International Journal of Pharmaceutics, 41 (1988) 121–129
Elsevier

IJP 01380

Kinetics of regeneration of metronidazole from hemiesters of maleic acid, succinic acid and glutaric acid in aqueous buffer, human plasma and pig liver homogenate

Claus Larsen, Peter Kurtzhals and Marianne Johansen

Royal Danish School of Pharmacy, Department of Pharmaceutics, Copenhagen (Denmark)

(Received 25 May 1987)
(Accepted 30 June 1987)

Key words: Hemimaleinate, hemisuccinate and hemiglutarate esters of metronidazole; Kinetics of hydrolysis; Release rate in human plasma; Release rate in pig liver homogenate; Water-soluble prodrug

Summary

Kinetics of regeneration of metronidazole from the corresponding monoesters of maleic acid, succinic acid and glutaric acid in aqueous buffer solution, 80% human plasma and pig liver homogenate has been investigated at 37 °C. In all cases the hydrolysis followed first-order kinetics and complete reversion to metronidazole was observed as determined by HPLC. Metronidazole, hemimaleinate and hemisuccinate were not subject to plasma enzyme-facilitated hydrolysis, whereas the cleavage of the hemiglutarate ester proceeds 50 times faster in plasma compared to aqueous buffer pH 7.4. A reversed order of reactivity of the three hemiesters in pig liver homogenate has been observed with the hemisuccinate ester being the best substrate for pig liver hydrolases. In 5% liver homogenate a half-life of hydrolysis of metronidazole monosuccinate of 12 min has been determined. In comparison the half-lives of the hemimaleinate and the hemiglutarate esters are approximately 2 h.

Introduction

Hemisuccinate ester prodrugs have been extensively used as a means of improving aqueous solubility of alcoholic drugs: methylprednisolone (Anderson and Taphouse, 1981; Anderson et al., 1984, 1985), chlorzoxazone (Johansen and Bundgaard, 1981), propranolol (Garceau et al., 1978), hexachlorophene (Higuchi et al., 1971), oxazepam (Salmona et al., 1974), suxibuzone (Marunaka et al., 1980; Yasuda et al., 1982), metronidazole (Johansen and Larsen, 1984), hydrocortisone (Garrett, 1962a; Mauger et al., 1969), acetaminophen (Dittert et al., 1968; Rattie et al., 1970), chloramphenicol (Brent et al., 1980; Wargin and Wurster, 1983). The bioavailability of such derivatives after parenteral administration varies considerably and different degrees of bioconversion have been reported for hemisuccinate esters of various steroids (Melby and St. Cyr, 1961; Narang et al., 1983; Antal et al., 1983; Derendorf et al., 1985a and b) and chloramphenicol (Nahata and Powell, 1981; Kauffmann et al., 1981; Burke et al., 1982; Kramer et al., 1984). Besides hemisuccinates other dicarboxylic acid hemiesters have been synthesized (Garrett, 1962b; Yamamoto et

---

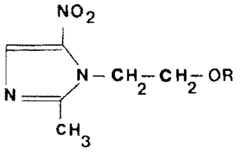
Correspondence: C. Larsen, Royal Danish School of Pharmacy, Department of Pharmaceutics, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.

al., 1971), but only sparse information is available concerning structural effects on the chemical stability of the derivatives.

As a part of our evaluation of the therapeutic potential of using dextrans as carriers for drug substances we have examined succinic acid (Larsen and Johansen, 1987a and b; Larsen, 1986), maleic acid and glutaric acid (to be published) as spacer arms to provide bioreversible attachment of alcoholic drugs (e.g. metronidazole) to dextrans. Since the degradation of the conjugates proceeds through parallel formation of metronidazole and the corresponding hemiesters, the aim of the present study has been to investigate the conversion of the latter hemiesters in aqueous buffer, human plasma and pig liver homogenate.

Materials and Methods

Metronidazole was a gift from DAK-Laboratories (Copenhagen, Denmark). Metronidazole monosuccinate (MMS), metronidazole monoglutarate (MMG) and metronidazole monomaleinate (MMM) were prepared according to a method of Vermeersch et al. (1985) with slight modifications. Metronidazole (11.7 mmol) was dissolved in 90 ml of acetonitrile and 11.7 mmol of the individual acid anhydride and 4-dimethylaminopyridine (0.6 mmol) were added. The mixtures were left at ambient temperature for 7 days. The solvent was removed in vacuo and in case of the metronidazole monoesters of succinic acid and glutaric acid the solid residue was recrystallized from ethanol–water resulting in crystals with m.p. of 106–107° C and 104–105° C, respectively. The brown semi-solid residue, obtained after evaporation in vacuo of the metronidazole monomaleinate reaction mixture, was dissolved in 25 ml of hot water. Upon addition of ethanol a brownish precipitate was formed. The decanted aqueous solution was concentrated under reduced pressure and left overnight at 5° C. The precipitate formed was filtered and dried over potassium hydroxide (m.p. 132–133° C). The structures of the metronidazole hemiesters were confirmed by microanalysis, ^{1}H-NMR and IR analysis. The solvents used in the mobile phases were of chromatographic grade. All other chemicals and buffer substances were of analytical or reagent grade.



R:

CO CH_2 CH_2 COOH	(HEMISUCCINATE)
CO CH_2 CH_2 CH_2 COOH	(HEMIGLUTARATE)
CO CH CH_2 COOH	(HEMIMALEINATE)
H	(METRONIDAZOLE)

Formula 1.

