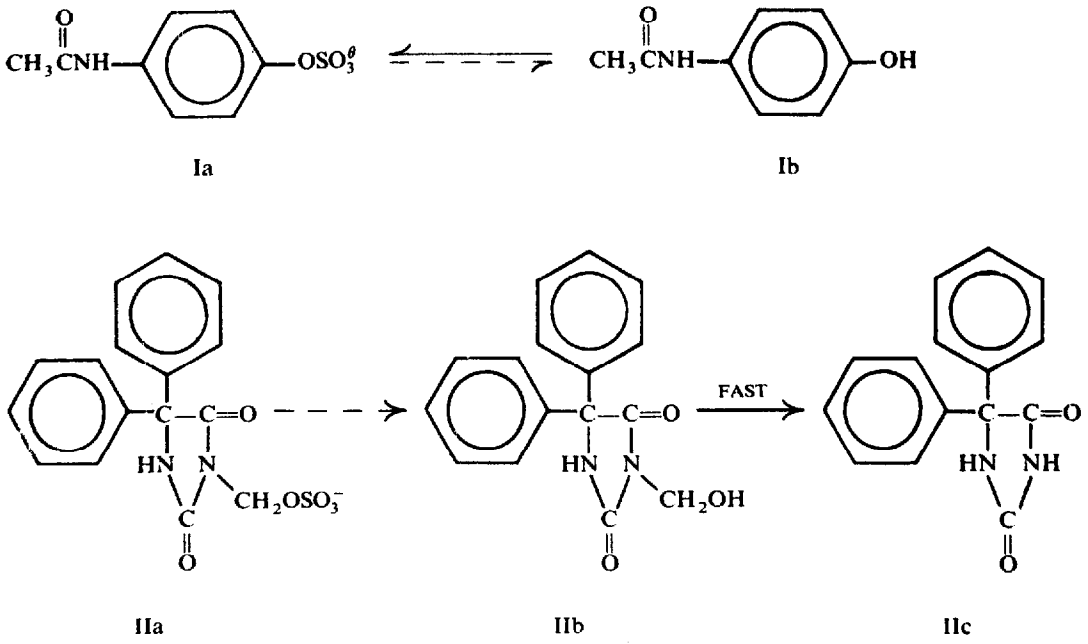

Ia Ib

IIa IIb IIc

Scheme I

Compound Ia is a natural metabolite of acetaminophen, while IIa is not a metabolite of phenytoin. If IIa were to be cleaved by sulfatases, it would generate IIb which rapidly and quantitatively eliminates formaldehyde with a half-life of ~ 2 s under physiological conditions to yield phenytoin (Bundgaard and Johansen, 1980). The solid arrows in Scheme I represent known chemical and biochemical reactions, the broken arrows represent the possible breakdown steps due to sulfatase activity.

Materials and methods

Acetaminophen (F.H. Faulding, Adelaide, South Australia) and phenytoin (Sigma Chemicals, St. Louis, MO) were used without further purification. The potential prodrugs, Ia and IIa, were synthesized as described below. All other chemicals were reagent grade and were used without further purification. Water was either distilled in an all-glass still or was double-deionized.

Potassium acetaminophen sulfate (Ia)

Compound Ia was synthesized as the potassium salt (monohydrate) from Ib according to a method described previously (Fendler and Fendler, 1968). Chloroform was used as the reaction solvent. Impure Ia was recrystallized twice from ethanol (95%) and activated charcoal to give a final yield of 42%. The melting point was higher than 265°C with slow decomposition. No Ib was observed when a solution of Ia was examined by HPLC and TLC. NMR (deuterium oxide) spectra were consistent with the structure of Ia. Elemental analysis for CHNS agreed closely within ±0.2% (for each element) with the formula $C_8H_8NO_5SK \cdot H_2O$ (Ia).

Potassium 3-sulfonyloxymethyl-5,5-diphenylhydantoin (IIa)

The synthesis of IIa involved previously reported procedures (Fendler and Fendler, 1968; Hearse et al., 1969) where the reactive alcohol was IIb prepared by a method similar to that already reported (Bundgaard and Johansen, 1980). A white solid (mp 192–194°C) was obtained with a yield of 30%.

No IIc was observed when a solution of IIa was examined by GC and HPLC. NMR (deuterium oxide) spectra were consistent with the structure of compound IIa. Elemental analysis for CHNS agreed within ±0.5% (for each element) with the formula $(C_{16}H_{13}O_6N_2SK)_2 \cdot 3H_2O$.

