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Synthesis, chemical and enzymatic hydrolysis, and bioavailability evaluation in rabbits of metronidazole amino acid ester prodrugs with enhanced water solubility

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Abstract

A series of amino acid esters (**3a–e**) have been synthesized and evaluated as potential prodrugs of metronidazole with the aim of improving aqueous solubility and therapeutic efficacy. The aqueous solubility and the lipophilicity (expressed as the log P value) of metronidazole and its esters were investigated. In general the prodrugs revealed enhanced water solubility compared with metronidazole. N,N-diethylglycinate hydrochloride (**3a**) and 4-ethylpiperazinoacetate (**3e**) derivatives displayed higher aqueous solubility, which exceeded that of the parent drug by factors of approximately 140 and 100, respectively. All the esters revealed lower log P values than metronidazole except for the 4-phenylpiperazinoacetate derivative (**3f**), which was 6.5-times more lipophilic than metronidazole. The hydrolysis kinetics of the esters were studied in aqueous phosphate buffer (pH 7.4) and 80 % human plasma at 37°C. In all cases the hydrolysis followed pseudo-first-order kinetics and resulted in a quantitative reversion to metronidazole as evidenced by HPLC analysis. The prodrugs exhibited adequate chemical stability (half-life, $t_{\frac{1}{2}}$, 4–16 h) in aqueous phosphate solution of pH 7.4. In 80 % human plasma they were hydrolysed within a few minutes to metronidazole. The esters **3d** (methylpiperazinoacetate derivative) and **3f** were exempted since their $t_{\frac{1}{2}}$ values were approximately 2.5 and 8.5 h, respectively. A comparative pH–rate profile study of N,N-diethylglycinate hydrochloride (**3a**) and 4-ethylpiperazinoacetate (**3e**) derivatives in aqueous buffer solution over the pH range 2.2–10 was investigated. The results indicated that **3a** showed marked stability at pH 2–6 followed by accelerated hydrolysis at pH 7.4. The basic ester **3e** was found to be less stable at lower pH values but exhibited comparative stability at physiological pH. Moreover, in-vivo experiments in rabbits revealed a higher metronidazole plasma level with sustained release characteristics within the prodrug-treated animals (10- and 2.5-fold) as compared with the parent drug-treated group. In conclusion, the designed amino acid esters **3a** and **3c–e** might be considered as good candidates for water-soluble prodrug forms of metronidazole.

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Introduction

In the course of our continued interest to improve the therapeutic properties of metronidazole, we have recently developed two series of identical and non-identical twin ester prodrugs for oral delivery (Mahfouz et al 1998; Aboul-Fadl & Mahfouz 1998). Those studies afforded a chemical delivery system that was characterized by

enhanced lipophilicity, adequate chemical stability and improved bioavailability of metronidazole. A challenging limitation for formulations of metronidazole in a simple injection dosage form is its relatively low water solubility (approximately 1.0%, w/v, at 25°C). The intravenous administration of the drug is restricted to the infusion of 100–200 mL every 8 h using 0.5% w/v aqueous solution to meet the required dose (Bundgaard et al 1984a; Jensen et al 1990).

Several reports have been published on overcoming metronidazole's solubility problem. Firstly, the use of dicarboxylic acid hemiesters e.g. hemisuccinate, hemiglutarate and hemimaleate was studied. However, those prodrugs showed limited stability in aqueous solution and were slowly and incompletely converted in-vivo to metronidazole (Larsen et al 1988; Vermeersch et al 1990). Alternatively, a water-soluble metronidazole phosphate ester prodrug with adequate chemical stability was reported by Cho et al (1982), but it was found that it was not rapidly or quantitatively converted to the parent drug in-vivo.

A third type of water-soluble ester prodrug had an ionizable amino function in the acid moiety, which revealed facile hydrolysis in human plasma as illustrated by propacetamol, 4-(acetamido) phenyl diethylaminoacetate HCl salt, which is an injectable water soluble form of paracetamol currently approved by the *European Pharmacopoeia* 2000. Application of this prodrug type on metronidazole has been reported (Bundgaard et al 1984a, b, 1989; Cho & Haynes 1985; Jensen et al 1990). This type of water-soluble ester prodrug was investigated mostly as the hydrochloride salt but unfortunately it showed high instability in aqueous solutions (Bundgaard et al 1989; Jensen et al 1990). It appeared from the values of pseudo-first-order hydrolysis rate constants that the protonated amino acid ester possessed very high susceptibility to undergo hydrolysis as compared with the free base form (Bundgaard et al 1984b).

