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Prodrugs as drug delivery systems XXVII. Chemical stability and bioavailability of a water-soluble prodrug of metronidazole for parenteral administration

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Summary

The hydrochloride salt of the N,N-dimethylglycine ester of metronidazole was in a previous report identificd as a prodrug candidate as a parenteral delivery form of metronidazole. In this study, the bioavailability of metronidazole from the ester after intravenous administration was evaluated in beagle dogs and, furthermore, the kinetics of degradation of the ester in aqueous solution was examined. The animal experiments showed that the ester is rapidly and completely converted to metronidazole after intravenous injection of a 13% w/v aqueous solution and that the metabolism and elimination pattern of the parent drug remain similar to that following administration of metronidazole per se. The kinetics of degradation was studied in aqueous solution over the pH range 1–10. The ester hydrolyzed quantitatively to metronidazole with maximum stability at pHs < 4. It would not be suitably stable for formulation as a ready-to-use solution but its stability at pH ~ 4.5 (corresponding to the pH of 5–20% w/v solutions) is compatible with its use as a formulation to be reconstituted as a solution within several hours prior to use.

Introduction

In the foregoing study (Bundgaard et al., 1984) 8 amino acid esters of metronidazole were prepared and evaluated as water-soluble prodrugs with the purpose of

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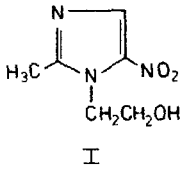
developing preparations suitable for intravenous injection. Due to its facile cleavage to metronidazole (I) in plasma, excellent solubility properties (> 50% w/v in water), and ease of synthesis and purification, the hydrochloride salt of metronidazole N,N-dimethylglycinate (II) appeared to be the most promising prodrug candidate as a parenteral delivery form of metronidazole. The present study was undertaken to evaluate the bioavailability of metronidazole from the ester II following intravenous administration and to provide information on the chemical stability of the ester in aqueous solution as a function of, e.g., pH and temperature. Beagle dogs were chosen for the bioavailability study since the previous study (Bundgaard et al., 1984) showed the rate of hydrolysis of the ester to be of the same magnitude in plasma derived from such animals and from humans.



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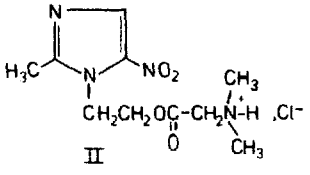


II

Materials and Methods

Kinetic measurements

The study of the chemical stability of the ester II was performed in aqueous buffer solutions at constant temperature. Hydrochloric acid, formate, acetate, phosphate, borate and carbonate were used as buffers: a constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

The hydrolysis of the ester was generally monitored by measuring the amount of metronidazole formed using the HPLC method described in the previous paper (Bundgaard et al., 1983). Most reactions were followed to completion, the initial ester concentration being about 0.02 $mg \cdot ml^{-1}$. 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. For slower reactions, the initial rate method was used. In these cases, an accurately weighed quantity of the metronidazole ester salt was dissolved in the buffer solution and metronidazole production was followed until 3–5% of the reaction was complete. In a few cases the reaction of the ester II was also followed by monitoring the disappearance of the ester using the HPLC method for analysis of metronidazole, but using a solvent system consisting of methanol–0.005 M acetate buffer, pH 4.5 (85 : 15 v/v). The rate constants obtained using these various procedures at similar reaction conditions agreed within ±5%.

Bioavailability studies in dogs

Two beagles weighing 11 and 12 kg were used in a cross-over study to compare the bioavailability of the ester II with that of metronidazole following intravenous

administration. The dogs received either 10 mg/kg of metronidazole in the form of a 0.5% w/v aqueous solution (~ 2 ml/kg) or metronidazole N,N-dimethylglycinate hydrochloride (52 mg/kg) in the form of a 13% w/v aqueous solution (~ 0.4 ml/kg). This dosis of the ester salt is equivalent to 30 mg/kg of metronidazole. The dosis given of metronidazole was lower than of the ester in order to avoid the administration of a large volume of injection solution. The intravenous injection was carried out in less than 1 min. After drug administration blood samples were withdrawn at appropriate times for up to 2 h and assayed as described below. A one-week period was allowed for the cross-over.