Aqueous hydrolysis of Ia and IIa

TLC analysis

Compound Ia (25 mg) was dissolved in sufficient water and 0.01, 0.1 or 1.0 M hydrochloric acid to produce 5 ml of solution. Aliquots (20 μl) were chromatographed (acetone–strong ammonia solution 9 : 1) at time zero and at regular time intervals and the plates examined to detect hydrolysis products. The R_f values in this system were 0.31 and 0.73 for Ia and Ib, respectively.

Spectrophotometric analysis

Solutions of Ia (0.418 mM) in 0.2 or 0.5 M hydrochloric acid, I = 0.5 M adjusted with sodium chloride, were maintained at 37.0 ± 0.1°C in a water bath. At regular time intervals, aliquots were removed from the solution and diluted with aqueous sodium hydroxide solutions to produce solutions with pH ~ 12.8.

First-order rate constants were calculated from plots of $\log(A - A_\infty)$ against time, where A and A_∞ were the absorbance of a pH 12.8 solution of the reaction mixture at time t and of an equimolar solution of Ib at pH 12.8, respectively.

Gas chromatographic (GC) analysis

Solutions of IIa in phosphate buffer, pH 7.42 and I = 0.5 M adjusted with potassium chloride, were maintained at 70.0 ± 0.1°C, 60.0 ± 0.1°C and 50 ± 0.1°C in a water bath. At regular intervals, 50 μl aliquots were removed from the solution and assayed for phenytoin by a GC method (Stella, 1977).

Anti-writhing studies of Ia and Ib [1]

The *p*-quinone-induced anti-writhing activity of Ia and Ib in mice was compared. A fresh solution of *p*-quinone was prepared immediately before use by dissolving 1 mg in 1 ml of 50% ethanol and diluting 10 ml with aqueous 0.9% sodium chloride. An intraperitoneal dose of 1 mg/kg was used. This dose was just sufficient to cause writhing in 100% of a normal group of 10 mice.

Compound Ia was dissolved in 0.9% sodium chloride solution for administration to mice at a dose volume of 0.1 ml/10 g body weight. The solution was administered either by the oral, intraperitoneal or intravenous routes.

[1] Bios (Consulting and Contract Research) Ltd., United Kingdom.

Compound Ib was suspended in aqueous 0.1% tragacanth and administered orally to mice. Each dose volume was 0.1 ml/10 g body weight. On a molar basis, 890 mg of Ia is equivalent to 500 mg of Ib.

Animals

Male albino mice of the LACA strain[1], weighing 18–20 g, from which food had been withheld overnight, were used. Groups of 10 animals were taken for dosing.

Experiment 1

To demonstrate a dose–response relationship for the anti-writhing activity of Ib, groups of 10 mice received oral doses of either tragacanth (0.1%, 0.1 ml/10 g body weight) or various doses of Ib suspended in 0.1% tragacanth (0.1 ml/10 g body weight). The animals were observed for 50 min for the appearance of writhing.

Experiment 2

Three groups of mice received the following oral doses: (a) tragacanth (0.1%, 0.1 ml per 10 g body weight); (b) compound Ia (890 mg/kg); and (c) compound Ib (500 mg/kg). After 30 min, each mouse received an intraperitoneal injection of *p*-quinone (1 mg/kg). Three observers, unaware of the dosing schedule, then continuously watched the mice (one observer per group) for 50 min. A record was made of: (i) the number of animals presenting with writhes; and (ii) the total number of writhes made in a group.

Experiment 3

Three groups of mice received the following doses: (a) and (b) as in experiment 1; and (c) compound Ia, intraperitoneal injection, 890 mg/kg. After 30 min, each mouse received *p*-quinone and the animals were observed as described for Experiment 1.

Experiment 4

Mice received an intravenous injection of aqueous 0.9% sodium chloride or Ia dissolved in 0.9% sodium chloride solution. In each case, the dosage volume was 0.1 ml/10 g body weight. After 5 min, the surviving animals [2] received *p*-quinone (1 mg/kg) by intraperitoneal injection. The mice were then observed for the appearance of writhing.

In vivo evaluation of IIa as a prodrug of phenytoin in the rat

To determine the availability of phenytoin after an intravenous dose of IIa, a study was carried out in two male Sprague–Dawley rats (Sasco, Omaha, NE). The rats were anesthetized with sodium pentobarbital (dose: 60 mg/kg). A dose of 25 mg phenytoin equivalents of IIa per kg of body weight was administered as an aqueous solution (volume ~ 300 μl) intravenously via the femoral vein. Blood samples (~ 350 μl at each time point) were withdrawn from the jugular vein at 15, 30, 45, 75,

[2]See Results and discussion.

