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Prodrugs as drug delivery systems XXVI. Preparation and enzymatic hydrolysis of various water-soluble amino acid esters of metronidazole

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Summary

Eight amino acid esters of metronidazole were synthesized and evaluated as potential water-soluble prodrugs with the aim of developing preparations suitable for intravenous injection. Hydrochloride salts of all the esters exhibited a water-solubility greater than 20%. Rates of hydrolysis of the esters at 37°C in 80% human plasma were studied. Complete reversion to metronidazole was observed as determined by HPLC and in all cases the hydrolysis followed strict first-order kinetics. The susceptibility of the various esters to undergo enzymatic hydrolysis varied widely, the observed half-lives in 80% human plasma being 8 min (N-propylglycinate), 12 min (N,N-dimethylglycinate), 30 min (4-morpholinoacetate), 41 min (glycinate), 46 min (3-dimethylaminopropionate), 207 min (3-aminopropionate), 334 min (4-dimethylaminobutyrate) and 523 min (4-methyl-1-piperazinoacetate). It was concluded that due to its facile enzymatic conversion in plasma or blood, excellent solubility properties (> 50% w/v) and ease of synthesis and purification, the hydrochloride of metronidazole N,N-dimethylglycinate appears to be the most promising prodrug candidate as a parenteral delivery form of metronidazole.

Introduction

Metronidazole (I) is a widely used drug for the prevention and treatment of infections caused by anaerobic bacteria. Although the drug is usually administered

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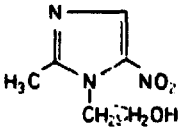
orally intravenous infusion providing rapid onset of action is often required. Parenteral dosage forms for a simple injection are not available, presumably because of the relatively low solubility of metronidazole in water (~ 1% w/v at 25°C). The intravenous administration of the drug is presently performed in the form of infusion, using 0.5% w/v aqueous solutions. To meet the required doses it is usually necessary to give 100–200 ml of such solutions every 8 h.

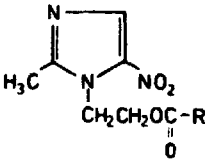
The present study was undertaken to overcome the solubility problem in formulating a parenteral solution to be administered by a single injection, using the prodrug approach. The alcoholic functional group in metronidazole is readily esterifiable and, therefore, it appeared to be possible to obtain ester derivatives providing the desired improvement in water-solubility and, at the same time, possessing a high susceptibility to undergo enzymatic hydrolysis in the body. Ideally, once the delivery function has been achieved only the parent drug should be present in the systemic circulation.

For some drugs with alcoholic functional groups, hemiesters of dicarboxylic acids like succinic acid have been used as prodrug forms with improved water-solubility properties, e.g. for various corticosteroids (Stella, 1975). In the case of metronidazole, however, the monosuccinate ester was found to be an unsuitable prodrug since it did not cleave rapidly and quantitatively to metronidazole upon parenteral administration (unpublished observation). A similar problem has been described with the monosuccinate ester of chloramphenicol (Burke et al., 1980; Kauffman et al., 1981; Nahata and Powell, 1981). Phosphate esters are freely soluble and stable in vitro at physiological pH 7.4 and in the past, several phosphate ester prodrugs have been used in preparing parenteral dosage forms of various drugs containing an alcoholic functional group (Sinkula and Yalkowsky, 1975). While this work was performed, a report describing metronidazole phosphate as a water-soluble prodrug appeared (Cho et al., 1982). Although such an ester possibly may turn out to be a clinically useful prodrug the data published indicate a rather slow rate of conversion of the ester to metronidazole in vivo. Furthermore, the bioavailability of the metronidazole following administration of the phosphate ester in rats was significantly lower than that obtained with metronidazole at the same dosage level, indicating the excretion of some unchanged phosphate ester.

A third type of water-soluble ester derivative is esters with an ionizable amino function in the acid portion. Although various amino acid esters have previously been proposed as water-soluble bioreversible derivatives of drugs containing an alcoholic hydroxy group such as oxazepam and lorazepam (Nudelman et al., 1974), hydrocortisone (Kawamura et al., 1971), bucetin (Kigasawa et al., 1979) and a pyridazin-3-one derivative (Fogt et al., 1980), only sparse information is available on the enzymatic hydrolysis of such esters. In a recent study (Johansen and Bundgaard, 1981) we observed a facile hydrolysis of the N,N-dimethylglycine ester of N-(hydroxymethyl)chlorzoxazone in human plasma. Therefore, it was decided to synthesize a series of amino acid esters of metronidazole and to evaluate their potentiality as water-soluble prodrug forms for metronidazole. It was hoped that a comparative study of the rates of enzymatic hydrolysis of these esters would also be of more general value, e.g. in the design of amino acid ester prodrugs of other drug substances.