Apparatus

High-performance liquid chromatography (HPLC) was done with a Waters Assoc. Model 6000A constant-flow pump equipped with a Waters Assoc. Model 450 variable-wavelength UV detector and a Rheodyne injection valve Model 7125 with a 20 μl loop. Readings of pH were carried out on a Radiometer Type pH M 26 meter at the temperature of study. Infrared spectra were recorded on a Unicam SP 200 spectrophotometer using the potassium chloride disc technique. ^{1}H-NMR spectra were run on a Jeol C-60-HL instrument. Melting points were taken in capillary tubes and are not corrected.

HPLC analysis

The various metronidazole hemiesters and parent metronidazole were determined by using two reversed-phase high-performance liquid chromatographic procedures. The column employed (250 × 4.5 mm) was packed with LiChrosorb RP-8 (7 μm particles) and was equipped with a small pre-column packed with Perisorb RP-8 (30–40 μm particles). For the separation of MMS and MMG from their product of hydrolysis, metronidazole, a mobile phase consisting of methanol–0.05 M citrate buffer pH 2.7 (4 : 6 v/v) was used. The degradation of MMM was followed by using a mobile phase consisting of methanol–0.05 M citrate buffer pH 2.7 (3 : 7 v/v). The latter eluent allowed determination of the 3 hemiesters and metronidazole, simultaneously. Employing this

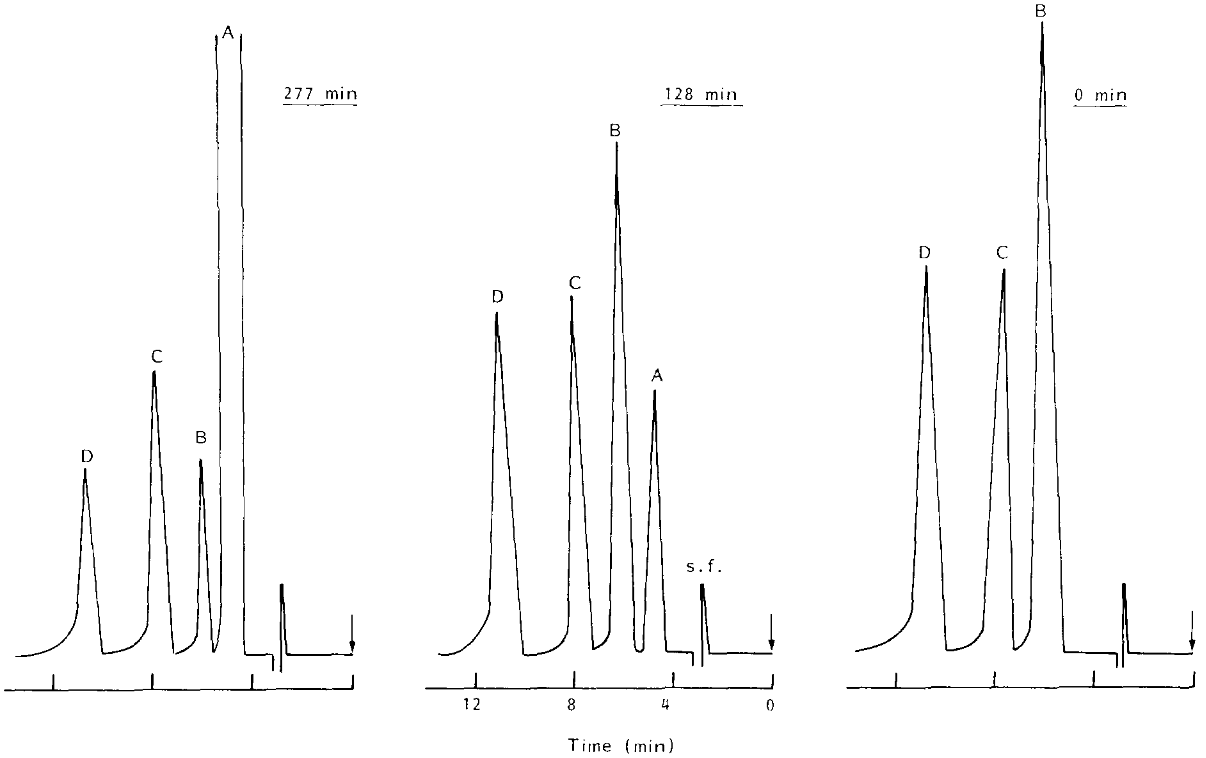


Fig. 1. Simultaneous determination of metronidazole (A), MMM (B), MMS (C) and MMG (D) by HPLC after incubation of the 3 hemiesters in 0.1 N HCl (37 °C).

system the following capacity factors were found: metronidazole (0.73); MMM (1.15); MMS (1.85) and MMG (3.0) (Fig. 1). The flow rate was 1.0 ml · min^{-1} and the effluent was monitored at 320 nm. Quantitation of the various compounds was done from peak height measurements in relation to those of standards chromatographed under the same conditions.

