J. Pharm. Pharmacol. 1996, 48: 479–485
Received September 4, 1995
Accepted January 19, 1996

Synthesis, Physical Properties, Toxicological Studies and Bioavailability of L-Pyroglutamic and L-Glutamic Acid Esters of Paracetamol as Potentially Useful Prodrugs

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Abstract

Paracetamol ester prodrugs with L-pyroglutamic and L-glutamic acid, biosynthetic precursors of glutathione, have been synthesized to reduce paracetamol hepatotoxicity and improve bioavailability.

The toxicological studies of paracetamol esters show that only L-5-oxo-pyrrolidine-2-paracetamol carboxylate reduces toxicity after administration of an overdose. The glutathione hepatic values in mice obtained by intraperitoneal injection of the ester are superimposable on controls and the oral LD50 was found to be greater than 2000 mg kg^{-1} and the intraperitoneal LD50 was 1900 mg kg^{-1}.

These results taken together with hydrolysis and bioavailability data show that ester is a potential candidate as a prodrug of paracetamol.

Paracetamol is a commonly used analgesic sold over the counter. It is remarkably safe at therapeutic doses. However, a number of investigators have reported that large overdoses of paracetamol can produce a fulminant hepatic and renal tubular necrosis which can be lethal both in man and animals (Boyer & Rouff 1971; Prescott et al 1971). Paracetamol is normally metabolized at the 4-OH position to glucuronide and sulphate conjugates with some 5% of the paracetamol going to -SH conjugates at the 3-OH position following P450 oxidation steps. Overall the proportion of paracetamol that is excreted as sulphur compounds varies with species and dose (Wong et al 1986). The toxicity of paracetamol has been attributed to the electrophilic metabolite *N*-acetyl-*p*-benzoquinonimine, which is formed by a microsomal cytochrome P450 mixed-function oxidase (Potter et al 1973; Miner & Kissinger 1979; Nelson et al 1980; Calder et al 1981). Following therapeutic doses, the reactive metabolite appears to be detoxified, at least in part, by its covalent interaction with glutathione (Wong et al 1979; Rosen et al 1984). At higher doses, glutathione becomes depleted, thus allowing the toxic metabolite to react extensively with other tissue molecules (Jollow et al 1973; Beierschmitt et al 1989). These interactions are believed to result in liver damage and correlate with the amount of covalent binding to tissue protein of radiolabelled paracetamol (Mitchell et al 1973; Fernando et al 1980). *N*-Acetylcysteine protects against paracetamol hepatotoxicity primarily by facilitating glutathione synthesis (Labadarios et al 1977). This has provided the rationale for administering precursors of glutathione, or sulphydryl-containing compounds, on the basis that these will increase hepatic stores of reduced glutathione. Moreover, biochemical studies have elucidated the enzymatic basis of the functions of glutathione (Meister 1973, 1988). The conversion of pyroglutamic acid (PGA) to glutamate catalysed by 5-oxoprolinase, and the two-step synthesis of glutathione from glutamate (Wellner et al 1974), suggests the use of PGA or L-glutamic acid (GA) for increasing cellular levels of glutathione. The purpose of this study was to synthesize three esters as potential prodrugs of paracetamol and investigate the influence of these esters on hepatic levels of glutathione to reduce hepatotoxicity and to improve bioavailability.

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Materials and Methods

Chemicals

Paracetamol, *N*-CBZ-L-pyroglutamic acid and *N*-CBZ-L-glutamic acid were purchased from Sigma (St Louis, MO, USA); triethylamine, trimethyl acetyl chloride and palladium/charcoal were obtained from Aldrich (Milwaukee, WI, USA); esterase from hog liver was obtained from Boehringer Mannheim (Germany). All other chemicals or solvents were reagent grade.

Analytical methods

IR spectra were recorded on a Perkin-Elmer 1600 Fourier transform spectrophotometer as KBr disks. Melting points were determined with a Büchi apparatus and are not corrected. ^{1}H NMR spectra [d (ppm) J(Hz)] were obtained on a Brüker AC300 (300 MHz) spectrometer (reference TMS). Mass spectra were determined on a Kratos MS25RF at 70 eV. Elemental analysis for C, H and N were obtained on a Carlo-Erba 1106 analyser and were within 0·4% of theoretical values. Thin-layer chromatography (TLC) was carried out on Merck Silica Gel 60 F_{254} (0·25 mm thickness). Column chromatography was performed by the flash procedure as described by Still et al (1978).

Synthesis of paracetamol esters

The synthesis of L-pyroglutamic and L-glutamic esters of paracetamol were performed following a new method of synthesis (Fig. 1) with respect to that proposed by Laruelle et al (1986).