Assay of blood samples

Since one of the purposes of the animal experiments was to determine the kinetics of disappearance of the intact ester II it was necessary to use conditions which minimized the enzymatic hydrolysis of the ester during sample preparation and assay. The following procedure was found to be satisfactory. The venous blood samples (2–3 ml) taken from the dogs were withdrawn into ice-cooled tubes containing 30 μl of a heparin solution (~ 150 I.E.) and 60 mg of sodium fluoride. After mixing the cooled blood samples were centrifuged within 20 min. A 200 μl sample of the plasma obtained was deproteinized with 2500 μl of acetonitrile. After centrifugation, 2000 μl of the supernatant was evaporated to dryness at 20°C under a current of nitrogen. The residue was finally dissolved in 200 μl of the appropriate HPLC eluent and injected on HPLC.

For the analysis of metronidazole and its metabolites a modification of the procedure described by Hackett and Dusci (1979) was used. The column, packed with a stationary phase of Spherisorb C_{18} (5 μm particles) was eluted at 37°C with a mobile phase consisting of methanol–0.02 M acetate buffer, pH 4.0 (30 : 70 v/v), the eluent being monitored by UV absorbance at 313 nm. For analysis of intact ester II, a solvent system consisting of methanol–0.02 M acetate buffer, pH 4.0 (90 : 10 v/v) was used. The limits of detection were 0.2 $\mu g \cdot ml^{-1}$ of metronidazole and its metabolites and 0.03 $\mu g \cdot ml^{-1}$ of ester II.

Results and Discussion

Kinetics and mechanism of hydrolysis of the N,N-dimethylglycinate ester II

The kinetics of hydrolysis of the N,N-dimethylglycinate ester (II) of metronidazole was studied in aqueous buffer solutions over the pH range 1–10.3. Under the experimental conditions used II hydrolyzed to yield metronidazole quantitatively as evidenced by the HPLC analysis. At constan pH and temperature the reactions displayed strict first-order kinetics.

The rates of hydrolysis were subject to a slight catalysis by most of the buffer substances used to maintain constant pH. Plots of the observed pseudo-first-order rate constants at each pH value (k_{obs}) against the total buffer concentration were linear over at least 3 buffer concentrations. Values of the buffer-independent first-order rate constant (k_{hyd}) were obtained from the intercepts of such linear plots.

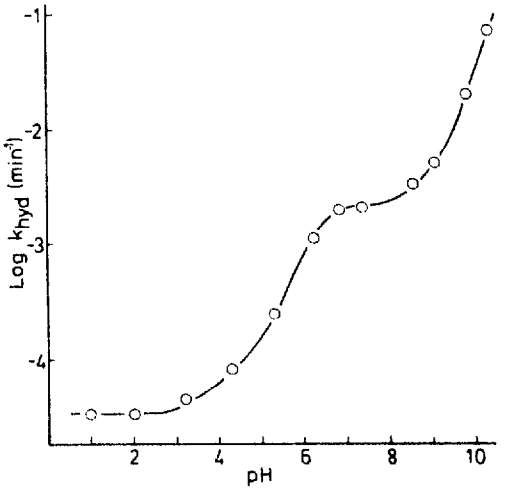
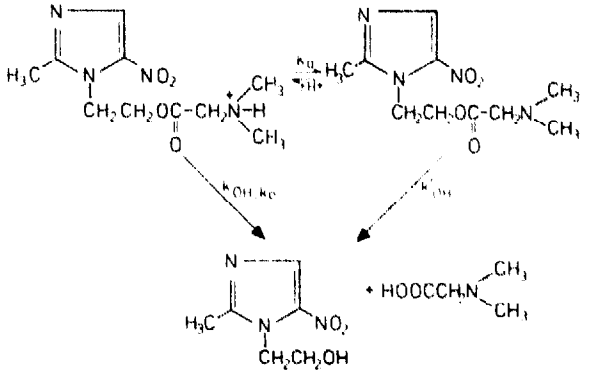


Fig 1. The pH–rate profile for the hydrolysis of the ester II at 37°C and $\mu = 0.5$ in aqueous solutions.

