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The absorption and elimination of metoclopramide in three animal species

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The absorption and elimination of metoclopramide have been studied in the rat, rabbit and dog. Thin-layer chromatography followed by photodensitometry was used for the analysis of the unchanged drug and its metabolites. *N*-De-ethylation is an important Phase I metabolic reaction and conjugation with glucuronic acid and sulphate is a major route of metabolism, particularly in the rabbit. The pharmacokinetic parameters after intravenous administration showed little interspecies variation. First order elimination kinetics with short half-lives and high apparent volumes of distribution ($>1{\cdot}1$ kg^{-1}) were observed. Major interspecies variations were seen after oral administration of high doses of the drug. Metoclopramide was eliminated slowly after oral administration to rats. The findings in the rabbit and in the dog suggest that the liver plays an active role reducing the systemic availability of unchanged metoclopramide after oral administration.

Most of the information on the pharmacokinetics of metoclopramide available in the literature have been obtained with colorimetric techniques for the analysis of the drug in tissues and body fluids (Arita, Hori & others, 1970 a, b; Donatelli, 1971). The procedures used involve diazotization followed by coupling with *N*-(1-naphthyl)-ethylenediammonium dichloride or other reagents and would not distinguish between metoclopramide and related aromatic amines.

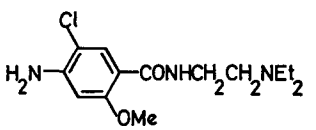
Arita & others (1970a) have demonstrated that two of the metabolites found in rabbit urine are aromatic amines and we have found at least three such metabolites in urine and plasma of animals given metoclopramide. It was therefore decided to reinvestigate the pharmacokinetics of this drug using an analytical method more selective than those used hitherto.

We have used thin-layer chromatography and photodensitometry for separate analysis of metoclopramide and its metabolites. The experiments demonstrate the existence of major interspecies differences in the pharmacokinetics of metoclopramide when the drug is given orally.

METHODS

Compounds

The hydrochloride of metoclopramide was obtained from A.M.S.A., Firenze, Italy, and its identity was confirmed using infrared spectrometry and mass spectrometry. Its purity ($>99\%$) was demonstrated by thin-layer chromatography (solvents 1 and 2, see below) and by gas chromatography of the trimethylsilyl derivative (3% OV-1 on Gas-Chrom Q).



Metoclopramide

Animal experiments

Male albino rats (200–300 g), female albino rabbits (2·0–3·3 kg) and mongrel dogs (11–23 kg) of either sex were used. Before experiments on the absorption of orally administered metoclopramide, all animals were fasted overnight. Rats were treated with a purified diet (Bakke, 1969) for three days, before being used in investigations of the urinary metabolites of metoclopramide.

For oral administration, metoclopramide hydrochloride was dissolved in water and 10 ml kg^{-1} was given by stomach tube. For intravenous administration the drug was dissolved in 0·9% NaCl solution and 1 ml kg^{-1} was injected into the tail vein in the rat, the marginal ear vein of the rabbit and a superficial vein of the forelimbs in the dog. The doses of metoclopramide hydrochloride corresponded to 5, 10 and 15 mg kg^{-1} of the base intravenously and 25, 50, 100 and 150 mg kg^{-1} of the base orally.

Heparinized blood was obtained from the jugular veins of groups of rats which were killed at different times after administration of the drug. In the rabbit, a cannula was inserted into the central artery of the ear, heparin (2000 units) was injected and blood samples were withdrawn at short time intervals. In the dog blood was obtained from a superficial vein of one forelimb through an indwelling heparinized cannula.

Urine was collected from rabbits and rats given metoclopramide hydrochloride orally at a dose corresponding to 100 mg kg^{-1} of the base. The rabbits were killed 4 h after dosing and the urine was obtained by bladder puncture. The rats were kept in metabolic cages and the urine was collected for 24 h following the administration of the drug.

Analytical methods

Metoclopramide was extracted with chloroform (5 ml) from plasma (0·5–1 ml) to which was added 0·1M borate buffer pH 8·0 (1 ml). The extraction was carried out in stoppered tubes shaken for 1 h. The phases were then separated by centrifugation, the chloroform was evaporated in a stream of nitrogen using gentle heat and the extracts taken up in 70 μl of chloroform and spotted on to precoated thin-layer plates (Silica gel 60 F_{254}, Merck, Germany).

The chromatograms were developed in butanol–acetic acid–water (4:1:1) (solvent 1) and scanned using a Vitatron photodensitometer (model TLD 100). Diminution of background fluorescence at 254 nm was measured using a 525 nm filter. The amount of metoclopramide in plasma was obtained from a calibration curve constructed using plasma samples to which known amounts of the drug (0·2–25 μg ml^{-1}) had been added. The variation coefficient obtained by repeated analysis (n = 21) of rabbit plasma to which had been added metoclopramide (5 μg ml^{-1}) was $\pm$ 9·2%. Since authentic reference compounds were not available for the metabolites, quantitative estimation of metabolite I was carried out using the calibration curve for metoclopramide (see Results).