Kinetic measurements

The kinetic studies in aqueous solution were conducted over the pH range 0.1–10.5 employing the following buffers: hydrochloric acid, citrate, acetate, phosphate, borate and carbonate. The various buffers were adjusted to an ionic strength of 0.5 by addition of a calculated amount of potassium chloride. Samples were maintained at 37 ± 0.2 °C in a constant-temperature water bath. For the fast degradation reactions the disappearance of the individual hemiester compound was monitored after adding 50–100 μl of a stock solution of the hemiester in methanol to 10 ml of the appropriate buffer to give an initial concentration of about $7 \cdot 10^{-5}$ M. All buffer solutions were preheated to the temperature of study. At suitable intervals aliquots were withdrawn and analyzed immediately. Whenever convenient, rate constants for the 3 ester derivatives were obtained simultaneously by adding the compounds to the same buffer solution (Fig. 1). Pseudo-first-order rate constants were calculated from the slopes of the logarithm of the concentration of intact hemiester versus time plots using linear regression.

For the slowly proceeding degradation reactions the pH dependent first-order rate constants were determined by monitoring the initial rates of product formation (Connors, 1973). Metronidazole concentrations were monitored versus time up to a total percentage of no more than 2% of the initial reactant concentration. The presence of pseudo-first-order hydrolysis kinetics was established by linear plots of the initial rates of product

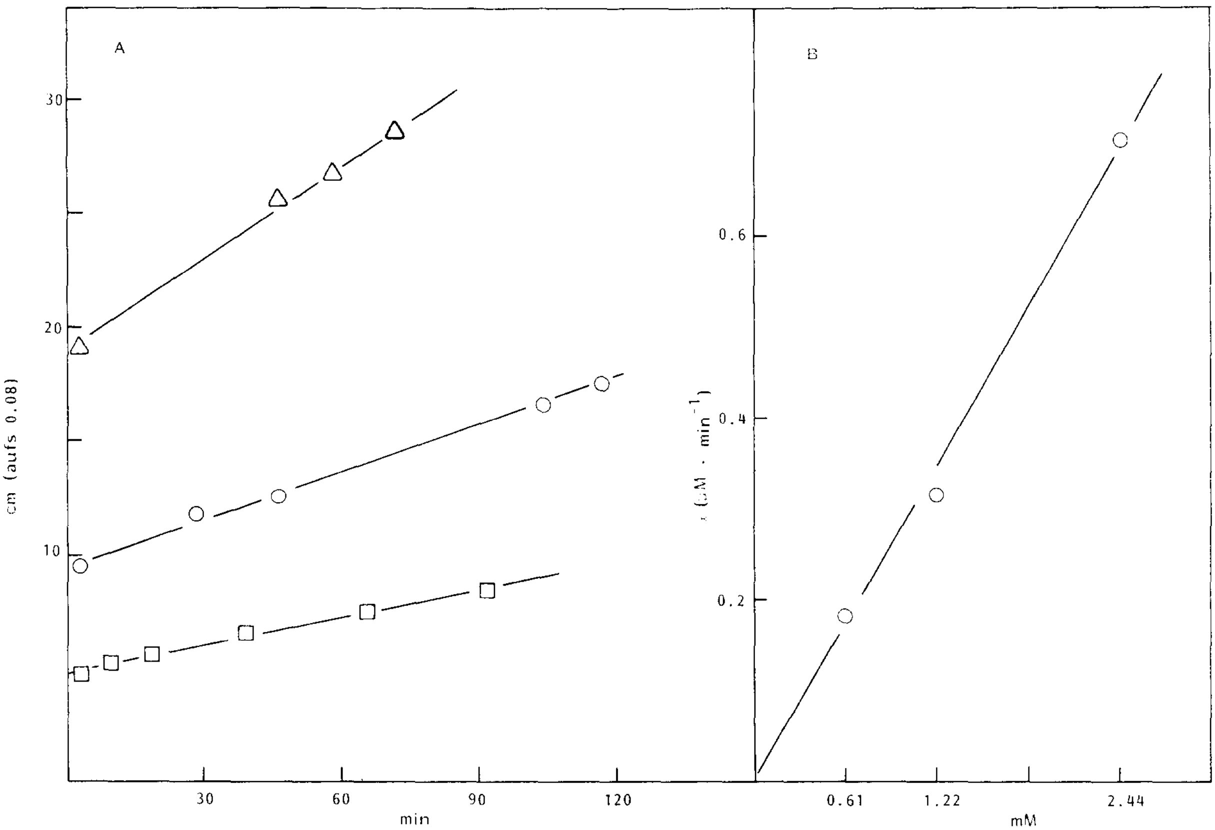


Fig. 2. A: initial rates of formation of metronidazole after incubation of various concentrations of MMM in 0.05 M borate buffer pH 9.45 (37 ° C): □, 0.61 mM; ○, 1.22 mM; and △, 2.44 mM. B: slopes of the lines in Fig. 2A plotted against the reactant concentration.

formation versus the concentration of the ester prodrug (Fig. 2).

Kinetics of hydrolysis of the ester derivatives in 80% human plasma were determined exploiting the initial rate method. Plasma samples maintained at 37 ° C were spiked with the prodrug (initial concentration $5 \cdot 10^{-3}$ M). 500 μl aliquots were withdrawn and deproteinized with 1500 μl of methanol. The mixture was vortexed and after centrifugation (2 min at 10,000 *g*) 20 μl of the clear supernatant was applied to the column. The formation of the parent metronidazole was followed for a time period resulting in an overall degradation of the ester compound of less than 3%. In case of MMG the first-order rate constant was also determined by following the disappearance of the ester prodrug. The rate constants derived by use of the two different methods agree within ±5%.