In this paper the synthesis of 8 amino acid esters of metronidazole (II–IV) is described along with the results of a study of their rates of hydrolysis in plasma or blood. In the following paper (Bundgaard et al., 1984) studies pertinent to the chemical stability and bioavailability of a selected prodrug candidate (compound II) are reported.





II: R = $-CH_2N(CH_3)_2$

III: R = $-CH_2NH_2$

IV: R = $-CH_2NH(CH_2CH_2CH_3)$

V: R = $-CH_2CH_2NH_2$

VI: R = $-CH_2CH_2N(CH_3)_2$

VII: R = $-CH_2CH_2CH_2N(CH_3)_2$

VIII: R = $-CH_2$-morpholino

IX: R = $-CH_2$-N-N-CH_3

Materials and Methods

Apparatus

High-performance liquid chromatography (HPLC) was done with a Spectra-Physics Model 3500 B instrument equipped with a variable-wavelength UV detector (8-μl 1 cm flow cells) and a 10-μl loop injection valve. Readings of pH were carried out on a Radiometer Type pH M 26 meter at the temperature of study. Infrared spectra were run 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 using tetramethylsilane as an internal standard. Melting points were taken in capillary tubes and are not corrected.

Synthesis of metronidazole esters

Preparation of intermediates

Metronidazole chloroacetate. To a stirred suspension of metronidazole (171.2 g, 1.0 mol) in a mixture of 91.0 g (1.15 mol) of pyridine and 1500 ml of methylene chloride was added chloroacetyl chloride (130 g, 1.15 mol) within 1.5 h and thereupon, the mixture was refluxed for 1 h. The resultant solution was washed with 3 × 500 ml of water, dried over sodium sulphate and evaporated to dryness under reduced pressure. Upon addition of 200 ml of ether while stirring, the residue

crystallized. Recrystallization from aqueous ethanol yielded 195.1 g (79%) of the title compound (m.p. 70–72°C).

Metronidazole bromoacetate. Bromoacetyl bromide (222.0 g, 1.1 mol) was added dropwise, while stirring and cooling to a mixture of metronidazole (171.2 g, 1.0 mol) in pyridine (87.0 g, 1.1 mol) and 1500 ml of methylene chloride. The suspension was stirred at room temperature for 3 h, filtered, washed with 3 × 600 ml of water, dried over sodium sulphate and evaporated in vacuo. The residue was dissolved in 300 ml of methanol and this solution was added dropwise to 1200 ml of ice-water with efficient stirring. After standing at 5°C for 20 h the mixture was filtered, giving 217.1 g (74%) of the title compound (m.p. 66–69°C).

Metronidazole iodoacetate. A mixture of metronidazole chloroacetate (37.2 g, 0.15 mol), potassium iodide (31.1 g, 0.19 mol) and acetone (700 ml) was refluxed for 20 h and then concentrated to about 1/4 in vacuo. After dilution with 450 ml of methylene chloride and filtration, the solution was washed with an aqueous sodium thiosulphate solution (7%, 150 ml) and water (150 ml). The organic phase was dried over magnesium sulphate and evaporated in vacuo. Upon addition of 40 ml of ether the residue crystallized. Recrystallization from ethanol–water afforded 27.0 g (53%) of metronidazole iodoacetate (m.p. 68–69°C).

The ^{1}H-NMR spectra (DMSO-d_6) of these compounds were in agreement with their structures.

Metronidazole N,N-dimethylglycinate hydrochloride (II)

Dicyclohexylcarbodiimide (26.6 g, 0.13 mol), N,N-dimethylglycine (12.4 g, 0.12 mol) and metronidazole (17.1 g, 0.10 mol) were added to 200 ml of dry pyridine. After stirring at room temperature for 4 days the mixture was filtered and evaporated to dryness under reduced pressure. The residue was suspended in 300 ml of ethyl acetate, cooled to 5°C and filtered. To the filtrate were added dropwise and under stirring 35 ml of 3 N hydrogen chloride in ethyl acetate. After standing for 1 h at 5°C, the precipitate formed (II) was filtered, washed with ethyl acetate and recrystallized from 2-methoxyethanol. Yield: 13.6 g (46%); m.p. 210–211°C. Analysis—calculated for $C_{10}H_{17}ClN_4O_4$: C, 41.03; H, 5.85; Cl, 12.11; N, 19.14%. Found: C, 41.00; H, 5.96; Cl, 12.12; N, 19.26%. ^{1}H-NMR (D_2O) δ: 2.88 (3H, s, $CC\underline{H}_3$); 3.33 (6H, s, $N(C\underline{H}_3)_2$); 4.55 (2H, s, $OOCC\underline{H}_2$); 4.9–5.2 (4H, m, $C\underline{H}_2C\underline{H}_2$); 8.32 (1H, s, C$\underline{H}$).