The effect of pH on the rates of hydrolysis at 37°C is shown in Fig. 1 in which the logarithm of k_{hyd} has been plotted against pH. At pH > 5 the pH–rate profile shows two linear segments with slopes of unity with a plateauing occurring between pH 7 and 9; at low pH the rate of hydrolysis becomes independent of pH. This pattern indicates that the free base and the protonated forms of the tertiary amino ester undergo hydrolysis with different rates and that the hydrolysis can be described in terms of specific base-catalyzed reactions of these species along with a spontaneous (pH-independent) reaction of the protonated ester (Scheme 1):

$$k_{hyd} = k_0 \frac{a_H}{a_H + K_a} + k_{OH} a_{OH} \frac{a_H}{a_H + K_a} + k'_{OH} a_{OH} \frac{K_a}{a_H + K_a} \qquad (1)$$

where a_{OH} and a_H refer to the hydroxide ion and hydrogen ion activity, respectively, $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of total ester in the protonated and free base form, respectively, and K_a is the apparent ionization constant of the dimethylammonium group in the ester. The rate constant k_0 refers to the pH-inde-



Scheme 1

pendent hydrolysis of the protonated form of the ester (equal to k_{hyd} at pHs < 2.5) while k_{OH} and k'_{OH} are the second-order rate constants for the apparent attack of hydroxide ion on the protonated and unprotonated ester species, respectively. Values of the latter constants were determined from the straight line portions of the pH–rate profile; the hydroxide ion activity was calculated from the measured pH at 37°C as described previously (Bundgaard et al., 1979). The line drawn for the pH–rate profile in Fig. 1 was constructed from Eqn. 1 and the following rate and ionization constants (37°C and $\mu = 0.5$):

$k_0 = 3.3 \times 10^{-5}\ min^{-1}$;

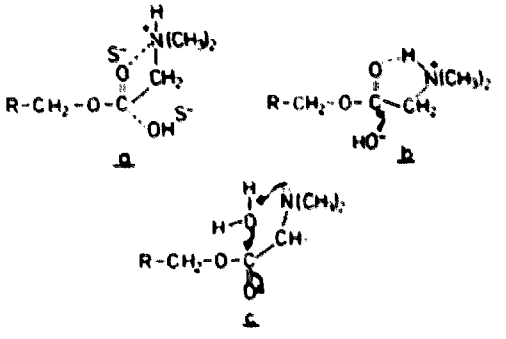
$k_{OH} = 5.4 \times 10^{4}\ M^{-1} \cdot min^{-1}$;

$k'_{OH} = 140\ M^{-1} \cdot min^{-1}$;

$K_a = 10^{-6.5}$.

By potentiometric titration at 37°C and $\mu = 0.5$ the pK_a of the ester was determined to be 6.55 which compares well with the kinetically obtained value. At 23°C a pK_a value of 6.80 was found.

It appears from the values of k_{OH} and k'_{OH} that the ester with a protonated dimethylamino moiety possesses an almost 400-fold greater susceptibility to undergo hydrolysis as compared to the free base form. Such rate differences have previously been observed for various amino acid esters of aliphatic alcohols or phenols (Wolfenden, 1963; Hay et al., 1966; Pilbrant, 1969; Hay and Morris, 1972; Kirby and Lloyd, 1976; Johansen and Bundgaard, 1981; Kovach et al., 1981; Caswell et al., 1981) and the increased rate of reaction of the protonated ester may be attributed to the positively charged nitrogen atom being situated in such a way that electrostatic interactions with the developing negative charge on the carbonyl oxygen atom may occur and cause stabilization of the transition state (a) (Scheme 2). However, a mechanism involving intramolecular general acid catalysis by the protonated dimethylamino group of the nucleophilic attack of hydroxide ions (b) has also to be considered. A kinetic equivalent of either of these mechanisms is intramolecular general base catalysis by the unprotonated dimethylamino group of



Scheme 2

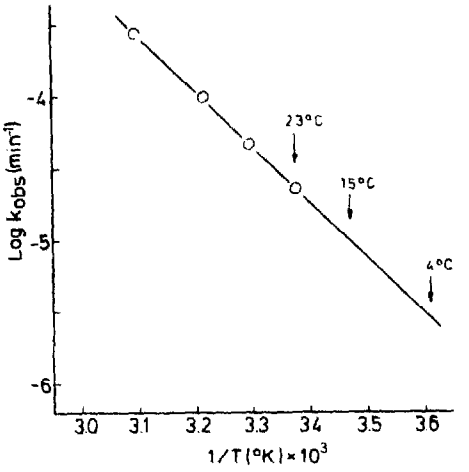


Fig. 2. Arrhenius plot for the hydrolysis of II to metronidazole in 0.05 M acetate buffer, pH 4.4, $\mu = 0.5$.

attack of water on the ester group (c). Such a mechanism has been suggested to be involved in the hydrolysis of various phenyl esters of 3-dimethylaminopropionic acid and of *p*-nitrophenyl-2-dimethylaminobenzoate (Kirby and Lloyd, 1976). The present kinetic data make it impossible to distinguish between these kinetically equivalent mechanisms. More than likely the major reactions taking place at pH < 2.5 and pH > 9 involve water attack on the protonated ester and hydroxide ion attack on the unprotonated ester, respectively.