The stability experiments in pig liver homogenates were performed essentially as described above for the kinetic runs in 80% human plasma. 100 μl portions of a stock solution of the individual prodrug (2.80×10^{-2} M) were added to 10.0 ml liver homogenate, preheated to 37 ° C. The time-dependence of disappearance of the ester was followed by HPLC. The pig liver was homogenized with one volume of 0.9% NaCl. After centrifugation the supernatant was frozen immediately in 2.5 ml centrifuge tubes. Prior to use the homogenate (50% w/v) was diluted with 0.2 M aqueous phosphate buffer pH 7.40 and eventually adjusted to pH 7.40 with a few drops of 0.1 M NaOH. No apparent change in pH of the diluted homogenates was observed during the degradation experiments.

Determination of ionization constants

By potentiometric titration according to Albert and Serjeant (1971) at 37 °C and $\mu = 0.5$ the ionization constants were determined to be: $10^{-3.14}$ (MMM); $10^{-4.58}$ (MMS); $10^{-4.59}$ (MMG)

Results and Discussion

Kinetics of hydrolysis of the three metronidazole hemiesters in aqueous buffer

The kinetics of hydrolysis of the metronidazole monoesters of maleic acid (MMM), succinic acid (MMS) and glutaric acid (MMG) was studied in aqueous buffer solutions over the pH range 0.1–10.5. General acid–base catalysis of hydrolysis by the involved buffer substances was negligible. Under the experimental conditions used the ester compounds hydrolyzed to yield metronidazole quantitatively as evidenced by HPLC analysis. At constant pH and temperature the reactions displayed strict first-order kinetics.

The effect of pH on the rates of hydrolysis of the water-soluble ester prodrugs (37 °C and $\mu = 0.5$) is given in Fig. 3 and Table 1. Regarding MMS and MMG the pH–rate profiles exhibit a typical U-shape characteristic for degradation reactions susceptible to specific acid–base catalysis obeying the general rate law:

$$k_{obs} = k_H a_H + k_0 + k_{OH} a_{OH} \qquad (1)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activity, respectively. k_H and k_{OH} are the second-order rate constants for specific acid and specific base catalysis, respectively. k_0 is the first-order rate constant for spontaneous or water-catalyzed hydrolysis. The activity a_H was calculated in accordance with Harned and Hamer (1933). The respective rate constants are presented in Table 2. The hydrolysis rate profile of MMM deviates from the simple U-shape (Fig. 3). The pH–rate profile shows a narrow pH-independent region between pH 1 and 2, representing the hydrolysis of acid ester. Below pH 1 significant exter-

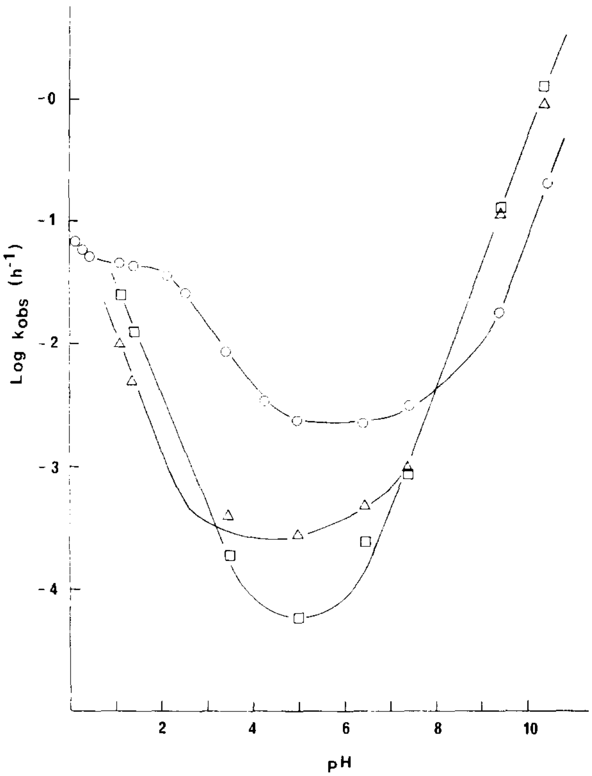


Fig. 3. pH–rate profiles for hydrolysis of MMM (○), MMS (△) and MMG (□) in aqueous solution at 37 °C and $\mu = 0.5$

TABLE 1

First-order rate constants for hydrolysis of the 3 metronidazole hemiesters in aqueous solution ($\mu = 0.5$ and 37 °C)

pH	k_{obs} (h^{-1})		
	Maleinate	Succinate	Glutarate
0.14	6.70×10^{-2}	n.d.	n.d.
0.27	5.96×10^{-2}	n.d.	n.d.
0.44	5.38×10^{-2}	n.d.	n.d.
1.14	4.63×10^{-2}	9.98×10^{-3}	2.58×10^{-2}
1.44	4.37×10^{-2}	5.01×10^{-3}	1.23×10^{-2}
2.14	3.55×10^{-2}	n.d.	n.d.
2.54	2.57×10^{-2}	n.d.	n.d.
3.48	8.64×10^{-3}	3.92×10^{-4}	1.84×10^{-4}
4.27	3.44×10^{-3}	n.d.	n.d.
5.00	2.20×10^{-3}	2.71×10^{-4}	5.90×10^{-5}
6.48	2.18×10^{-3}	4.66×10^{-4}	2.40×10^{-4}
7.41	3.07×10^{-3}	9.78×10^{-4} *	8.52×10^{-4}
9.45	1.77×10^{-2}	1.07×10^{-1}	1.25×10^{-1}
10.48	2.07×10^{-1}	1.08	1.27

n.d. = not determined.