The compound was also prepared according to the following procedure. Dimethylamine (38 g, 0.84 mol), cooled to −30°C, was added while stirring to an ice-cooled solution of metronidazole bromoacetate (116.8 g, 0.40 mol) in dry acetone (750 ml). The mixture was stirred at 5°C for 3 h. The dimethylammonium bromide formed was filtered and the filtrate evaporated in vacuo. The residue was dissolved in 400 ml of methylene chloride and the solution washed with 3 × 150 ml of water, dried over magnesium sulphate and evaporated in vacuo. To a solution of the residue in 700 ml of ethyl acetate was added dropwise while stirring and cooling 3.3 M hydrogen chloride in ethyl acetate (123 ml), the stirring being continued for 1 h at 5°C after all had been added. The crystalline compound formed was filtered, washed with ethyl acetate and recrystallized from methanol–ethyl acetate–ether. Yield: 85.5 g (73%), m.p. 210–211°C.

Metronidazole glycinate hydrochloride (III)

Metronidazole (8.6 g, 0.05 mol), N-tert-butoxycarbonylglycine (10.5 g, 0.06) and dicyclohexylcarbodiimide (13.4 g, 0.065 mol) were added to 100 ml of dry pyridine. After stirring for 24 h at room temperature the mixture was filtered and evaporated to dryness in vacuo. The residue was suspended in 100 ml of methylene chloride and filtered. The filtrate was washed with a 5% aqueous solution of sodium bicarbonate (2 × 50 ml) and water (50 ml), dried over magnesium sulphate and evaporated in vacuo. The semi-solid residue (metronidazole N-tert-butoxycarbonylglycinate) was dissolved in 50 ml of methylene chloride and 50 ml of trifluoroacetic acid was added dropwise while stirring and cooling. The solution was stirred at room temperature for 30 min and then evaporated in vacuo. To a solution of the residue in 200 ml of ethyl acetate was added 3.0 N hydrogen chloride in ethyl acetate (17 ml) while stirring and cooling to 5°C. The obtained solid was recrystallized from ethanol–ethyl acetate–ether, giving 5.5 g of metronidazole glycinate hydrochloride. Titration with 0.1 N sodium hydroxide and 0.1 N perchloric acid showed the product to be a mixture of mono- and dihydrochloride salt. ^{1}H-NMR (DMSO-d_6 + D_2O) δ: 2.47 (3H, s), 3.70 (2H, s), 4.3–4.7 (4H, m) and 7.95 (1H, s).

Metronidazole N-propylglycinate hydrochloride (IV)

A mixture of metronidazole iodoacetate (10.2 g, 0.03 mol) *n*-propylamine (3.8 g, 0.065 mol) and 250 ml of dry dioxane was stirred at room temperature for 75 min and then evaporated in vacuo. The residue was taken up in methylene chloride (200 ml) and washed with a saturated sodium chloride solution (3 × 75 ml). The organic phase was dried and evaporated to dryness. To a solution of the residue in 100 ml of ethyl acetate was added 3.0 N hydrogen chloride in ethyl acetate (10 ml) while stirring and cooling. The crystalline precipitate was recrystallized from ethanol–ethyl acetate–ether, giving 5.3 g (58%) of the title compound, m.p. 150–160°C. ^{1}H-NMR (DMSO-d_6 + D_2O + DCl) δ: 1.03 (3H, t), 1.5–2.1 (2H, m), 2.80 (3H, s), 3.07 (2H, t), 4.03 (2H, s), 4.5–5.0 (4H, m) and 8.6 (1H, s).