N-CBZ-L-5-oxo-pyrrolidin-2-tert-butoxycarbonyloxycarbonyl anhydride **3**. A mixture of *N*-CBZ-L-pyroglutamic acid **1** (500 mg, 1·89 mmol), dry toluene (10 mL) and dry triethyla-

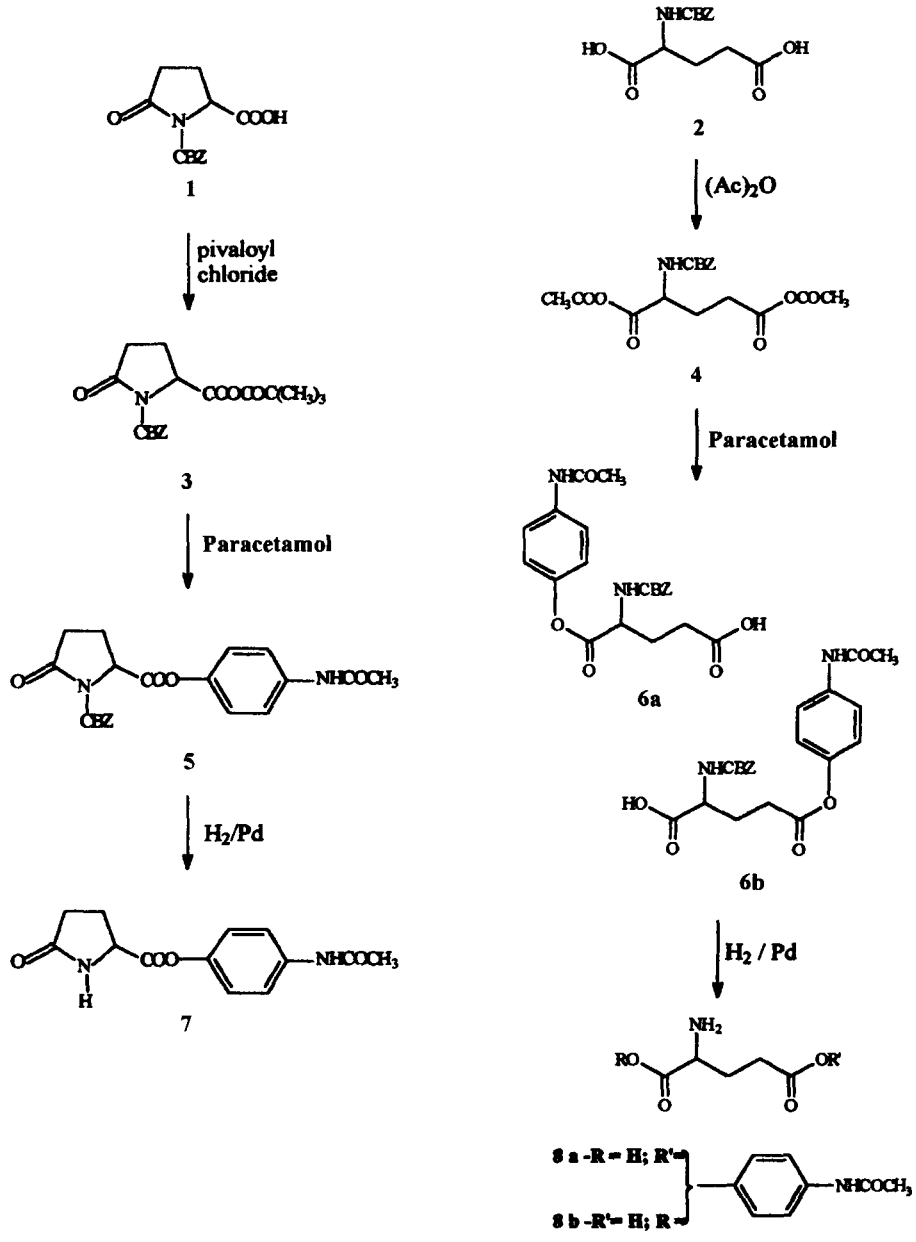

FIG. 1. Synthesis of paracetamol esters.

mine (264 μL, 2 mmol) was cooled to − 5°C. Pivaloyl chloride (234 μL, 2 mmol) was added and the solution was stirred for 90 min. The reaction was monitored by TLC with eluent ethyl acetate:hexane (7:3 v/v) and the precipitate formed was removed by suction filtration. The solvent was removed yielding anhydride as a white solid (589 mg, yield 90%), m.p. 59–61°C. IR (cm^{-1}) ν_{max}: 1810–1825 (−CO−O−CO), 1790 (CO−NH), 1760 (CO, lactamic). ^{1}H-NMR (DMSO, d6): δ 7·41–7·30 (m, 5H, ArH); 5·32–5·29 (q, J = 7 Hz, 2H, $-O-CH_2$); 4·88 (q, J = 8 Hz, 1 H, −CH−); 2·32–2·24 (m, 4H, $-CH_2-$); 1·23–1·19 (m, 9H, CH_3).