* Previously: 1.09×10^{-3} h^{-1} (Johansen and Larsen, 1984).

TABLE 2

The calculated values of the rate constants given in Eqn. 1 and Eqn. 2 for the hydrolysis of the metronidazole hemiesters in aqueous solution at 37 °C and $\mu = 0.5$

	k_H $(M^{-1} \cdot h^{-1})$	k_{H_2O} $(M^{-1} \cdot h^{-1})$	k_{OH} $(M^{-1} \cdot h^{-1})$
MMM	4.4×10^{-2} [a]	3.9×10^{-5}	2.7×10^{2}
MMS	1.4×10^{-1}	4.9×10^{-6}	1.6×10^{3}
MMG	3.5×10^{-1}	1.1×10^{-6}	1.7×10^{3}

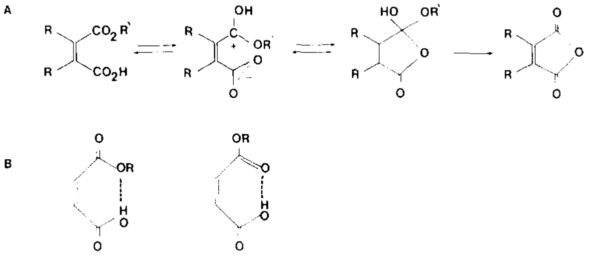
[a] k_{acid} (h^{-1}).

nal acid catalysis of hydrolysis is observed with the slope of the pH–rate profile being close to 0.5.

The pH-dependence of hydrolysis of metronidazole monomaleinate for pH above 1 might be expressed by the rate equation:

$$k_{obs} = k_{acid} \cdot \frac{a_H}{a_H + K_a} + (k_0 + k_{OH} \cdot a_{OH}) \cdot \frac{K_a}{a_H + K_a} \tag{2}$$

where $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of total ester in free acid and anion form, respectively, and K_a is the apparent ionization constant of MMM $(10^{-3.14})$, k_{acid} represents the rate constant for degradation of the acid ester form. The obtained rate constants (Eqn. 2) are shown in Table 2. A similar pH–rate profile has been found for the hydrolysis of methyl hydrogen dialkylmaleates (Aldersley et al., 1974) for pH below 8 and it was suggested, that the dialkylmaleic anhydride was the initial product of hydrolysis of the acid form of the ester (Scheme 1A).



Scheme 1.

Taking into consideration that the efficiency of intramolecular ring closure reactions of dicarboxylic acid monoesters is favored by the presence of α-β alkyl substituents (Bruice and Pandit, 1960) and low pK_a-value of the leaving group (Thanassi and Bruice, 1966; Gaetjens and Morawetz, 1960) another possible mechanism might be operating at low pH for less substituted maleinates. In a study of the hydrolysis of ethyl hydrogen maleinate (Pekkarinen, 1954, 1957) it was observed that the rate of hydrolysis was proportional to the concentration of the undissociated ethyl ester acid at pH < 2. The latter author has ascribed this behaviour to the breakdown of cyclic structures formed by intramolecular hydrogen bonds (Scheme 1B).

Regeneration of metronidazole in plasma

The rates of liberation of metronidazole from the hemiester prodrugs in 80% human plasma are presented in Table 3 together with the rate constants for hydrolysis of the compounds in aqueous buffer pH 7.40. Since the aim of the experiments primarily has been to gain information on the influence of the pro-moiety structures on the relative rates of prodrug bioconversion, the substrate

TABLE 3

Hydrolysis rates of the metronidazole hemiesters in 0.05 M phosphate buffer pH 7.40 and 80% human plasma at 37 °C

	80% Plasma		0.05 M phosphate pH 7.40		$t_{1/2}$ (buffer)
	k_{obs} (h^{-1})	$t_{1/2}$ (h)	k_{obs} (h^{-1})	$t_{1/2}$ (h)	$t_{1/2}$ (plasma)
MMM	2.0×10^{-3}	345	3.1×10^{-3}	226	0.7
MMS	1.1×10^{-3} *	631 *	9.8×10^{-3}	709	1.1
MMG	4.2×10^{-3}	16.4	8.5×10^{-4}	813	50.0

* From a previous study (Johansen and Larsen, 1984).