The imidazole ring in metronidazole is a weak base, the pK_a value being 2.6 (Cho et al., 1982). Esterification of the hydroxyl group is not expected to change this pK_a value and since no break is observed in the pH–rate profile for the ester II at pH 2–3, protonation of the imidazole ring has apparently no significant influence on the rate of ester hydrolysis.

Solubility and stability of compound II

The hydrochloride salt of the N,N-dimethylglycinate ester of metronidazole displayed a high aqueous solubility. The solubility in water was found to exceed 50% w/v or 1.7 M at 20°C, the pH of 1–20% w/v solutions being 4.4–4.6. The solubility of metronidazole in aqueous solutions of pH > 3 and at 25°C is 1% w/v or 0.058 M (Cho et al., 1982).

The effect of temperature on the rate of hydrolysis of the ester II was studied in 0.05 M acetate buffer of pH 4.5 over the range 23–50°C. From the slope of a plot of the logarithm of the observed pseudo-first-order rate constants against the reciprocal of absolute temperature (Fig. 2) an activation energy of 17.7 kcal · mol^{-1} was calculated. From this Arrhenius-type plot the stability of aqueous solutions of ester II may be predicted at various temperatures. Thus, the times in which 10% of the ester have degraded ($t_{10\%}$) are found to be 73 h at 23°C, 166 h at 15°C and 24 days at 4°C. These values are for a pH of 4.4 which corresponds to the value of aqueous solutions of the hydrochloride salt of II.

In considering the stability of concentrated solutions of the ester prodrug it may

be important to recognize not only the loss of the ester per se but also the gradual formation of metronidazole and its subsequent precipitation as the saturation solubility of the drug is reached. The formation of a saturated solution of metronidazole will be a function of the initial concentration of the ester ($[II]_0$) so the initial rate of formation of metronidazole (M) would be:

$$\left(\frac{d[M]}{dt}\right)_i = k[II]_0 \quad (2)$$

where k is the observed pseudo-first-order rate constant for the hydrolysis of II to metronidazole under the designated experimental conditions. At pH 4.4–4.6, corresponding to the pH range of 1–30% aqueous solutions of the hydrochloride salt of II, and 23°C, k is approximately 1.4×10^{-3} h^{-1}. The time for metronidazole to potentially begin nucleating from solutions of II hydrochloride salt (t_{pt}) can be calculated from Eqn. 2, knowing that the solubility of metronidazole is 10 $mg \cdot ml^{-1}$. Table 1 gives the calculated zero-order rates of formation of metronidazole as a function of the initial ester concentration at 23°C. Since $t_{10\%}$ for the ester is 73 h at 23°C the results given in Table 1 indicate that the stability of aqueous solutions of the prodrug ester will only be limited by the potential precipitation of metronidazole formed upon hydrolysis in those cases where the concentration of the ester in aqueous solutions exceeds about 20%.

On the basis of the kinetic data, it is apparent that II would not be sufficiently stable for formulation as a ready-to-use solution even at refrigerated temperatures. However, its stability at pH ~ 4.5 is compatible with its use as a formulation to be reconstituted as a solution within several hours prior to use.

Animal studies

To test the prediction that the ester II would be a suitable water-soluble prodrug of metronidazole for parenteral administration, metronidazole and II were given intravenously to two beagle dogs in a cross-over study. Fig. 3 shows the plasma levels of metronidazole, its major metabolite, 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole, as well as of intact ester obtained after administration of the

TABLE 1

APPARENT ZERO-ORDER INITIAL RATES OF FORMATION OF METRONIDAZOLE (M) FROM ITS PRODRUG II AND TIMES FOR M TO BEGIN PRECIPITATION (t_{pt}) FROM SOLUTIONS OF II AS CALCULATED FROM EQN. 2 (AT 23°C)

Concentration of II, hydrochloride salt ($mg \cdot ml^{-1}$)	$(d[M]/dt)_i$ ($mg \cdot ml^{-1} \cdot h^{-1}$)	t_{pt} (h)
50	0.042	238
100	0.084	119
200	0.168	60
300	0.252	40

Fig. 3.