L-5-oxo-pyrrolidine-2-paracetamol-carboxylate **7** *(paracetamol-PCA).* Anhydride **3** (500 mg, 1·44 mmol) and paracetamol (218 mg, 1·44 mmol) in dry toluene (20 mL) containing triethylamine (2 mmol) were kept under agitation overnight (TLC eluent diethylether:methanol:ethyl acetate 75:5:20 v/v). The mixture was diluted with CH_2Cl_2 and the organic layer was washed with saturated $NaHCO_3$ and water, dried (Na_2SO_4) then evaporated giving 364 mg of **3** (yield 63·6%). M.p. 178–180°C. IR (cm^{-1}) ν_{max}: 1795 (−NH−CO), 1765 (=N−C=O), 1710 (−CO ester), 1675 (C=O lactamic). ^{1}H-NMR (DMSO, d6): δ 9·87 (br, s, 1H, NH); 7·62–6·79 (m, 9H, ArH); 5·32–5·26 (q, J = 8 Hz, 2H, $-O-CH_2$); 4·79 (q, J = 7·5 Hz, 1H, −CH−); 2·57 (s, 3H, CH_3); 2·24–2·07 (m, 4H, $-CH_2-$). The *N*-CBZ-L-5-oxo-pyrrolidin-2-paracetamol-carboxylate **5** (200 mg, 0·5 mmol) was removed from the protecting group in dry methanol at 60°C for 30 min by treatment with H_2 and palladium on carbon (0·65 g) after filtration, the filtrate was purified by flash chromatography (diethylether:methanol:ethyl acetate 75:5:20 v/v) to give L-5-oxo-pyrrolidine-2-paracetamol-carboxylate **7** as white crystal (345 mg, 91·3%). M.p. 185–

186°C. IR (cm^{-1}) ν_{max}: 3265 (−NH−amidic); 1720 (C=O ester); 1700 (C=O lactamic); 1660 (CO−NH). ^{1}H−NMR (DMSO, d6): δ 9·54 (s, 1H, −NH); 8·19 (br, s, 1H, =NH); 7·29–6·71 (m, 4H, ArH); 4·88 (q, J = 8 Hz, 1H, −CH−); 2·32–2·24 (m, 4H, $-CH_2$); 2·18 (s, 3H, CH_3). MS m/z: 262 (M^+), 247, 194, 151.

L-Glutamic acid γ- and α-4 (acetylamino-phenyl) ester **8a** and **8b.** *N*-CBZ-L-glutamic acid **2** (1 g, 3·5 mmol), was acetylated with anhydride (1·2 mL, 10·5 mmol), the mixture was heated to 50°C and stirred for 1 h until solubilization then kept at room temperature (21°C) overnight (TLC eluent ethyl acetate:benzene 7:3 v/v). The reaction was quenched with ice and the precipitate was filtered and washed with diethyl ether to give a *N*-CBZ-L-glutamic acid α- and γ-diacetyl-anhydride **4** as a white solid (715 mg, 55·9%). M.p. 79–81°C. IR (cm^{-1}) ν_{max}: 3330 (NH–CBZ); 1870 (CO−O−CO), 1695 (C=O amidic). The anhydride (500 mg, 1·37 mmol) was suspended in CH_2Cl_2 and added slowly to an aqueous solution containing (4 mmol) of paracetamol sodium salts at 0°C; after 50 min the mixture was kept at room temperature overnight. The aqueous layer was acidified with 5M HCl, the α and γ esters were precipitated and separated by flash chromatography (ethyl acetate:butanol 4:1 v/v) to give 379 mg of esters (137 mg of α-ester, m.p. 165–168°C; 242 mg of γ-ester; m.p. 155–157°C). IR (cm^{-1}) ν_{max}: 3430 (br, −OH); 1740 (C=O ester); 1725 (C=O amidic); 1710 (C=O acid). The compounds were deprotected starting from α-ester **6a** (100 mg) and γ-ester **6b** (200 mg) by the same procedure described for the preparation of L-5-oxo-pyrrolidine-2-paracetamol-carboxylate **7** (yield 63%).

L-Glutamic acid γ-(4-acetylamino-phenyl) ester **8a** (γ-paracetamol-GA). M.p. 170–172°C. IR (cm^{-1}) ν_{max}: 3430 ($-NH_2$); 3300 (br, OH); 1735 (C=O ester); 1710 (C=O acid). ^{1}H−NMR (D_2O): δ 7·24–7·00 (m, 4H, ArH); 4·35 (m, 1H, −CH); 3·10 (m, 2H, CH_2); 2·25 (m, 2H, $-CH_2$); 1·95 (s, 3H, CH_3).

L-Glutamic acid α-(4-acetylamino-phenyl) ester **8b** (α-paracetamol-GA). M.p. 181–188°C. IR (cm^{-1}) ν_{max}: 3580 ($-NH_2$); 3510 (br, −OH); 1750 (C=O ester); 1705 (C=O acid). ^{1}H−NMR (D_2O): δ 7·27–7·12 (m, 4H, ArH); 4·53 (m, 1H, −CH); 3·27 (m, 2H, CH_2); 2·22 (m, 2H, $-CH_2$); 1·98 (s, 3H, CH_3).