concentrations employed have been far below K_m thus providing strict first-order release kinetics. It is apparent that MMM and MMS are not substrates for plasma esterases, or preferably ester hydrolases, whereas the conversion of MMG is facilitated by catalysis of plasma enzymes. In general ester derivatives containing a terminal carboxy or sulfonate group are poor substrates for plasma enzymes (Krisch, 1971; Anderson et al., 1985). Therefore the observed 50-fold increase in hydrolysis rate of MMG in plasma compared to aqueous buffer appears interesting. Previous data related to the degradation of hemiester prodrugs in plasma [chlorzoxazone (Johansen and Bundgaard, 1981); metronidazole (Johansen and Larsen, 1984); acetaminophen (Dittert et al., 1968); chloramphenicol (Schmidt and Vömel, 1965); methylprednisolone (Anderson et al., 1985)] along with the results of the present study suggest, that at least 3 factors may influence the susceptibility of such derivatives to undergo plasma enzyme-catalyzed cleavage. First, the distance between the terminal ionized carboxy group and the ester bond to be hydrolyzed is of importance, and it appears that a decrease in chain length of the pro-moiety affords a reduction in cleavage rate. Second, the size of the drug may affect the accessibility of enzymes to the ester bond to be disrupted. Third, we have speculated that also the pK_a value of the drug functional hydroxy group may be a determining factor. The ratio of the half-lives of acetaminophen hemisuccinate (a phenol ester) in aqueous buffer pH 7.4 and 2% human plasma is 60 (Dittert et al., 1968). The corresponding ratio for the monosuccinate ester of *N*-hydroxymethyl chlorzoxazone is close to 2 (Johansen and Bundgaard, 1981). The pK_a value of the *N*-hydroxymethyl compound is 13.1 (Johansen and Bundgaard, 1979). In case of chloramphenicol monosuccinate (Schmidt and Vögel, 1965) and metronidazole monosuccinate (Johansen and Larsen, 1984) it has been observed that the compounds were not subject to plasma enzyme-mediated hydrolysis. The latter substances possess an aliphatic alcoholic group with an expected pK_a value close to that of ethanol, i.e. 15.9 (Ballinger and Long, 1960). The trend, which with caution may be derived from the above-mentioned reports, shows resemblance to the increasing efficiency of intramolecular catalyzed hydrolysis of dicarboxylic hemiesters with decreasing pK_a value of the leaving group (Gaetjens and Morawetz, 1960; Thanassi and Bruice, 1966).

Hydrolysis in pig liver homogenate

The rates of hydrolysis of MMM, MMS and MMG in pig liver homogenate are given in Table 4. Quantitative conversion to metronidazole for the 3 compounds was evidenced by HPLC analysis. Compared to the conversion rates in human plasma a reversed order of reactivity of the ester prodrugs is observed in pig liver homogenate. Metronidazole monosuccinate is approximately 10 times more sensitive towards enzyme-catalyzed cleavage than the two other compounds. Even in this dilute homogenate preparation (5% w/v) liberation of metronidazole from MMS proceeds 3.5×10^3 times faster in comparison to aqueous buffer pH 7.4. Using a fixed substrate concentration (MMG) it is found that the regeneration rate increases linearly with the content of homogenate in the reaction solution. This observation is in accordance with Michaelis-Menten kinetics at low substrate concentrations. By use of purified liver

TABLE 4

Reconversion rates of metronidazole from the corresponding hemiesters in various pig liver homogenates at 37 °C

A fixed substrate concentration (2.8×10^{-4} M) was employed in all the degradation experiments.

Homogenate (% w/v)	k_{obs} (h^{-1})			$t_{1/2}$ (h)		
	MMM	MMS	MMG	MMM	MMS	MMG
5	0.35	3.50	0.30	2.0	0.2	2.3
10	–	–	0.66	–	–	1.1
20	–	–	1.32	–	–	0.53

esterase preparations it has been reported, that hemisuccinate esters were not substrates for the isolated enzymes (Levy and Ocken, 1969; Schöttler and Krisch, 1974). More recent experiments, however, have revealed that the liver of various animals contains enzyme systems capable of splitting steroid hemisuccinate esters (Oshima et al., 1980; Hattori et al., 1981; Ali et al., 1985). Data from the latter studies also indicate the presence of two or more steroid hormone ester hydrolyzing enzymes. The dissimilarity of the patterns of reactivity of MMM, MMS and MMG may suggest that quite different ester hydrolases are operating in plasma and the liver.

References

Albert, A. and Serjeant, E.R., *Determination of Ionization Constants,* 2nd edn., Chapman and Hall, London, 1971.

Aldersley, M.F., Kirbye, A.J. and Lancaster, P.W., Intramolecular displacement of alkoxide ions by the ionised carboxygroup: hydrolysis of alkyl hydrogen dialkylmaleates. *J. Chem. Soc. Perkin Trans. II,* (1974) 1504–1510.

Ali, B., Kaur, S., James, E.C. and Parmar, S.S. Identification and characterization of hepatic carboxylesterases hydrolyzing hydrocortisone esters. *Biochem. Pharmacol.,* 34 (1985) 1881–1886.

Anderson, B.D. and Taphouse, V., Initial rate studies of hydrolysis and acyl migration in methylprednisolone 21-hemisuccinate and 17-hemisuccinate. *J. Pharm. Sci.,* 70 (1981) 181–186.

Anderson, B.D., Conradi, R.A. and Lambert, W.J., Carboxyl group catalysis of acyl transfer reactions in corticosteroid 17- and 21-monoesters. *J. Pharm. Sci.,* 73 (1984) 604–611.

Anderson, B.D., Conradi, R.A., Spilman, C.H. and Forbes, A.D., Strategies in the design of water-stable, water-soluble prodrugs III: Influence of the pro-moiety on the bioconversion of 21-esters of corticosteroids. *J. Pharm. Sci.,* 74 (1985) 382–387.