However, we reported a series of bucetin ester prodrugs bearing a nonprotonated α-amino acid moiety. Some of those derivatives exhibited enhanced water solubility, good stability in aqueous buffer solutions and rapid bioconversion (El-Faham & Mahfouz 1994).

This study reports on a series of metronidazole amino acid esters as water-soluble prodrugs. The physicochemical properties, kinetics of hydrolysis and bioavailability in rabbits of the synthesized metronidazole amino acid ester prodrugs were investigated. A comparative study of the pH–rate profiles for a salt and basic form of the prodrug was undertaken also.

Materials and Methods

Chemistry

Metronidazole was kindly provided by the Alexandria Co. for drug and chemical industries, Alexandria, Egypt. All the other chemicals and reagents were of reagent grade and those for kinetic studies were of analytical grade. Freshly double-distilled water was used in the preparation of the solutions.

Melting points were determined on a Stuart scientific capillary melting point apparatus and are uncorrected. Thin layer chromatography was performed using DC-Alufolein (Kieselgel 60G, F 254, 0.25 mm). Chloroform/methanol (9 : 1) was used for the developing system and the spots were visualized by UV at 254 nm. Elemental analyses were performed at the Chemistry Department, Faculty of Science, Assiut University (Assiut, Egypt). IR spectra (KBr disc) were recorded on an IR-470 Shimadzu spectrometer (Japan). ^{1}H NMR spectra were scanned on a Varian EM-360 L NMR spectrometer (60 MHz; USA). Chemical shifts were expressed in δ (ppm) relative to TMS (tetra methylsilane) as an internal standard.

Solubility and partition coefficients were determined using a shaker with a thermostatically controlled water bath (Unitronic 320 OR-Selecta, Spain). All the UV measurements were performed on a Shimadzu UV 150-02 double beam spectrometer (Tokyo, Japan).

Synthesis of metronidazole chloroacetate (2)

To a stirred suspension of metronidazole (**1**), 5.13 g (30 mmol) in 50 mL methylene chloride, dried pyridine 2.74 g (35 mmol) was added followed by dropwise addition of chloroacetyl chloride 3.96 g (35 mmol) within 30 min. The mixture was refluxed for 2 h. The resulting solution was cooled, washed with water (3×100 mL) and dried over anhydrous sodium sulfate. The filtrate was evaporated to dryness under reduced pressure and crystallized from ether to yield 85% metronidazole chloroacetate (**2**), mp 71–73°C, reported 70–72°C (Bundgaard et al 1984a). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.4 (s, 3H, CH_3); 3.9 (s, 2H,CH_2Cl), 4.5 (m, 4H, NCH_2CH_2O) and 7.7 (s, 1H, imidazole).

General method for synthesis of amino acid ester prodrugs (3a–f)

A mixture of the respective amine (20 mmol) and metronidazole chloroacetate (**2**), 1.2 g (4 mmol) in 50 mL dry dioxane, was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure. The

residue was dissolved in 100 mL methylene chloride, washed with a saturated solution of sodium chloride (3×50 mL), dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated under reduced pressure and the residue recrystallized from the proper solvent to give the title ester prodrugs. Compound **3a** was obtained as the hydrochloride salt.

*Metronidazole N,N-diethylglycinate hydrochloride (**3a**)*
Yield 85%, mp 173–181°C (ether/ethanol). ^{1}H NMR ($CDCl_3$/DMSO-d_6) δ (ppm): 1.3 (t, J = 7 Hz, 6H, $2CH_2\underline{CH_3}$), 2.6 (s, 3H, CH_3); 3.2 (q, J = 7 Hz, 4H, $2\underline{CH_2}CH_3$), 4.0 (m, 4H, NCH_2CH_2O), 11.5 (broad exchangeable s, 1H, protonated diethylamino) and 8.0 (1H, s, imidazole). Anal. calcd. for $C_{12}H_{21}N_4O_4Cl$: C 44.93, H 6.60, N 17.47%. Found: C 44.54, H 6.44, N 17.32.