Toxicological studies

Determination of reduced glutathione in the liver. Male mice were used in the experiments. Four to six hours before treatment, food was withdrawn from all animals but water was freely available. Glutathione was determined by HPLC with an electrochemical detector as described by Bousquet et al (1989) in liver extracts in five groups of four mice (Swiss male mice, 26–30 g). Four groups were treated intraperitoneally with 3·3 mmol kg^{-1} paracetamol, α-paracetamol-GA, γ-paracetamol-GA and paracetamol-PCA respectively in 0·5% aqueous methylcellulose. The glutathione levels were determined four hours after administration.

Acute toxicity in mice. The determination of LD50 was as described by Boyd & Bereczky (1966) following administration of paracetamol and paracetamol-PCA by intragastric cannula and intraperitoneal injection.

Statistical analysis. Data are presented as means ± standard deviations. Two-sided Student's *t*-test of differences between two sample means was used to assess the significance between groups. The significance level was set at $P < 0.05$.

In-vitro experiments

Determination of apparent lipophilic index. The lipophilic index of paracetamol-PCA ester prodrug was evaluated by reverse-phase HPLC capacity factor (k') (Brent et al 1983) using the following equation:

$$\log K = \log [(t_r - t_o)/t_o] \quad (1)$$

where t_r is the retention time of a retained peak and t_o is the retention time of an elution solvent.

Chemical hydrolysis. The hydrolysis of paracetamol-PCA prodrug was studied at pH 1·1 using a buffer solution of HCl and glycine and in pH 5·5 and 7·4 phosphate buffers. A constant ionic strength of 0·5 μ at 37°C was maintained in both buffers by adding a calculated amount of potassium chloride. Buffer solutions containing the ester prodrug (5×10^{-4} M) were kept at a constant temperature of 37 ± 0·1°C in a water bath and at appropriate time intervals samples were taken and analysed immediately by HPLC for paracetamol and the remaining ester prodrug. A reverse-phase C18 column (LiChrospher 100 RP-18 5 μm 250 mm × 4 mm i.d., obtained from Hewlett Packard, Milano, Italy) equipped with a direct-connect guard column (LiChrospher 100 RP-18 5 μm 4 × 4 mm, obtained from Hewlett Packard, Milano, Italy) was used in conjunction with the HPLC apparatus. Chromatographic separations were achieved using a mobile phase of CH_3CN (15%) and water (85%) at a flow rate of 1·0 mL min^{-1} with an injection volume of 20 μL. Detection was carried out at 254 nm and external standardization was used. Under these conditions the retention times for paracetamol and paracetamol-PCA ester were 4·13 and 8·72 min, respectively. Fig. 2 shows a representative chromatogram of paracetamol and paracetamol-PGA prodrug. The standard curve was linear over the range 0·030–100 μg mL^{-1}. The equation for the standard curve relating the paracetamol concentration (y, μg mL^{-1}) to peak area (A) in this range was $y = 2.758 \times 10^{-5} \times A - 0.054989$. The correlation coefficient of the standard curve was 0·9999. The sensitivity of the assay was determined by analysing progressively lower concentrations and was found to be 30 ng for a signal/noise ratio of 3:1.

Enzymatic hydrolysis. The rate of enzymatic hydrolysis of paracetamol-PCA ester prodrug was studied in a pH 7·4 isotonic phosphate buffer at 37 ± 0·1°C. The reaction was initiated by adding 10 μL stock solution of the prodrug in CH_3CN to 10 mL preheated buffer solution containing the appropriate amount of esterase. The final concentrations in the reaction mixture were 1 μM and 1 int. unit mL^{-1} for the substrate and for the enzyme respectively. At appropriate time intervals samples were taken and immediately analysed for paracetamol and the remaining prodrug by HPLC.

In-vivo experiments

Bioavailability studies. Male rabbits, 2–3 kg, were individually housed in cages in an air-conditioned room and maintained on a

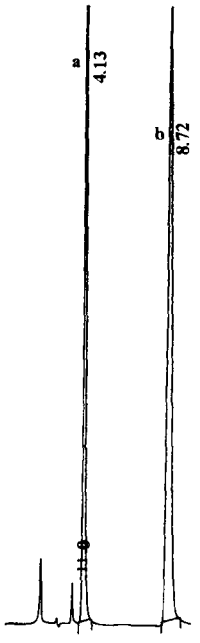

FIG. 2. Representative chromatogram of paracetamol (a) and L-pyroglutamic acid ester of paracetamol (b) in the hydrolysis studies.