Metronidazole 3-aminopropionate hydrochloride (V)

This compound was obtained from metronidazole (8.6 g, 0.05 mol), N-tert-butoxycarbonyl-β-alanine (11.4 g, 0.06 mol) and dicyclohexylcarbodiimide (13.4 g, 0.065 mol) using the procedure described for the preparation of III. Recrystallization from ethanol–ethyl acetate–ether yielded 3.6 g. The product was shown to be a mixture of mono- and dihydrochloride salt. ^{1}H-NMR (DMSO-d_6 + D_2O) δ: 2.5–3.0 (4H, m), 2.63 (3H, s), 4.45 (2H, t), 4.72 (2H, t) and 8.50 (1H, s).

Metronidazole 3-dimethylaminopropionate hydrochloride (VI)

The title compound was obtained by condensing metronidazole (5.1 g, 0.030 mol) and 3-dimethylaminopropionic acid (4.2 g, 0.036 mol) with dicyclohexylcarbodiimide (8.0 g, 0.039 mol) in pyridine as described for the preparation of II. Recrystallization from ethanol–ethyl acetate–ether yielded 3.0 g (33%), m.p. 163–164°C. ^{1}H-NMR (DMSO-d_6) δ: 2.47 (3H, s), 2.6–3.3 (4H, m), 2.67 (6H, s), 4.57 (t) and 4.48 (t) (4H), 7.93 (1H, s) and 11.3 (1H, b).

Metronidazole 4-dimethylaminobutyrate hydrochloride (VII)

This compound was prepared by a procedure similar to the one described in the synthesis of II, using metronidazole (11.1 g, 0.065 mol), 4-dimethylaminobutyric acid (10.2 g, 0.078 mol) and dicyclohexylcarbodiimide (17.4 g, 0.085 mol). The product obtained was recrystallized from ethanol–ethyl acetate–ether, giving 5.6 g (27%), m.p. 127–132°C. ^{1}H-NMR (DMSO-d_6 + D_2O) δ: 1.5–2.2 (2H, m), 2.40 (t) and 2.48 (s) (5H), 2.75 (6H, s), 3.03 (2H, t), 4.40 (t) and 4.58 (t) (4H) and 7.98 (1H, s).

Metronidazole 4-morpholinoacetate (VIII)

Morpholine (40,0 g 0.46 mol) was added to a solution of metronidazole chloroacetate (24.8 g, 0.10 mol) in 500 ml of dry dioxane. The mixture was stirred at room temperature for 28 h, filtered and evaporated in vacuo. The residue was taken up in 200 ml of methylene chloride, washed with a saturated sodium chloride solution (3 × 100 ml), dried over sodium sulphate and evaporated to dryness. The residue was recrystallized from cyclohexane, giving 19.7 g (66%) of the title compound (m.p. 77–78°C). ^{1}H-NMR ($CDCl_3$) δ: 2.3–2.7 (m) and 2.52 (s) (7H), 3.13 (2H, s), 3.5–3.8 (4H, m), 4.45 (t) and 4.58 (t) (4H) and 7.88 (1H, s).

Metronidazole 4-methyl-1-piperazinoacetate (IX)

This compound was prepared by a procedure similar to the one described in the synthesis of VIII, using metronidazole chloroacetate (40.0 g, 0.16 mol) and 1-methylpiperazine (73.1 g, 0.73 mol). The product obtained was recrystallized from cyclohexane, giving 41.2 g (82%) (m.p. 96–99°C). ^{1}H-NMR ($CDCl_3$) δ: 2.25 (3H, s), 2.3–2.7 (m) and 2.52 (s) (11H), 3.15 (2H, s), 4.45 (t) and 4.60 (t) (4H) and 7.92 (1H, s).

Analysis of metronidazole by HPLC

The product of hydrolysis of the various esters, metronidazole, was determined by using a reversed-phase high-performance liquid chromatographic procedure. A column, 25 × 4.5 cm, packed with LiChrosorb RP-8 (7 μm particles) and equipped with a small pre-column was eluted with a solvent system consisting of methanol–0.005 M acetate buffer pH 4.5 (4 : 6 v/v). The flow rate was 1.6 ml · min^{-1} and the column effluent was monitored at 315 nm. Under these conditions metronidazole had an elution time of 2.5 min while the various ester derivatives were retained on the column for more than 20 min. Quantitation of metronidazole was done from measurement of the peak height in relation to that of a standard chromatographed under the same conditions.

Kinetic measurements

The conversion of the esters to metronidazole in 0.05 M phosphate buffer, pH 7.40, containing 80% plasma obtained from human subjects or various animals was studied at 37°C. The reactions were initiated by adding an aqueous or ethanolic solution of the esters to the pre-equilibrated plasma solutions to give an initial concentration of about 0.2 mg · ml^{-1}. At appropriate intervals 200 μl samples were withdrawn and added to 1000 μl of ethanol in order to deproteinize the plasma.