Antal, E.J., Wright, C.E., Gillespie, W.R. and Albert, K.S., Influence of route of administration on the pharmacokinetics of methylprednisolone. *J. Pharmacokin. Biopharm.,* 11 (1983) 561–576.

Ballinger, P. and Long, F.A., Acid ionization constants of alcohols II. Acidities of some substituted methanols and related compounds. *J. Am. Chem. Soc.,* 82 (1960) 795–798.

Brent, D.A. Chandrasurin, P., Ragouzeos, A., Hurlbert, B.S. and Burke, J.T., Rearrangement of chloramphenicol-3-monosuccinate. *J. Pharm. Sci.,* 69 (1980) 906–908.

Bruice, T.C. and Pandit, U.K., The effect of geminal substitution ring size and rotamer distribution on the intramolecular nucleophilic catalysis of the hydrolysis of monophenyl esters of dibasic acids and the solvolysis of the intermediate anhydrides. *J. Am. Chem. Soc.,* 82 (1960) 5858–5865.

Burke, J.T., Wargin, W.A., Sheretz, R.J., Sanders, K.L., Blum, M.R. and Sarubbi, F.A., Pharmacokinetics of intravenous chloramphenicol sodium succinate in adult patients with normal renal and hepatic function. *J. Pharmacokin. Biopharm.,* 10 (1982) 601–614.

Connors, K.A., *Reaction Mechanisms of Organic Analytical Chemistry*, Wiley, New York, 1973, pp. 41–110.

Derendorf, H., Rohdewald, P., Möllmann, H., Rehder, J., Barth, J. and Neveling, D., Pharmacokinetics of prednisolone after high doses of prednisolone hemisuccinate. *Biopharm. Drug Disp.,* 6 (1985a) 423–432.

Derendorf, H., Möllmann, H., Rohdewald, P., Rehder, J. and Schmidt, E.W., Kinetics of methylprednisolone and its hemisuccinate ester. *Clin. Pharmacol. Ther.,* 37 (1985b) 502–507.

Dittert, L.W., Caldwell, H.C., Adams, H.J., Irvin, G.M. and Swintosky, J.V., Acetaminophen prodrugs 1, Synthesis, physicochemical properties and analgesic activity. *J. Pharm. Sci.,* 57 (1968) 774–780.

Gaetjens, E. and Morawetz, H., Intramolecular carboxylate attack on ester groups. The hydrolysis of substituted phenyl acid succinates and phenyl acid glutarates. *J. Am. Chem. Soc.,* 82 (1960) 5328–5335.

Garceau, Y., Davis, I. and Hasegawa, J., Plasma propranolol levels in beagle dogs after administration of propranolol hemisuccinate ester. *J. Pharm. Sci.,* 67 (1978) 1360–1363.

Garrett, E.R., Prediction of stability in pharmaceutical preparations X. Alkaline hydrolysis of hydrocortisone hemisuccinate. *J. Pharm. Sci.,* 51 (1962a) 445–455.

Garrett, E.R., Solvolysis of 21-hydrocortisone esters and hemiesters. *J. Med. Pharm. Chem.,* 5 (1962b) 112–133.

Harned, H.S. and Hamer, W.J., The ionization constant of water and the dissociation of water in potassium chloride solutions from electromotive forces of cells without liquid junction. *J. Am. Chem. Soc.,* 55 (1933) 2194–2206.

Hattori, K., Kamio, M., Nakajima, E., Oshima, T., Satoh, T. and Kitagawa, H., Characterization of steroid hormone ester hydrolyzing enzymes in liver microsomes. *Biochem. Pharmacol.,* 30 (1981) 2051–2056.

Higuchi, T., Takechi, H., Pitman, I.H. and Fung, H.L., Intramolecular bifunctional facilitation in complex molecules. Combined nucleophilic and general acid participation in hydrolysis of hexachlorophene monosuccinate. *J. Am. Chem. Soc.* 93 (1971) 539–540.

Johansen, M. and Bundgaard, H., Prodrugs as drug delivery systems XVI. Novel water-soluble pro-drug types for chlorzoxazone by esterification of the N-hydroxymethyl derivative. *Arch. Pharm. Chemi Sci. Edn.,* 9 (1981) 43–54.

Johansen, M. and Larsen, C., Stability and kinetics of hydrolysis of metronidazole monosuccinate in aqueous solution and in plasma. *Int. J. Pharm.,* 21 (1984) 201–209.

Johansen, M. and Bundgaard, H., Pro-drugs as drug delivery systems VI. Kinetics and mechanism of the decomposition of N-hydroxymethylated amides and imides in aqueous solution and assessment of their suitability as possible pro-drugs. *Arch. Pharm. Chem. Sci. Edn.,* 7 (1979) 175–192.

Kauffmann, R.E., Miceli, J.N., Strebel, L., Buckley, J.A., Done, A.K. and Dajarii, A.S., Pharmacokinetics of chloramphenicol succinate in infants and children. *J. Pediatr.*, 98 (1981) 315–320.

Kramer, W.G., Rensimer, E.R., Ericsson, C.D. and Pickering, L.K., Comparative bioavailability of intravenous and oral chloramphenicol in adults. *J. Clin. Pharmacol.*, 24 (1984) 181–186.

Krisch, K., Carboxylic ester hydrolases. In Boyer, P.D. (Ed.), *The Enzymes, Vol. 5*, 3rd edn., Academic, New York, 1971, pp. 43–69.