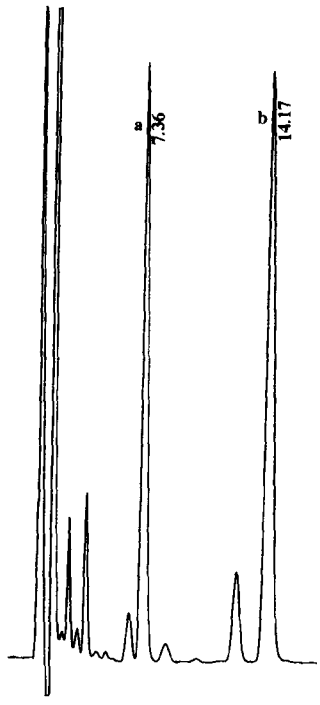

FIG. 3. Typical chromatogram of plasma sample of treated animals: paracetamol (a) and internal standard (b).

standard laboratory diet for about 2 weeks before the experiments. The rabbits were kept without food 12 h before the experiments with free access to water. During the trials, all rabbits were kept in restraining boxes in a normal upright posture. Fifteen minutes before oral and endovenous administration the rabbits were cannulated via a central ear artery using polyethylene tubing. Oral bioavailability was evaluated in two groups of six male rabbits. A dose of 17·27 mg kg^{-1} paracetamol-PGA prodrug suspension in 0·5% aqueous methylcellulose was administered orally to the first group of animals by gastric gavage. The second group was treated in the same way with an equivalent molar amount of paracetamol (10 mg kg^{-1}) and PGA (8·50 mg kg^{-1}) in the same vehicle. Intravenous bioavailability was estimated in two groups of six male rabbits. A dose of 17·27 mg kg^{-1} paracetamol-PGA prodrug in a Tween 80 15% water solution (freshly prepared), was injected into the marginal ear vein. The second group was treated in an equivalent manner with an equivalent molar amount of paracetamol (10 mg kg^{-1}) and PGA (8·50 mg kg^{-1}) in the same vehicle. At preset interval times 3 mL blood samples were collected into heparinized tubes for both experiments. The volume of blood aspirated was replenished with saline through an intravenous drip during the experiment.

Determination of paracetamol plasma concentrations. Plasma samples (200 μL) were treated with 200 μL 2% $ZnSO_4 \cdot 7H_2O$ solution in methanol:water (30:70) containing an appropriate amount of the internal standard to deproteinize the plasma. This mixture was vortexed for 1 min and centrifuged at 6000 rev min^{-1} for 10 min (Centrifugette 4206, ALC, Milano, Italy). The resulting supernatant was filtered through a 0·20 μm Teflon membrane (Spartan-3 Schleicher Schuell, Keene, NH, USA) using a 500 μL gas-tight syringe and 100 μL of the fluid was analysed by HPLC. The recovery efficiency from blank rabbit plasma to which had been added 20 μg mL^{-1} and 1 μg mL^{-1} paracetamol standard was 95·25 ± 4·86% and 93·50 ± 6·30%, respectively. A reverse-phase column (Hypersil ODS; 5 μm, 150 mm × 4·6 mm i.d., obtained from Alltech) equipped with a direct-connect guard column (Hypersil ODS; 5 μm of 10 mm × 4·6 mm obtained from Alltech) was used in conjunction with the HPLC apparatus described above. The HPLC conditions were as follows: mobile phase, CH_3CN: CH_3COONa 0·39 M (pH 5·50 with CH_3COOH) 1:99; flow rate 1 mL min^{-1}. Detection was carried out at 254 nm and internal standardization was used. The sensitivity of the assay was determined by analysing progressively lower concentrations and was found to be 35 ng for a signal/noise ratio of 3:1. Fig. 3 shows a representative chromatogram of paracetamol and internal standard (hippuric acid) in the plasma sample. No interfering peaks were observed in the blank plasma chromatogram.

Results and Discussion

Toxicological studies

Determination of reduced glutathione in liver. The groups of mice treated intraperitoneally with 3·3 mmol kg^{-1} paracetamol or an equivalent molar dose of α- and γ-paracetamol-GA showed significantly lower ($P < 0·01$) glutathione levels in comparison with the control group with a decrease of 35% (Table 1), while the group treated with an equivalent dose of paracetamol-PGA maintains glutathione levels superimposable with the control group ($P > 0·05$). The α- and γ-paracetamol-GA groups showed behaviour similar to that of paracetamol, as L-glutamic acid released from the hydrolysis of the prodrugs followed the primary route of catabolism via α-ketoglutarate and therefore the glutathione levels did not increase (Wilson & Koeppe 1961). The groups of mice treated with paracetamol-PGA maintained glutathione levels superimposable with the

Table 1. Hepatic glutathione levels in liver extracts obtained from five groups of four male mice after intraperitoneal administration of 3·3 mmol kg^{-1} of paracetamol, paracetamol-α-GA, paracetamol-γ-GA and paracetamol-PGA in 0·5% aqueous methylcellulose. The glutathione levels were determined four hours after administration by HPLC with electrochemical detection.

Experiment	Glutathione (μmol (g tissue)$^{-1}$)
Control	6·4 ± 0·9
Paracetamol	4·3 ± 1·0*
Paracetamol-α-GA	4·1 ± 0·8*
Paracetamol-γ-GA	4·2 ± 0·8*
Paracetamol-PGA	6·6 ± 1·1**

*$P < 0·01$, **$P > 0·05$ compared with control.

control, because the PGA formed in-vivo by the hydrolysis, specifically enhanced the biosynthesis of reduced glutathione in liver (Wellner et al 1974). In view of these results only the paracetamol-PGA ester was investigated for acute toxicity, for chemical and enzymatic hydrolysis and for the bioavailability study.