105 and 270 min after dosing. The blood samples were transferred to containers (Vacutainers 2 ml, Becton-Dickinson, Rutherford, NJ) containing 3 mg of ethylenediamine tetraacetic acid. Two-hundred μl of the blood sample were analyzed for phenytoin by gas chromatography (Stella, 1977). Aliquots of 100 μl of the whole blood were also incubated overnight, at 37°C, with 200 μl of a commercial sulfatase enzyme preparation (Glusulase, Endo Laboratories, Garden City, NY) in order to convert whole blood IIa to phenytoin. The treated samples were then analyzed for total phenytoin, i.e. phenytcin plus intact ester, IIa, by gas chromatography (Stella, 1977) and the IIa concentration determined by difference.

Results and discussion

In neutral solutions (pH 7.4) at 37°C, there was no detectable hydrolysis of Ia to acetaminophen as determined by TLC and spectrophotometric analysis over 9 days. The hydrolysis of IIa to phenytoin (pH 7.42) was observed at 70, 60 and 50°C when followed by an assay specific for phenytoin. An Arrhenius plot was linear giving an energy of activation of 23.8 kcal/mol which is consistent with that found (Kawamura et al., 1971) for the hydrolysis of hydrocortisone-21-sulfate. The half-life of IIa at 25 and 37°C was ~ 230 days and 49 days, respectively.

Compound Ia hydrolyzed with half-lives of 25 h and 8.8 h under acidic pH conditions, 0.2 M HCl and 0.5 M HCl, respectively. It appears, therefore, that Ia and IIa are chemically stable under the neutral pH conditions studied and at least in the case of Ia, significant cleavage would not occur in an acid environment similar to that found in the stomach.

To determine if Ia exhibits any in vivo analgesic activity relative to acetaminophen after oral and intraperitoneal administration, the anti-writhing activity of both compounds was studied in *p*-quinone-induced writhing experiments (Experiments 1–3) in mice. In all the anti-writhing experiments summarized in Tables 1–3, Ia exhibited no activity after oral and intraperitoneal administration while oral acetaminophen produced the expected suppression of writhing. If anything, Ia appeared to exacerbate the writhing relative to controls (Table 2).

The toxicity of Ia was obvious in the intravenous dosing experiment (Experiment 4). Injection of normal saline had no apparent adverse effect on the animals. However, compound Ia proved to possess a lethal action after intravenous administration. At a dose of 890 mg/kg, all 10 mice died within 30 s of injection. Death was preceded by a convulsive episode terminating in dyspnea and respiratory depression. At a dose of 445 mg/kg, 8 out of 10 mice died within 30 s of injection. Both surviving mice showed a positive writhing response within 50 min of receiving *p*-quinone. After a dose of 222.5 mg/kg, all animals appeared normal without the appearance of convulsive episodes and none of them died. However, following *p*-quinone administration, all animals gave a positive writhing response. All mice receiving intravenous normal saline also writhed after the administration of *p*-quinone.

An attempt was made to determine the rate of hydrolysis of IIa to IIc, phenytoin,

TABLE 1

COMPARISON OF DOSE OF COMPOUND Ib AND PERCENTAGE OF MICE WRITHING

Dose (mg/kg)	Number of mice writhing (%)
0 [a]	100
62.5	100
125	80
250	60
500	40
1000	0

[a] Tragacanth, 0.1% suspension, dose volume 0.1 ml/10 g body weight. All doses of Ib were made in tragacanth suspension, and administered at 0.1 ml/10 g body weight.

TABLE 2

NUMBER OF WRITHES PER 10 MICE AT DIFFERENT TIMES AFTER ORAL (EXPERIMENT 1) AND INTRAPERITONEAL (EXPERIMENT 3) ADMINISTRATION OF COMPOUND Ia, AND ORAL ADMINISTRATION OF TRAGACANTH SUSPENSION AND COMPOUND Ib

Time (min)	Number of writhes per group					
	Experiment 1			Experiment 3		
	Tragacanth [a]	Ia [b]	Ib [c]	Tragacanth [a]	Ia [b]	Ib [c]
0–10	30	32	0	102	140	6
0–20	106	124	0	216	294	44
0–30	170	196	24	306	368	84
0–40	228	244	26	316	412	96
0–50	256	262	26	328	424	98

[a] 0.1% suspension, dose volume 0.1 ml/10 g body weight.
[b] 890 mg/kg, equivalent to 500 mg/kg of Ib.
[c] 500 mg/kg, as a suspension.

TABLE 3

PERCENTAGE OF MICE WRITHING FOLLOWING DOSING WITH TRAGACANTH, COMPOUND Ia AND COMPOUND Ib

Treatment for each group	Route of administration	% of mice writhing
Tragacanth [a]	oral	100
Ia [b]	oral	100
	intraperitoneal	100
Ib [c]	oral	40
	intraperitoneal	60

[a] 0.1% suspension, dose volume 0.1 ml/10 g body weight.
[b] 890 mg/kg, equivalent to 500 mg/kg of Ib.
[c] 500 mg/kg as a suspension.

in the plasma of rats, dogs and humans, and rat liver and intestinal homogenates (Varia, 1981). No detectable cleavage was observed in these tissues. Compound IIa did undergo rapid and quantitative hydrolysis to phenytoin on incubation with the commercial sulfatase enzyme preparation.