*Metronidazole 1-piperidinoacetate (**3b**)*
Yield 85%, mp 111–114°C (ether). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.5 (m, 6H, piperidino $CH_2CH_2CH_2$), 2.5 (m, 7H, CH_3 + piperidino CH_2NCH_2), 3.15 (s, 2H, $COCH_2N$), 4.5 (m, 4H, NCH_2CH_2O) and 8.0 (s, 1H, imidazole). Anal. calcd. for $C_{13}H_{20}N_4O_4$: C 52.69, H 6.80, N 18.91%. Found: C 52.49, H 6.80, N 18.78.

*Metronidazole 1-morpholinoacetate (**3c**)*
Yield 87%, mp 75–77°C (ether/ethylacetate), reported 77–78°C (Bundgaard et al 1984a). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.5 (m, 7H, $CH_3 + CH_2NCH_2$), 3.2 (s, 2H, $COCH_2N$), 3.6 (t, J = 5 Hz, 4H, CH_2OCH_2) 4.5 (m, 4H, NCH_2CH_2O) and 7.9 (s, 1H, imidazole).

*Metronidazole 4-methyl-1-piperazinoacetate (**3d**)*
Yield 86%, mp 97–99°C (ether), reported 97–99°C (Bundgaard et al 1984a). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.15 (s, 3H, NCH_3), 2.4 (broad s, 11H, CH_3 + piperazine protons), 3.0 (s, 2H, $COCH_2N$), 4.5 (m, 4H, NCH_2CH_2O) and 7.7 (s, 1H, imidazole).

*Metronidazole 4-ethyl-1-piperazinoacetate (**3e**)*
Yield 90%, mp 64–65°C (ether/petroleum ether). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.07 (t, J = 7 Hz, 3H, $CH_2\underline{CH_3}$) 2.5 (m, 13H, $CH_3 + \underline{CH_2}CH_3$ + piperazine protons), 3.15 (s, 2H, $COCH_2N$), 4.6 (m, 4H, NCH_2CH_2O) and 8.0 (s, 1H, imidazole). Anal. calcd. for $C_{14}H_{23}N_5O_4$: C 51.68, H 7.13, N 21.53%. Found: C 52.12, H 7.00, N 21.00.

*Metronidazole 4-phenyl-1-piperazinoacetate (**3f**)*
Yield 85%, mp 88–90°C (ether/ethanol). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.6 (m, 7H, CH_3 + piperazine CH_2NCH_2), 3.2 (m, 6H, $COCH_2N + CH_2NCH_2$), 4.5 (m, 4H, NCH_2CH_2O) 6.9 (d, J = 8 Hz, 3H, phenyl $H_{3,4,5}$), 7.25 (t, J = 8 Hz, 2H, phenyl $H_{2,6}$), and 7.9 (s, 1H, imidazole). Anal. calcd. for $C_{18}H_{24}N_5O_4$: C 57.74, H 6.46, N 18.71%. Found: C 58.14, H 6.30, N 18.74.

Aqueous solubility

The aqueous solubility of metronidazole and its amino acid ester prodrugs (**3a–f**) was determined at 25°C. An excess amount of the respective compound was added to 2 mL water in a screw-capped test tube. The suspension was rotated on a thermostatically controlled mechanical shaker switched on 30 strokes min^{-1} for 24 h to attain equilibrium. The mixture was filtered, and a portion was diluted with an appropriate amount of water and analysed for metronidazole or its amino acid ester prodrug content spectrophotometrically at 320 nm.

Lipophilicity

The partition coefficients of metronidazole and its prodrugs (**3a–f**) were determined in an n-octanol/water system at 25°C. The two phases had been previously saturated with each other and then separated. To ensure solubility an accurately weighed amount of each compound was added to the aqueous phase. A 3-mL sample of this solution was transferred to a 25-mL stoppered bottle and 3 mL of the saturated n-octanol was added. The bottle was then shaken for 4 h at 35 strokes min^{-1}. The aqueous layer was carefully separated and 1 mL of this layer was suitably diluted with water. The concentration of each compound was determined spectrophotometrically at $\lambda_{max} = 320$ nm by virtue of calibration curves correlating substance absorbance and known concentrations. All experiments were conducted in triplicate and the mean values were taken. The partition coefficients were calculated.