Acute toxicity in mice. The oral and the intraperitoneal LD50 of paracetamol-PGA was found to be 2000 mg kg^{-1} and 1900 mg kg^{-1} respectively, while that of paracetamol was 1200 mg kg^{-1} orally and intraperitoneally.

In-vitro release data analysis and lipophilic data

It was expected that the paracetamol-PGA ester prodrug would be hydrolysed in-vivo before, during or after absorption to release free paracetamol which would then exert its characteristic pharmacological actions. Therefore it was desirable to determine the relative susceptibility of these compounds in a simulated gastric juice (glycine buffer, pH 1·1) and at pH 5·5 and 7·4 in a phosphate buffer with and without the presence of hydrolytic enzyme. The paracetamol-PGA ester was found to be hydrolysed quantitatively to the parent phenol derivative in both enzymatic and chemical hydrolysis as revealed by HPLC analysis. The time courses for paracetamol and paracetamol-PGA ester during all hydrolysis experiments are shown in Fig. 4.

The progress of hydrolysis of paracetamol-PGA ester, at the initial concentrations studied, followed pseudo first-order kinetics, in both chemical and enzymatic trials over several half-lives as illustrated in Fig. 5.

Pseudo-first rate constants (k) were calculated from the slopes of linear plots of the logarithm of residual paracetamol-PGA ester vs time and the corresponding half-life obtained from the equation:

$$t_{\frac{1}{2}} = 0 \cdot 693/k \qquad (2)$$

The results are shown in Table 2. The influence of pH on the degradation of paracetamol-PGA ester is typical of a carboxylic ester with a specific base-catalysed hydrolysis. Inspection of data in Table 2 shows that the paracetamol-PGA ester was hydrolysed more slowly at pH 5·5 and 1·1 than at pH 7·4 with half-lives of 257·2, 246·65 and 16·26 min at pH 5·5, 1·1 and 7·4, respectively. At pH 7·4 in the presence of esterase the hydrolysis rate was more rapid, with a 2·6-fold change in $t_{\frac{1}{2}}$.

These results suggest that paracetamol-PGA ester prodrug would be hydrolysed to release paracetamol following oral or