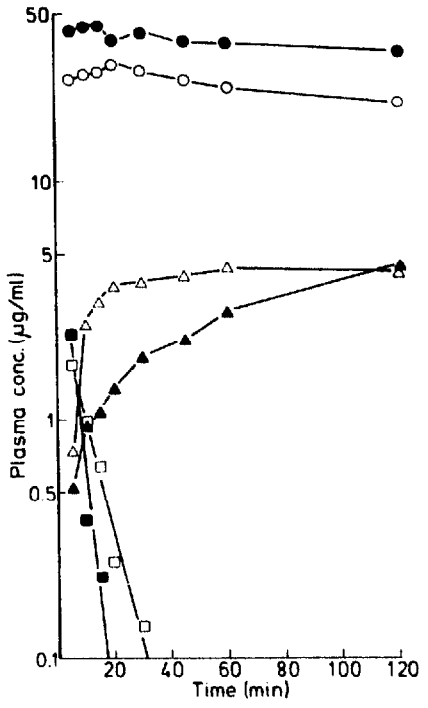


Fig. 4.

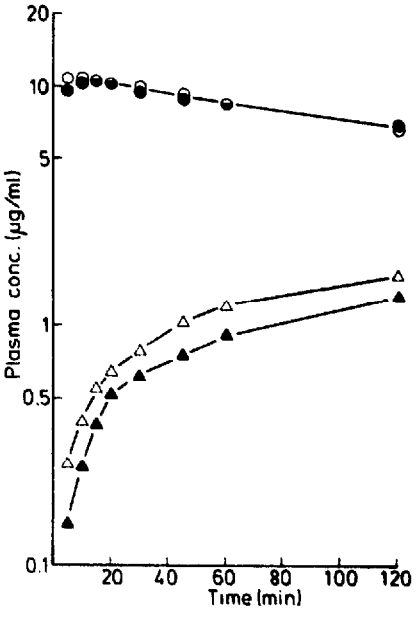


Fig. 3. Plasma concentrations in dog 1 (open symbols) and dog 2 (filled symbols) of metronidazole (circles), 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole (triangles) and intact ester II (squares) after intravenous injection of the ester II at a dose of 30 mg/kg metronidazole equivalents.

Fig. 4. Plasma concentrations in dog 1 (open symbols) and dog 2 (filled symbols) of metronidazole (circles) and 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole (triangles) after intravenous administration of metronidazole at a dose of 10 mg/kg.

hydrochloride salt of ester II. The plasma concentration versus time data observed after administration of metronidazole are shown in Fig. 4. 2-Methyl-5-nitroimidazole-1-acetic acid, another main metabolite of metronidazole (Stambaugh et al., 1968), could not be detected in measurable concentrations in any cases.

The results obtained show that the ester II is hydrolyzed rapidly in the dogs. The rate of disappearance follows good first-order kinetics as seen from Fig. 3, the half-lives being approximately 3 and 7 min in the two dogs. This observed rate of elimination of the ester may reflect the sum of two processes, conversion to the parent drug and loss of prodrug by other routes (Notari, 1981). As noted below, the rapid and quantitative formation of metronidazole seen after administration of the ester indicate, however, that the rate of ester disappearance is solely or predominantly due to the hydrolytic conversion. It was previously found that the half-life of hydrolysis of II in 80% dog plasma at 37°C in vitro is 25 min (Bundgaard et al., 1983). Since the corresponding half-life of hydrolysis in human plasma or blood is 9–12 min (Bundgaard et al., 1984) the ester would probably have an even shorter lifetime in humans than that observed in the dogs.

A comparison of the plasma concentration–time curves for metronidazole indicates that the ester is quantitatively and rapidly hydrolyzed to metronidazole. Besides, the pattern of metabolite formation following administration of the ester is quite similar to that observed after injection of metronidazole. As seen from Fig. 3 nearly maximal plasma concentrations of metronidazole are obtained already after 5 min following the administration of the ester II. The hydroxy metabolite of metronidazole is readily detectable after 5 min. Two hours after the prodrug administration, the concentration of the metabolite corresponds to about 20% of the concentration of metronidazole; the corresponding figure after administration of metronidazole is about 22%.

In conclusion, the animal experiments indicate that the ester II may be a potentially useful prodrug of metronidazole for intravenous administration. It is rapidly and completely converted to metronidazole and the metabolism and elimination pattern of the parent compound appear to be similar to that following administration of metronidazole per se. Thus, it appears that in dogs the prodrug ester and the parent drug may be regarded as nearly indistinguishable from a pharmacokinetic point of view. No signs of pain or local toxicity were observed in the dogs after the intravenous injection of the ester preparation.

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