After mixing and centrifugation, 10 µl of the clear supernatant was injected on HPLC and analyzed for free metronidazole. Pseudo-first-order rate constants were calculated from the slopes of linear plots of log ($M_\infty - M_t$) against time, where M_∞ and M_t are the metronidazole concentrations at infinity and at time t, respectively.

The hydrolysis of compound II was also studied in undiluted heparinized human blood, the conditions being identical to those described for the plasma solutions. Blood samples were removed at various times and immediately centrifuged for 2 min at 15,000 × *g*. The plasma fraction thus obtained was assayed for metronidazole by HPLC.

Non-enzymatic rates of hydrolysis were determined in 0.05 M phosphate buffer solutions (pH 7.4) at 37°C. The initial ester concentration was about 0.02 mg · ml^{-1} and the reaction progress was followed by measuring the amount of metronidazole formed using the HPLC method described above.

Results and Discussion

Eight amino acid esters of metronidazole were synthesized by standard procedures as indicated in the experimental section. The synthesis involved either condensation of the amino acid (free or protected with the N-tert-butoxycarbonyl group) with metronidazole in the presence of dicyclohexylcarbodiimide or reaction of an amine with an α-halogenacetate ester of metronidazole. All compounds were found to conform to the indicated structures as evidenced by proton nuclear magnetic resonance and infrared spectroscopy as well as by elemental analysis (of compound II). The purity of the esters was assessed by HPLC and TLC. For some compounds small amounts (< 0.1%) of metronidazole were detected. The esters VIII and IX were isolated in their free base forms but it was found that both form crystalline salts with hydrochloric acid, fumaric acid or maleic acid.

Hydrochloric acid salts or other acid addition salts of the amino ester derivatives showed, as expected, high solubilities in water. For all compounds the water-solubility of the HCl-salts was higher than 20% w/v. The hydrochloride salt of the N,N-dimethylglycinate ester (II) was especially very soluble (> 50% w/v at 20°C), the pH of 1–20% w/v solutions being 4.4–4.6.

Enzymatic hydrolysis of metronidazole esters

For the esters II–IX to behave as true prodrugs of metronidazole, they must undergo cleavage to metronidazole under in vivo conditions. Therefore, the rates of hydrolysis of the compounds were studied in human plasma solutions at 37°C to give indications as to their in vivo behaviour. At initial concentrations ranging from 0.05 to 0.3 mg · ml^{-1} all the esters underwent complete hydrolysis as indicated by the quantitative formation of metronidazole, and in all cases the hydrolysis exhibited strict first-order kinetics for more than 3 half-lives as shown in Fig. 1 for the N,N-dimethylglycinate ester (II). The half-lives for the hydrolysis in 80% human plasma solutions are given in Table 1 along with the corresponding data for hydrolysis in pure buffer solutions at similar pH and temperature.

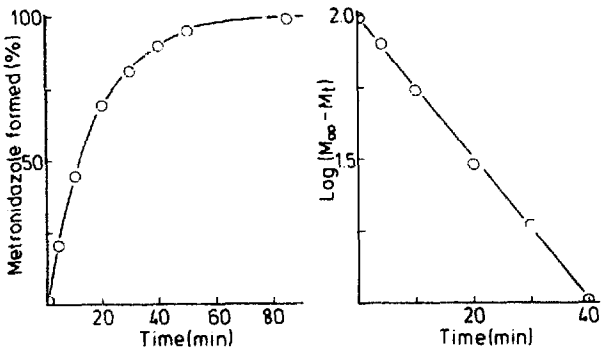


Fig. 1. Plots showing the first-order kinetically formation of metronidazole by hydrolysis of the N,N-dimethylglycinate ester II in 80% human plasma.

Inspection of the rate data in Table 1 shows clearly that the susceptibility of the various amino acid esters to undergo enzymatic hydrolysis varies widely. The esters derived from amino-substituted propionic and butyric acid (V, VI and VII) shows only a small acceleration in the cleavage rate in the presence of plasma and likewise, the hydrolysis of the 4-methyl-1-piperazino acetate ester is catalyzed to only a minor extent. A reasonably facile cleavage in plasma is observed for esters II, IV and VIII and consequently, these compounds appear to be suitable candidates as prodrugs of metronidazole. For the sake of comparison it is of interest to note that the half-life for the hydrolysis of the benzoate ester of metronidazole in 80% human plasma is 3.5 min (Bundgaard et al., 1983).