Hydrolysis kinetics

The kinetic studies were carried out using an HPLC system. This consisted of a pump (Knauer pump 64, Berlin, Germany), a Knauer variable-wavelength detector, a reversible-phase C18 column (Eurospher 80 RP-18, 25×0.5 cm i.d.) equipped with a cartridge guard column, a Shimadzu C–R 6A chromatopac recording integrator and a 20-μL injection loop. Chromatographic separations were achieved using a mobile phase of methanol/aqueous phosphate buffer mixture containing (% w/v) potassium dihydrogen phosphate (0.43 g) and

disodium hydrogen phosphate (0.37 g). The relative ratio of methanol and phosphate aqueous solution was adjusted in each case to attain complete separation of the peaks of the prodrug, parent drug and the expected degradation products. All measurements were carried out at $\lambda_{max} = 320$ nm, and the flow rate of 1.0 mL min^{-1} was adjusted for all prodrugs except the ester **3c**, where the flow rate was set at 1.5 mL min^{-1}. The kinetic data were the average of at least three experiments.

In aqueous buffers

The hydrolysis kinetics of metronidazole amino acid ester prodrugs (**3a**–**f**) was studied at pH 7.4. In addition to this, the pH–rate profile of the prodrugs **3a** and **3e** was determined in aqueous buffer solutions over the pH range 2.2–10, using McIlvaine buffer (pH 2.2–8) and Clark and Lubs buffer (pH 7.8–10). The total buffer concentration was 0.02 M and a constant ionic strength (μ) of 0.5 for each buffer was maintained by adding a calculated amount of potassium chloride. A sample (250 μL) of the methanolic solution of the respective prodrug (2×10^{-3} M) was added to 4.75 mL preheated buffer solution in screw-capped test tubes. The solutions were kept at 37°C in a water bath and at appropriate time intervals 20-μL samples were withdrawn and analysed by HPLC for the remaining ester prodrugs. Least-square equations, derived by correlating areas in HPLC chromatograms to known concentrations of each compound were used for calculation of the residual ester concentrations in the studied samples. The correlation coefficients of the standard curves were 0.999. Pseudo-first-order-rate constants for the hydrolysis were then obtained from linear plots of the log concentration of remaining ester vs time.

In human plasma

The rate of enzymatic hydrolysis of the ester prodrugs **3a**–**f** in human plasma diluted to 80% with 0.02 M phosphate buffer pH 7.4 was determined. Samples of stock solution (2×10^{-4} M; 200 μL) of the respective prodrug were incubated with 1.80 mL 80% human plasma at 37°C. At appropriate time intervals 100-μL samples of plasma/buffer mixture were withdrawn, deproteinated by addition of 200 μL methanol, immediately mixed and centrifuged at 1×10^4 rev min^{-1} for 10 min. Samples (20 μL) of the resulting clear supernatants were withdrawn and analysed for the remaining prodrugs by an HPLC method. The pseudo-first-order-rate constants for the hydrolysis and the half-lives ($t_{\frac{1}{2}}$) of prodrugs were calculated from linear plots of the log concentrations of the remaining prodrugs vs time.

Bioavailability study in rabbits

Rabbits (approximately 2 kg), from the authorized animal house (Faculty of Medicine, Assiut University), were divided into three groups of three animals each. The rabbits were used to compare the bioavailability of the prodrugs **3a** and **3e** with that of metronidazole following intravenous administration in the marginal ear vein. In group one, each rabbit received a dose of 2 mL kg^{-1} 0.5% w/v aqueous solution of metronidazole. An equivalent amount (0.5 mL kg^{-1}) of an aqueous solution (3.8%, w/v) of the prodrug **3a** or **3e** was administered to the rabbits of groups two and three, respectively. At appropriate time intervals up to 3 h, 2-mL blood samples were withdrawn from each rabbit into heparinized tubes and centrifuged for 15 min to separate the plasma. Plasma samples (100 μL) were diluted with an equivalent volume of methanol, and treated as detailed above for the hydrolysis in human plasma and assayed for metronidazole content. The mean concentration of metronidazole at the respective time interval in each group was taken.