Urine samples (5 ml) were adjusted to approximately pH 5 with 0·2 N hydrochloric acid and 2 ml of 0·1M acetate buffer pH 4·8 was added. After addition of 50 μl of β-glucuronidase/aryl sulphatase (Boehringer, Germany) the samples were incubated for 20 h at 40°. These hydrolysed samples and samples of non-hydrolysed urine were percolated through a column containing Amberlite XAD-2 resin (Rohm and Haas, Philadelphia, U.S.A.).

After washing the resin twice with 10 ml of water the columns were eluted with 15 ml of methanol and the extracts were evaporated to dryness not exceeding 60° using a rotary evaporator. The extracts were taken up in 0·4 ml of methanol and examined by thin-layer chromatography in solvent 1 and in isopropanol–NH_4OH–water (80:4:5) (solvent 2). The R_F value of metoclopramide in solvents 1 and 2 was 0·29 and 0·55, respectively.

Metoclopramide and its metabolites were visualized under ultraviolet light (254 nm) as dark quenching spots and by diazotization followed by coupling with *N*-(1-naphthyl)-ethylenediammonium dichloride. The plates were sprayed with 1% sodium nitrite in 1N HCl and then with 0·4% of the coupling agent in methanol. Strong red to violet colours developed with metoclopramide derivatives which contained aromatic amino groups.

A major metabolite (I) was identified in extract of urine from rats given metoclopramide. Preparative thin-layer chromatography was carried out in solvent 1, the metabolite was eluted from the plates with methanol and purified by chromatography in the solvents 1 and 2. After removing the methanol from the final eluate using a rotary evaporator, the residue was taken up in chloroform and introduced into the mass spectrometer (Hitachi-Perkin-Elmer RMU 6H) through the direct insertion probe.

Calculation of pharmacokinetic parameters

The closeness of fit of the data from the intravenous experiments with a first order elimination process was tested by plotting the results on semilogarithmic plasma concentration-time graphs. The regression line of the logarithm of the concentration of unchanged metoclopramide versus time and the correlation coefficient (r) of linear elimination was calculated by the method of least squares. The apparent volume of distribution (V_d) was calculated by dividing the dose by the extrapolated concentration at zero time (C_o). The half-life of the plasma concentration ($T\frac{1}{2}$) and the elimination contant (K_e) were calculated from the slope of the regression line (Gibaldi, 1971).

RESULTS

Comparison of the chromatograms of extracts of urine and plasma in different solvent systems showed that none of the metabolites interferred with unchanged metoclopramide in solvent 1. When groups of rats were studied the variability of blank plasma obtained from different animals did not permit accurate estimation of concentration below 0·5 μg ml^{-1}. When blank plasma was available from the same animal (rabbit and dog) the limit of detection was approximately 0·2 μg ml^{-1}. The error of analysis ($\pm$9·2%) was obtained with one technician carrying out the entire analytical procedure including extraction, chromatography and measurement of peak areas.

When equimolar amounts of various 2-methoxy-4-amino-5-chlorobenzamides available in this laboratory were analysed by thin-layer-chromatography and photodensitometry it was found that substitution of the diethylaminoethyl group of metoclopramide by other alkyl groups did not alter the response (Segura, unpublished results). Metoclopramide was therefore used as a reference compound for the quantitative analysis of metabolite I which was not available as an authentic standard. The results obtained using this method were confirmed by photodensitometry after diazotization and reaction with the coupling agent.

In the three animal species similar plasma concentration curves were observed after intravenous injection of the drug (Fig. 1). With doses of 10 mg kg^{-1} (rat and dog) and 5, 10 and 15 mg kg^{-1} (rabbit) the concentrations found initially (5–10 min) did not exceed 9 $\mu g\ ml^{-1}$. The amounts decreased rapidly to less than 1 $\mu g\ ml^{-1}$ after 60–120 min. Using this route of administration small quantities of metabolite I at R_F 0·38 (solvent 1) were found only in dog plasma (Fig. 1C).

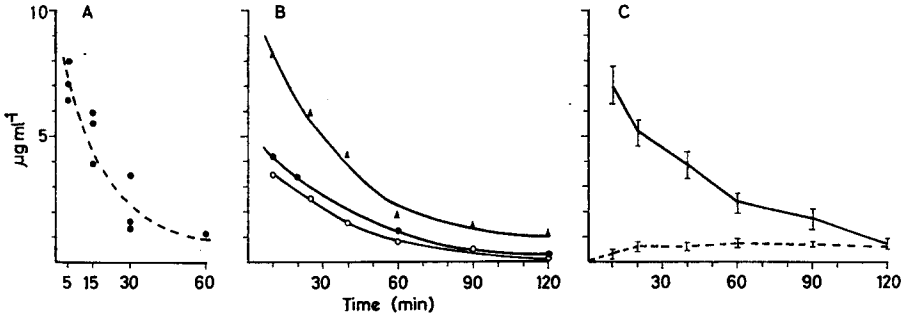


FIG. 1. Concentrations in plasma after intravenous injection of metoclopramide. A: Metoclopramide in plasma from individual rats killed at different times after injection of 10 mg kg^{-1} of the drug. B: Metoclopramide in plasma in rabbits given 5 (○ — ○), 10 (● — ●), and 15 mg kg^{-1} (▲ — ▲) of the drug. C: Metoclopramide (——) and metabolite I (– – –) in plasma after injection of 10 mg kg^{-1} of the drug in the dog. Each point represents the mean concentration of 4 dogs and the vertical bars indicate the standard error of the mean.