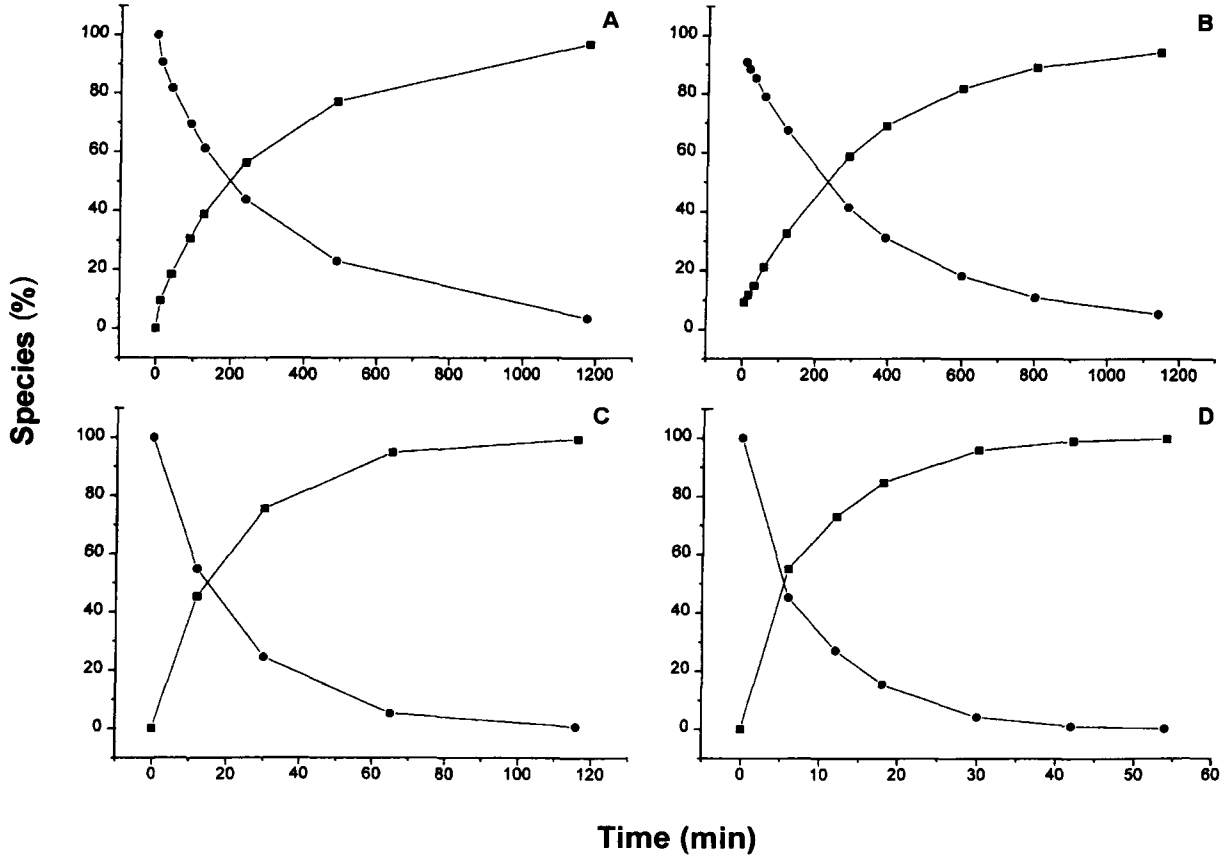

FIG. 4. Time course for the paracetamol (■) and L-pyroglutamic acid ester of paracetamol (●) during the hydrolysis of the prodrug at 37°C in a pH 1·1 buffer (A), in a pH 5·5 buffer (B), in a pH 7·4 buffer (C) and in a pH 7·4 buffer with esterase (D).

Table 2. Kinetic data for the hydrolysis of paracetamol-PGA ester prodrug. The hydrolysis was performed at pH 1·1, 5·5, 7·4 and 7·4 with esterase from hog liver. Buffer solutions, with a constant ionic strength of 0·5 μ, containing the ester prodrug (5×10^{-4}M) were kept at a constant temperature of 37 ± 0·1°C. In the enzymatic hydrolysis the final concentrations in the reaction mixture were 1 μM and 1 int. unit mL^{-1} for the substrate and for the enzyme respectively.

	pH			
	1·1	5·5	7·4	7·4 (enzymatic)
k (min^{-1})	$2{\cdot}81 \times 10^{-3}$	$2{\cdot}69 \times 10^{-3}$	$42{\cdot}60 \times 10^{-3}$	$110{\cdot}31 \times 10^{-3}$
t½ (min)	246·6	257·2	16·3	6·3

parenteral administration in animals and man. The water solubility of paracetamol-PGA ester and the octanol-water partition (log P) were not determined due to the poor stability of the ester in water. The lipophilicity of paracetamol-PGA ester was evaluated by means of reverse-phase HPLC capacity factor (k′) since a linear relationship between log k′ and log P has been observed for many types of compounds (Hafkenscheid & Tomlinson 1983). As expected, in CH_3CN:water (15:85) as mobile phase, the ester prodrug was more lipophilic than paracetamol with a log k′ value of 0·89 compared with 0·50 for the free drug.

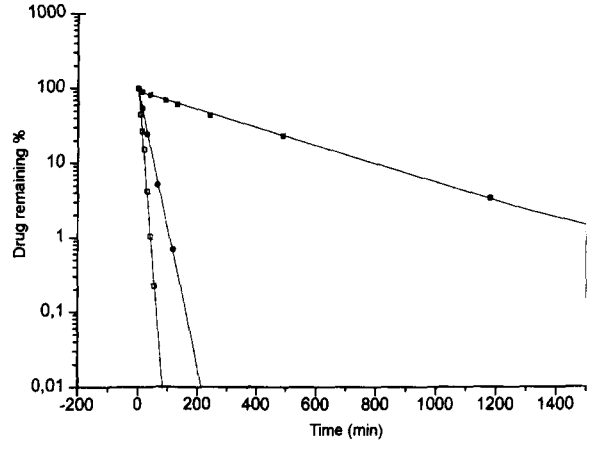

FIG. 5. Plots showing the first-order kinetics of hydrolysis of L-pyroglutamic acid ester of paracetamol at 37°C in pH 7·4 isotonic phosphate buffer with esterase (□), in pH 7·4 phosphate buffer (●) and in pH 1·1 (HCl, glycine) buffer solution (■).

In-vivo data analysis

For the oral bioavailability study the plasma paracetamol concentration vs time profile was characterized with several different parameters for the dosage form studied. The maximum drug concentration (C_{max}) and the time at which this sample was drawn (t_{max}) were obtained directly from individual concentration-time data. The elimination rate constants (k_{el}) were estimated by least squares regression of concentration–time profile data in the terminal log-linear region of the curves and in addition the half-lives were calculated. Finally the area under curve, concentrations vs time profile from time 0 to 8 h (AUC_{0-8}) was calculated using the trapezoidal rule (Gibaldi 1982). Absolute oral bioavailability was determined as the AUC

Table 3. Pharmacokinetic parameters for the oral route. A first group of six male rabbits was treated with a dose of 17·27 mg kg^{-1} of paracetamol-PGA prodrug suspension in 0·5% aqueous methylcellulose and a second group with an equivalent molar of paracetamol (10 mg kg^{-1}) and PGA (8·50 mg kg^{-1}) in the same vehicle.