Results and Discussion

Chemistry

The reaction sequence used for the preparation of compounds **3a–f** was achieved in two steps (Figure 1). The precursor metronidazole chloroacetate (**2**) was obtained by refluxing metronidazole with chloroacetyl chloride in methylene chloride using pyridine as acid

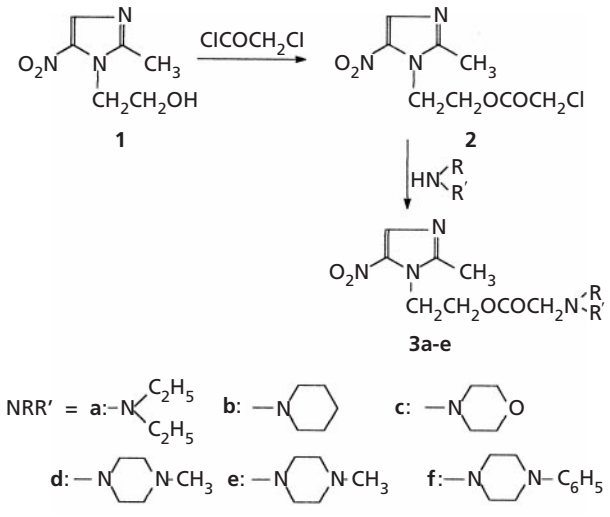

Figure 1 The synthesis of the metronidazole ester prodrugs **3a**–**f**.

scavenger. Subsequent condensation of the intermediate (**2**) with the respective amine at room temperature gave the title compounds **3a–f** in good yield. The purity was assessed by TLC and HPLC, and the assignments of the structures were based on elemental and spectroscopic methods of analyses. The IR spectral data revealed the characteristic carbonyl stretching vibration bands at 1722–1755 cm^{-1} of the ester groups in addition to the ether bands at approximately 1170 and 1255 cm^{-1}. In the ^1H NMR spectra the signals of the respective protons of the prepared esters were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed a singlet signal at $\delta \sim$ 2.5 ppm correlated to the methyl group located at C-2 of the imidazole nucleus; four methylene protons of metronidazole appeared as multiplet at $\delta \sim 4.5$ ppm in addition to a singlet signal at $\delta \sim 3$ ppm corresponding to the $COCH_2N$ group. This signal was shifted downfield to $\delta \sim 4$ ppm in compound **4a** due to the withdrawing effect of the protonated amino group. A downfield shifted signal at $\delta \sim 7.9$ ppm was assigned to the imidazole ring proton. The protons of the amine moiety were resonated at different δ-values of approximately 1.2–4.3 ppm and a downfield signal at $\delta \sim 7$ ppm was due to the aromatic protons of the phenyl group of the ester **3f**.

Physicochemical properties

The aqueous solubility and partition coefficient (log P) of metronidazole (**1**) and its amino acid ester prodrugs (**3a–f**) are listed in Table 1. It is evident that esterification of the hydroxyl group of metronidazole with different amino acids provided an additional handle to modify the physicochemical properties of the parent drug. It was observed as a general pattern that the synthesized prodrugs possessed higher aqueous solubility compared with metronidazole. The hydrochloride salt of metronidazole N,N-diethylglycinate ester (**3a**) displayed a higher aqueous solubility that exceeded the solubility of the parent drug in water by a factor of approximately 140. In addition, the solubility of the piperazine derivatives (**3d–f**) paralleled with the availability of the lone pair of electrons on the N^4 atom of the piperazine moiety. Accordingly, the ethyl piperazine derivative (**3e**) showed higher aqueous solubility in contrast to the phenyl piperazine derivative (**3f**), which displayed poor water solubility. The solubility of **3f** was even poorer than that of the parent drug. Generally, the observed increase in aqueous solubility of the investigated prodrugs was accompanied by a decrease in lipophilicity relative to the parent drug as illustrated by log P values (Table 1). The prodrug **3f**, derived from 4-phenylpiperazinoacetate, showed an expected extremely high partition coefficient, higher than metronidazole (4.73 and 0.74, respectively). This enhanced lipophilicity of **3f** represented a basis for its consideration as a prodrug form for oral, dermal and rectal delivery. The pH values of an aqueous solution (2.5–20 %, w/v) of N,N-diethylglycinate hydrochloride salt (**3a**) and the base 4-ethyl-1-piperazinoacetate (**3e**) were determined and found to be 1.5–2.2 and 9–9.5, respectively.

In-vitro stability

Aqueous buffers

Hydrolysis kinetics of metronidazole amino acid ester prodrugs **3a–f** were studied in aqueous buffer solution at pH 7.4. Under the experimental conditions used the

Table 1 Physicochemical and kinetic data* of metronidazole and its prodrugs (**3a–f**) in aqueous phosphate buffer solution and 80 % human plasma.