Larsen, C. and Johansen, M., Macromolecular prodrugs IV. Kinetics of hydrolysis of metronidazole monosuccinate dextran ester conjugates in aqueous solution and in plasma-sequential release of metronidazole from the conjugates at physiological pH. *Int. J. Pharm.*, 35 (1987a) 39–45.

Larsen, C. and Johansen, M., Macromolecular prodrugs V. Simultaneous determination of high-molecular-weight dextran metronidazole monosuccinate ester prodrugs and the hydrolysis products metronidazole and the corresponding monosuccinate ester on Nucleosil Diol. *J. Chromatogr.*, 389 (1987) 227–235.

Larsen, C., Macromolecular prodrugs VII. Hydrolysis of dextran metronidazole monosuccinate ester prodrugs. Evidence for participation of intramolecularly catalyzed hydrolysis of the conjugate metronidazole–succinic acid ester by the neighbouring dextran hydroxy groups. *Acta Pharm. Suec.*, 23 (1986) 279–288.

Levy, M. and Ocken, P.R., Purification and properties of pig liver esterase. *Arch. Biochem. Biophys.*, 135 (1969) 259–264.

Marunaka, T., Shibata, T., Minami, Y., Umeno, Y. and Shindo, T., Simultaneous high-performance liquid chromatographic determination of suxibuzone and its metabolites in plasma and urine. *J. Pharm. Sci.*, 69 (1980) 1258–1261.

Mauger, J.W., Paruta, A.N. and Gerraughty, R.J., Consecutive first-order kinetic consideration of hydrocortisone hemisuccinate. *J. Pharm. Sci.*, 58 (1969) 574–578.

Melby, J.C. and St. Cyr, M., Comparative studies on absorption and metabolic disposal of water-soluble corticosteroid esters. *Metab. Clin. Exp.*, 10 (1961) 75–82.

Nahata, M.C. and Powell, D.A., Bioavailability and clearance of chloramphenicol after intravenous chloramphenicol succinate. *Clin. Pharmacol. Ther.*, 30 (1981) 368–372.

Narang, P.K., Wilder, R., Chatterji, D.C., Yeager, R.L. and Gallelli, J.F., Systemic bioavailability and pharmacokinetics of methylprednisolone in patients with rheumatoid arthritis following "high-dose" pulse administration. *Biopharm. Drug Disp.*, 4 (1983) 233–248.

Oshima, T., Nakajima, E. and Hattori, K., Hydrolysis of methylprednisolone hemisuccinate to methylprednisolone by a nonspecific carboxylesterase. *Pharmacol. Res. Commun.*, 12 (1980) 139–146.

Pekkarinen, L., A kinetic study of the hydrolysis of some cis-trans isomeric aliphatic esters. *Ann. Acad. Sci. Fennicae*, Ser. A.II, No. 62 (1954) 7–77.

Pekkarinen, L., The hydrolysis of alkyl hydrogen maleates and orthophthalates. The effect of the alcohol component and solvent. *Ann. Acad. Sci. Fennicae*, Ser. A.II. No. 85 (1957) 3–31.

Rattie, E.S., Shami, E.G., Dittert, L.W. and Swintosky, J.V., Acetaminophen prodrugs III. Hydrolysis of carbonate and carboxylic acid esters in aqueous buffers. *J. Pharm. Sci.*, 59 (1970) 1738–1741.

Salmona, M., Saronio, C., Bianchi, R., Marcucci, F. and Mussini, E., In vitro hydrolysis of oxazepam succinate half-ester by a stereospecific soluble esterase from different animal species. *J. Pharm. Sci.*, 63 (1974) 222–225.

Schmidt, F.H. and Vömel, W., Über die Spaltung von Chloramphenicol-succinat durch tierische und menschliche Gewebe. *Klin. Wochenschr.*, 43 (1965) 535–539.

Schöttler, C. and Krisch, K., Hydrolysis of steroid hormone esters by an unspecific carboxylesterase from pig liver chromosomes. *Biochem. Pharmacol.*, 23 (1974) 2867–2875.

Thanassi, J.W. and Bruice, T.C., Neighbouring carboxyl group participation in the hydrolysis of monoesters of phthalic acid. The dependence of mechanisms on leaving group tendencies. *J. Am. Chem. Soc.*, 88 (1966) 747–752.

Vermeersch, J., Vandoorne, F., Permentier, D. and Schacht, E., Macromolecular prodrugs of metronidazole 1, Esterification of hydroxyl containing polymers with metronidazole monosuccinate. *Bull. Soc. Chim. Belg.*, 94 (1985) 591–596.

Wargin, W.A. and Wurster, D.E., Determination of the rearrangement kinetics of chloramphenicol-3-monosuccinate using an automated HPLC system. *Int. J. Pharm.*, 15 (1983) 37–48.

Yasuda, Y., Shindo, T., Mitani, N., Ishida, N., Oono, F. and Kageyama, T., Comparison of the absorption, excretion and metabolism of suxibuzone and phenylbutazone in humans. *J. Pharm. Sci.*, 71 (1982) 565–572.

Yamamoto, R., Fujisawa, S. and Kawamura, M., Pharmaceutic studies on water-soluble corticosteroid derivatives 1. Stability of hydrocortisone 21-hemiesters in solution. *Yakugaku Zasshi*, 91 (1971) 855–862.