In order to determine if IIa was hydrolyzed on in vivo administration, a 25 mg/kg phenytoin equivalent dose of IIa was administered intravenously to rats as an aqueous solution. The blood samples did not show any phenytoin over a 4.5 h period. The sulfate ester, IIa, was rapidly cleared from the blood by apparent first-order kinetics. Least-squares analysis of the whole blood concentration versus time profile ($r = 0.968$) showed IIa to have a $t_{1/2}$ of 34 min, a volume of distribution of 2.6 l/kg and a total body clearance (KV_d) of 53 ml/min/kg. This rapid rate of elimination of the ester with no apparent appearance of phenytoin suggests that the ester is cleared much faster than its in vivo cleavage rate by sulfatase enzymes.

In conclusion, Ia and IIa do not appear to behave as prodrugs of acetaminophen and phenytoin, respectively. This is probably due to the inability of these sulfate esters to be rapidly cleaved relative to their in vivo elimination rate, a conclusion consistent with the results seen in a recent pharmacokinetic study with dexamethasone-21-sulfate in man (Miyabo et al., 1981). Thus, it appears that the choice of sulfate esters as prodrugs of compounds containing an alcohol or phenolic functional group, with the possible exception of estrogens (Dolly et al., 1971; Powell and Olavesen, 1981) are generally not appropriate as they do not appear to revert to the parent compound in vivo.

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References

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

Conolly, M.E., Davies, D.S., Dollery, C.T., Morgan, C.D., Paterson, J.W. and Sandler, M., Metabolism of isoprenaline in dog and man. Br. J. Pharmacol., 46 (1972) 458–472.

Conway, W.D., Minatoya, H., Lands, A.M. and Shekosky, J.M., Absorption and elimination profile of isoproterenol. III. The metabolic fate of dl-isoproterenol-7-^{3}H in the dog. J. Pharm. Sci., 57 (1968) 1135–1141.

Dolly, J.O., Curtis, C.G., Dodgson, K.S. and Rose, F.A., Metabolism of sodium oestrone [^{35}S]-sulfate in the rat. Biochem. J., 123 (1971) 261–266.

Fendler, E.J. and Fendler, J.H., Hydrolysis of nitrophenyl and dinitrophenyl sulfate esters. J. Org. Chem., 33 (1968) 3852–3859.

Hearse, D.J., Olavesen, A.H. and Powell, G.M., The preparation and characterization of a series of [^{35}S]-labelled aryl esters for metabolic studies. Biochem. Pharmacol., 18 (1969) 173–180.

Kawamura, M., Yamamoto, R. and Fujisawa, S., Pharmaceutical studies on water-soluble corticosteroid derivatives. III. Stability of hydrocortisone 21-sulfobenzoates and 21-sulfate in solution. Yakugaku Zasshi, 91 (1971) 871–878.

Levy, G. and Matsuzawa, T., Pharmacokinetics of salicylamide elimination in man. J. Pharmacol. Exp. Ther. 156 (1967) 285–293.

Miyabo, S., Nakamura, T., Kuwazima, S. and Kishida, S., A comparison of the bioavailability and potency of dexamethasone phosphate and sulfate in man. Eur. J. Clin. Pharmacol., 20 (1981) 277–282.

Nilsson, H.T., Persson, K. and Tegner, K., The metabolism of terbutaline in man. Xenobiotica, 2 (1972) 363–375.

Powell, G.M. and Olavesen, A.H., The fate of sulfate esters in vivo. In G.J.Mulder (Ed.), Sulfation of Drugs and Related Compounds, CRC Press, Florida, 1981, pp. 187–212.

Roy, A.B., The synthesis and hydrolysis of sulfate esters. Adv. Enzymol., 22 (1960) 205–235.

Sinkula, A.A. and Yalkowsky, S.H., Rationale for design of biologically reversible drug derivatives: prodrugs. J. Pharm. Sci., 64 (1975) 181–210.

Stella, V.J., Use of trimethylanilinium hydroxide-tetramethylammonium hydroxide as on-column methylating agent for GLC analysis of phenytoin. J. Pharm. Sci., 66 (1977) 1510–1511.

Varia, S.A., Water-soluble prodrugs of 5,5-diphenylhydantoin, Ph.D. dissertation, University of Kansas, 1981.

Yoshimura, H., Morphine conjugates as pharmacologically active metabolites. J. Pharmacobio-Dynamics, 3 (1980) S6.