The hydrolysis of the ester II was also determined in whole human blood. At an initial concentration of 0.2 mg · ml^{-1} a first-order kinetic and complete conversion to metronidazole with a half-life of 9.5 min was observed. This is close to the half-life observed in plasma and thus, no significant loss of enzyme activity towards the metronidazole ester occurs during the separation of the plasma from the whole blood. It was also found that the enzyme activity towards the ester does not vary

TABLE 1

HALF-LIVES FOR THE HYDROLYSIS OF THE METRONIDAZOLE ESTERS II–IX TO METRONIDAZOLE IN 80% HUMAN PLASMA (pH 7.4) AND 0.05 M PHOSPHATE BUFFER pH 7.40 AT 37°C

Ester	$t_{1/2}$ in human plasma (min)	$t_{1/2}$ in buffer (min)
N,N-Dimethylglycinate (II)	12	250
Glycinate (III)	41	115
N-Propylglycinate (IV)	8	90
3-Aminopropionate (V)	207	315
3-Dimethylaminopropionate (VI)	46	52
4-Dimethylaminobutyrate (VII)	334	580
4-Morpholinoacetate (VIII)	30	1880
4-Methyl-1-piperazinoacetate (IX)	523	1720

TABLE 2

HALF-LIVES FOR THE HYDROLYSIS OF METRONIDAZOLE N,N-DIMETHYLGLYCINATE (II) IN 80% PLASMA FROM VARIOUS SPECIES (AT 37°C)

Species	$t_{1/2}$ (min)
Human	12
Dog	25
Rat	20
Mouse	1.9
Rabbit	2.6
Guinea pig	2.9

significantly between individuals. Thus, the half-lives of hydrolysis in plasma obtained from 3 individuals were found to be identical to within ±15%.

Before initiating studies in animals on, e.g., bioavailability and toxicity of a prodrug ester candidate it was of interest to compare the cleavage rate in plasma from various species with that observed in human plasma. The results of such an examination using the ester II are given in Table 2. The differences in the relative cleavage rates indicate that the overall esterase activity present in plasma varies between the animal species which is consistent with several previous findings involving other types of esters (e.g. Dittert et al., 1968; Daehne et al., 1971; Dow and Benedetti, 1982).

Apparently, no comparative study of the influence of the structure of the acid portion of amino acid esters of aliphatic hydroxyl group containing drugs or other compounds on the rates of hydrolysis in plasma or blood has previously been described. Therefore, the data presented for the various metronidazole esters may possibly be of more general interest, e.g. in the design of similar water-soluble derivatives of other drug substances. A priori, one should expect a similar relative enzymatic lability for the same series of esters with the alcohol function being derived from compounds other than metronidazole. A testing of this prediction is currently being carried out in our laboratories. It may be of interest to note a recent study of the enzymatic hydrolysis of various amino acid derivatives of benzocaine (Slojkowska et al., 1982). The nature of the amino acid moiety in these amides had a marked influence on the rates of hydrolysis in plasma or in various types of tissue. In contrast to the present results concerning esters, the N,N-dimethylglycyl derivative of benzocaine was found to be a very poor substrate for the hydrolyzing enzymes and it was hydrolyzed at slower rates than the corresponding glycyl derivative. The highest rate of hydrolysis was observed with DL-leucylbenzocaine.

Selection of an appropriate prodrug candidate

Since the aim of the present prodrug approach was to overcome the solubility problem in formulating parenteral solutions of metronidazole, an important property of the prodrug should be the capability to undergo rapid conversion to the parent drug following parenteral administration. Whereas all the esters prepared

provided the desired solubility properties, the esters II and IV appear to be the most promising prodrug candidates as regards rate of reversion to metronidazole under conditions similar to those encountered in vivo (cf. Table 1). In the selection of the optimal prodrug derivative, several other criteria should also be considered, such as the in vitro stability in bulk form and in aqueous solution, toxicity of the prodrug as well as of the pro-moiety relea ed from the derivative, and ease of synthesis and purification. On the basis of these criteria the N,N-dimethylglycinate ester II (hydrochloride salt) was chosen as the potentially most suitable prodrug of metronidazole and accordingly, it was subjected to more extensive testing including examination of its stability in aqueous solution and bioavailability following parenteral administration. The results of such studies are reported in the accompanying paper (Bundgaard et al., 1984).

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