	Paracetamol-PGA	Paracetamol
t_{max} (min)	50 ± 5	20 ± 3
C_{max} (μg mL^{-1})	9·2 ± 1·3	20·1 ± 2·70
k_{el} (h^{-1})	0·3096	0·3456
t½ (h)	2·24	2·01
AUC (μg mL^{-1} h)	33·60 ± 2·06	30·00 ± 1·55
Systemic availability (%)	89·0	82·5

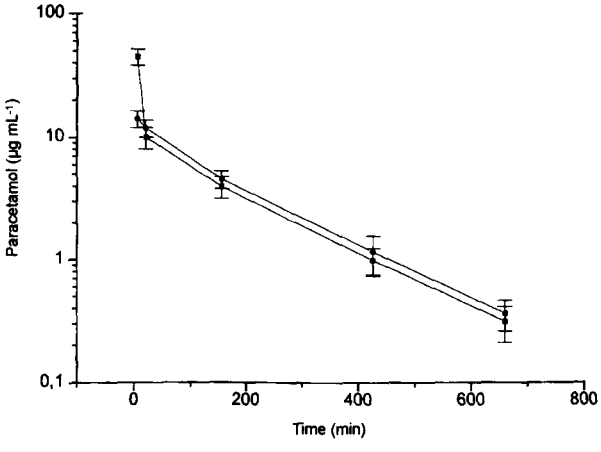

FIG. 6. Paracetamol plasma concentration–time curves after oral administration of L-pyroglutamic acid ester of paracetamol (●) and an equivalent dose of paracetamol (■).

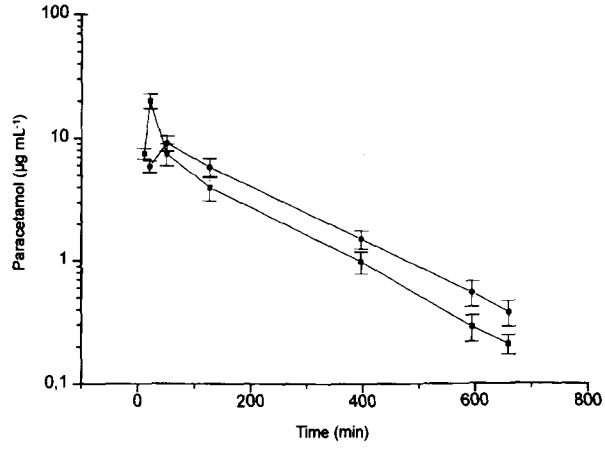

FIG. 7. Paracetamol plasma concentration–time curves after intravenous administration of L-pyroglutamic acid ester of paracetamol (●) and an equivalent dose of paracetamol (■).

Table 4. Pharmacokinetic parameters for the intravenous route. A first group of six male rabbits was treated with a dose of 17·27 mg kg^{-1} paracetamol-PGA prodrug in a Tween 80 15% water solution (freshly prepared), and a second group with an equivalent molar of paracetamol (10 mg kg^{-1}) and PGA (8·50 mg kg^{-1}) in the same vehicle.

	Paracetamol-PGA	Paracetamol
k_{el} (h^{-1})	0·3230	0·3235
t½ (h)	2·145	2·142
AUC ($\mu g\ mL^{-1}\ h$)	37·73 ± 3·42	36·38 ± 5·43

following oral administration divided by the AUC following intravenous dosage. The endovenous time course was characterized by means of different parameters such as the elimination rate constants (K_{el},), the half-lives (t½) and the AUC from 0 to 8 h. The oral and endovenous concentration–time profiles are shown in Figs 6 and 7 and the pharmacokinetics data are presented in Tables 3 and 4.

For the oral route peak plasma concentrations (C_{max}) following paracetamol-PGA ester prodrug administration were significantly lower ($P < 0·001$) than those for the paracetamol with a twofold decrease (9·2 ± 1·3 $\mu g\ mL^{-1}$ compared with 20·1 ± 2·7 $\mu g\ mL^{-1}$). For all the other time points the values with paracetamol-PGA prodrug were significantly higher ($P < 0·05$) and consequently the apparent rate of plasma clearance was slower than that observed with paracetamol as demonstrated by the increase in the t½ (from 121 to 144 min) and the decrease in K_{el} (from 0·3456 to 0·3096 min^{-1}). In addition the time to peak concentration (t_{max}) was increased from 20 min in the paracetamol group to 50 min in the paracetamol-PGA group. Furthermore the relative systemic availability values were significantly greater ($P < 0·01$) for the paracetamol-PGA group than for the paracetamol group (33·6 ± 2·06% compared with 30·0 ± 1·14%) with an increase in the the absolute systemic availability from 82·5 to 89·0%. Taken together these results suggest that the oral absorption for the paracetamol-PGA prodrug group ended later than the paracetamol group due to the more lipophilic character of the prodrug with respect to paracetamol that presumably decreased the dissolution rate of the ester suspension and hence prolonged or delayed the absorption during the period in which the plasma concentration was falling (Gibaldi 1982). For the intravenous route, the tested compound showed a similar trend in the plasma concentration–time profile and the pharmacokinetic parameters considered were very similar.

These experimental data may be explained by the fact that the paracetamol-PGA ester was administered to the rabbits in solution and not as a suspension, as was used for the oral route, and by its rapid in-vivo hydrolysis rate. These results are in accordance with the in-vitro release data where the enzymatic hydrolysis at pH 7·4 had a half-life of 6·13 min.

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