Compound	Solubility (mg mL^{-1})	log P	pH 7.4		Plasma	
			$k \times 10^{-3}$	$t_{\frac{1}{2}}$ (h)	k	$t_{\frac{1}{2}}$ (min)
Metronidazole (**1**)	10.5	0.75	–	–	–	–
3a	1448.4	0.03	2.846	4.06	0.021	33.56
3b	3.5	0.51	4.836	2.39	0.014	49.51
3c	337.4	0.27	1.488	7.76	0.013	52.59
3d	98.6	0.29	0.954	12.11	0.001	511.2
3e	994.2	0.51	0.691	16.73	0.015	46.52
3f	1.19	4.73	1.536	7.52	0.005	138.0

*Average of three experiments.

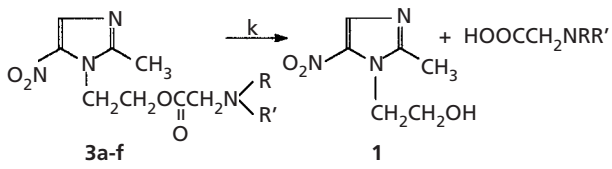

Figure 2 The degradation of the metronidazole ester prodrugs **3a–f**.

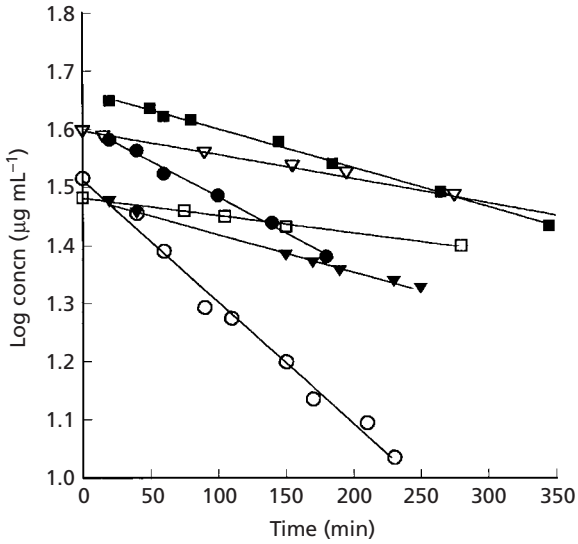

Figure 3 First-order plots for the hydrolysis of metronidazole ester prodrugs (**3a** ●; **3b** ○; **3c** ▼; **3d** ▽; **3e** □; **3f** ■) in phosphate buffer (pH 7.4). Each value is the average of three experiments.

target compounds hydrolysed to release the parent drug (Figure 2) as evidenced by HPLC analysis. At constant pH and temperature, the reaction displayed strict first-order kinetics.

The rate constants (k_{obs}) and the corresponding half-lives ($t_{\frac{1}{2}}$) for the respective prodrugs were calculated from the linear regression equations correlating the log concentration of the residual prodrug vs time. The results are summarized in Table 1 and Figure 3. The kinetic data revealed that the susceptibility of various amino acid esters to undergo hydrolysis in isotonic phosphate buffer solution varied appreciably. Unexpectedly the ester derived from 1-piperidinoacetate (**3b**) showed a reasonable facile cleavage at pH 7.4 ($t_{\frac{1}{2}}$ approximately 2.5 h). This is analogous to the previously reported data for bucetin 1-piperidinoacetate (El-Faham & Mahfouz 1994). Generally, it could be postulated that the rate of hydrolysis of the amino acid esters **3a–f** at pH 7.4 (as illustrated in Table 1) was mainly

Table 2 pH rate profile data* for half-lives ($t_{\frac{1}{2}}$) of the prodrugs **3a** and **3e** in aqueous buffer solutions (pH 2.2–10) at 37°C.

pH	3a		3e	
	k_{obs}	$t_{\frac{1}{2}}$ (h)	k_{obs}	$t_{\frac{1}{2}}$ (h)
2.2	1.157×10^{-3}	9.98	1.114×10^{-3}	10.37
3.0	1.316×10^{-3}	8.78	0.191×10^{-3}	60.60
4.0	0.917×10^{-3}	12.61	0.369×10^{-3}	31.27
5.0	1.439×10^{-3}	8.03	0.372×10^{-3}	31.07
6.0	2.035×10^{-3}	5.68	1.064×10^{-3}	10.86
7.4	2.846×10^{-3}	4.06	0.669×10^{-3}	16.73
8.0	6.708×10^{-3}	1.72	1.480×10^{-3}	7.81
9.0	9.652×10^{-3}	1.19	8.557×10^{-3}	1.35
10.0	0.012	1.00	0.019	0.60

*Average of three experiments.

related to some electronic and steric properties of the amine moiety. The observed higher susceptibility of the diethylamino and piperidino derivatives **3a** and **3b** was clearly attributed to the higher basic character of the amine partial structure of the molecule relative to the morpholino and piperazino derivatives **3c–f**. The effect of the electronic properties on the rate of hydrolysis at the studied pH has been emphasized by comparison of the k_{obs} values of the hydrochloride salt **3a** and the base **3b**, whereby **3a** exhibited relative less susceptibility ($k_{obs} = 2.85$) for hydrolysis than **3b** ($k_{obs} = 4.84$). On the basis of this kinetic data, it was apparent that the basic form of esters **3c–f** were stable enough for several hours at pH 7.4 to be used as water soluble formulations.

pH–rate profile

The esters **3a** and **3e**, as representatives for salt and basic forms of the prodrugs, were selected for the pH–rate profile study over the pH range 2.2–10 at 37°C. The investigation aimed to determine the optimum pH for maximum stability. The values of the pseudo-first-order-rate constants (k_{obs}) for hydrolysis of both compounds at the respective pH value and their corresponding $t_{\frac{1}{2}}$ are given in Table 2 and the pH–rate profiles are shown in Figure 4.

The results indicated that the hydrochloride salt **3a** revealed a marked stability at the pH range 2–6, which corresponded to the pH of its aqueous solution at concentrations ranging from 2.5 to 20 %, w/v. However, this prodrug showed an accelerated degradation rate near the physiological pH (U shape segment 6–8). These observations coincided with previously reported results of the pH-rate profile of metronidazole N,N-dimethyl-

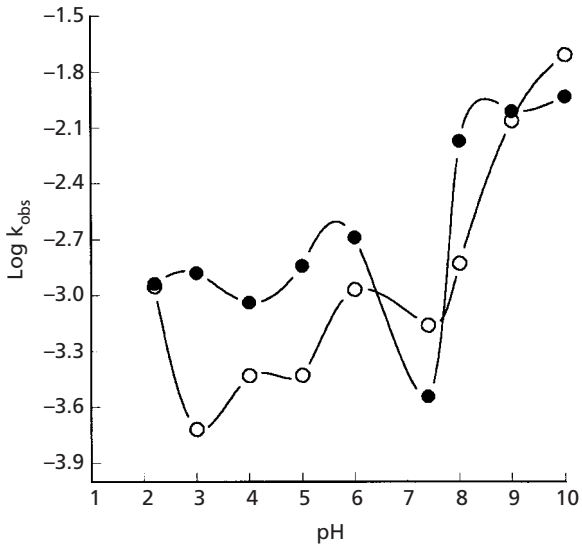

Figure 4 The pH–rate profiles for the hydrolysis of metronidazole prodrugs (**3a** ●; **3e** ○) at 37°C in aqueous buffers. Each value is the average of three experiments.

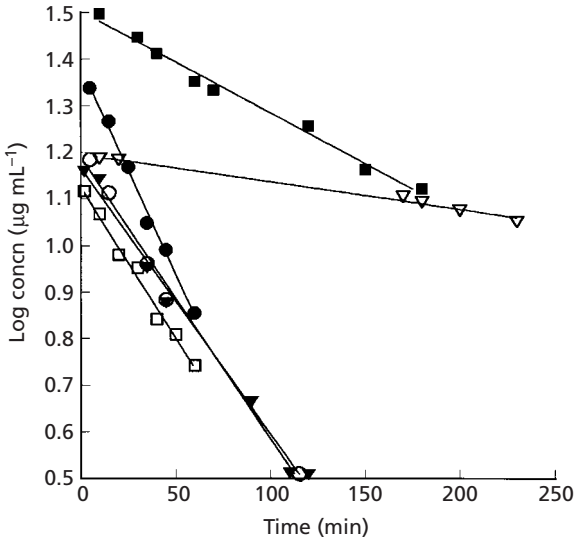

Figure 5 First-order plots for the hydrolysis of metronidazole ester prodrugs (**3a** ●; **3b** ○; **3c** ▼; **3d** ▽; **3e** □; **3f** ■) in 80% human plasma. Each value is the average of three experiments.

glycinate hydrochloride (Bundgaard et al 1984b). Consequently, the hydrochloride salt of metronidazole N,N-diethylglycinate could be formulated to be reconstituted as a solution for parenteral administration before use.

The basic ester prodrug **3e** was found to be less stable at lower pH (2–6), but exhibited marked stability relative to the hydrochloride salt **3a** at physiological pH. Formulations of this prodrug in aqueous solution of pH 7.4 attained a non-irritating stable parenteral preparation.

The observed enhanced hydrolysis susceptibility above the physiological pH might be explained on the basis of two factors; the elevated pOH value of the medium and the intramolecular nucleophilic catalysis by the lone pair of electrons of the amine moiety (Jensen et al 1990).

Human plasma

The rates of hydrolysis of the target esters were studied in 80% human plasma (pH 7.4) at 37°C as predictive for their in-vivo behaviour. The bioconversion proceeded in a similar sequence as in the case of the aqueous buffer solution. The rate constants (k_{obs}) for the individual reactions were calculated from the linear regression equations correlating log residual concentrations of the prodrugs vs time. The corresponding half-life ($t_{\frac{1}{2}}$) for the respective prodrug was then calculated (Table 2). The rate data clearly revealed that the susceptibility for enzymatic hydrolysis of the studied metronidazole amino acid ester prodrugs varied widely (Figure 5). The ester derived from 4-phenyl piperazinoacetate (**3f**) showed only a small acceleration in the cleavage rate in the presence of plasma. Likewise, the hydrolysis of the 4-methyl piperazinoacetate (**3d**) was catalysed to only a minor extent. In contrast, a reasonable rapid cleavage in plasma was obtained for the esters **3a–c** and **3e**. Consequently, these compounds were good substrates for plasma enzymes and appeared to be suitable candidates as prodrugs for metronidazole. It was interesting to observe from the kinetic study that metronidazole N,N-diethylglycinate hydrochloride (**3a**) was found to be much more readily hydrolysed in 80% human plasma and aqueous buffer solution (pH 7.4) than the investigated basic amino acid esters (**3b–f**).

Bioavailability

The amino acid ester prodrugs **3a** and **3e** were chosen for in-vivo evaluation as representative examples for the studied water soluble prodrugs of metronidazole for parenteral administration. Metronidazole and the prodrugs **3a** and **3e** were given intravenously to rabbits and at different time intervals; the concentration of metronidazole was estimated in plasma (Figure 6). A comparison of the plasma concentration–time curves of metronidazole indicated that the esters were rapidly hydrolysed to the parent drug. Maximum plasma concentrations of the liberated metronidazole from the esters **3a** and **3e** were attained after approximately 9 and

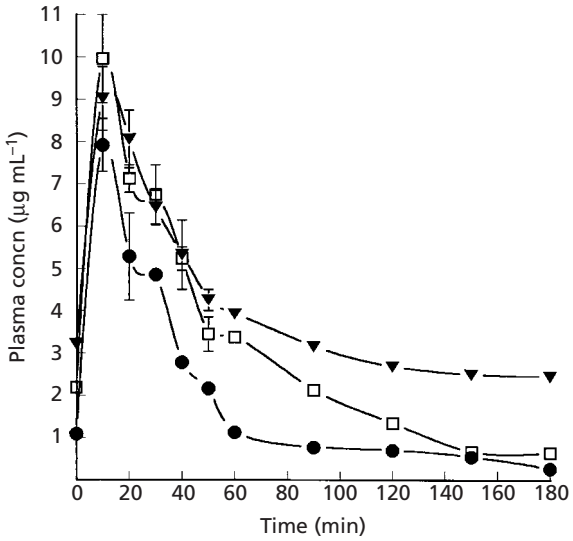

Figure 6 Plasma levels of metronidazole in rabbits after intravenous administration of the prodrugs (**3a** ▼; **3e** □; metronidazole ●). Each value represents the mean ± s.e. (n = 3).

10 min, respectively, compared with 8 min for the parent-drug-treated animals.

At all time intervals, the concentrations of metronidazole in the prodrug-treated animals were relatively higher compared with the animals receiving the parent drug. Furthermore, 3 h after administration of **3a** the metronidazole level was 10-times higher compared with the parent drug, indicating a sustained release. The prodrug **3e** resulted in a 2.5-fold increase in the metronidazole level after 3 h. In conclusion, the in-vivo evaluation study indicated that the amino acid esters **3a** and **3e** might be considered as potential biolabile water-soluble prodrug forms of metronidazole.

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