When the concentrations of unchanged metoclopramide in plasma after intravenous injection were plotted versus time on semilogarithmic graphs, no consistent deviations from linearity were observed in any of the species. The data fitted the equation for first order elimination kinetics (Gibaldi, 1971) with correlation coefficients in the range of 0·974 to 0·999 (Table 1). The mean half-lives of metoclopramide in plasma in the phase studied were 20·0, 27·9 and 36·1 min in the rat, the rabbit and the dog, respectively. The corresponding values for the apparent volume of distribution were 1·13, 1·46 and 1·27 l kg^{-1}.

Table 1. *Pharmacokinetic parameters for unchanged metoclopramide after intravenous injection in different animal species*

Expt	Body weight	Dose mg kg^{-1}	C_o $\mu g\ ml^{-1}$	V_d l kg^{-1}	K_e h^{-1}	$T\frac{1}{2}$ min	r
Rat (n=10) M	200–300 g	10	7·88	1·13	2·081	20·0	0·975
Rabbit F	2·8 kg	5	4·73	1·06	1·518	27·4	0·992
Rabbit F	2·0 kg	10	5·46	1·83	1·552	26·8	0·999
Rabbit F	3·3 kg	15	10·06	1·49	1·410	29·5	0·976
Rabbit				1·46	1·493	27·9	
(mean ± s.e.m.)				±0·22	±0·042	±0·8	
Dog F	14·4 kg	10	6·45	1·56	1·392	29·9	0·999
Dog M	11·0 kg	10	9·23	1·08	1·426	29·2	0·974
Dog M	15·0 kg	10	9·46	1·06	1·079	38·6	0·981
Dog M	21·0 kg	10	7·28	1·37	0·893	46·6	0·984
Dog			8·11	1·27	1·197	36·1	
(mean ± s.e.m.)			±0·74	±0·12	±0·128	±4·1	

C_o = Extrapolated concentration at zero time. V_d = Apparent volume of distribution. K_e = Elimination constant. $T\frac{1}{2}$ = Half-life of the plasma concentration. r = Correlation coefficient of linear elimination.

Major interspecies differences in the plasma concentration curves were observed after oral administration. In rats given 100 mg kg^{-1} of metoclopramide in solution, concentrations of approximately 5 μg ml^{-1} were found after only 5 min (Fig. 2A) and the highest concentrations were found between 30 min and 3 h. The concentrations had decreased slightly at 5 and 7 h. Metabolite I could be detected in plasma after 5 min and the amount both of this and of two additional metabolites (II and III) increased with time. The R_F values of the metabolites II and III in solvent I were 0·63 and 0·70, respectively.

In the urine of rats given metoclopramide orally, unchanged metoclopramide was found together with conjugates and 3 unconjugated metabolites (I, II and III) which all gave the characteristic reaction of aromatic amines after diazotization and coupling with *N*-(1-naphthyl)ethylenediammonium dichloride. The amounts of these metabolites as well as of metoclopramide increased substantially when the urines were incubated with β-glucuronidase and sulphatase.

The mass spectrum of the major metabolite (I) in rat urine contained a molecular ion at *m/e* 271 as compared to *m/e* 299 with metoclopramide. Common ions at *m/e* 184 and at *m/e* 201 occurred in the spectra of both of the compounds. These large fragments probably arise from cleavage of the amide bond and rupture of the adjacent N–C bond with hydrogen rearrangement.

The most abundant peaks in the spectrum of metclopramide occurred at *m/e* 99 $[CHCH_2N(C_2H_5)_2]^+$ and at *m/e* 86 $[CH_2N(C_2H_5)_2]^+$.

These ions were absent in the spectrum of the metabolite but important peaks appeared at *m/e* 71 and at *m/e* 58, i.e. 28 mass units less than those of the parent compound. The finding that both the molecular ion and the ions arising from the side-chain of the metabolite, are all 28 mass units less than the corresponding fragments in the mass spectrum of metclopramide suggested that metabolite I was the *N*-de-ethylated derivative.

This interpretation was confirmed when the mass spectrum of authentic *N*-(ethylaminoethyl)-2-methoxy-4-amino-5-chlorobenzamide became available, which was identical with that of metabolite I isolated in the present study (A. H. Beckett & G. Huizing, personal communication).

The plasma concentration curves following oral administration of metoclopramide in the rabbit (Fig. 2B) were different from those observed in the rat and in the dog.

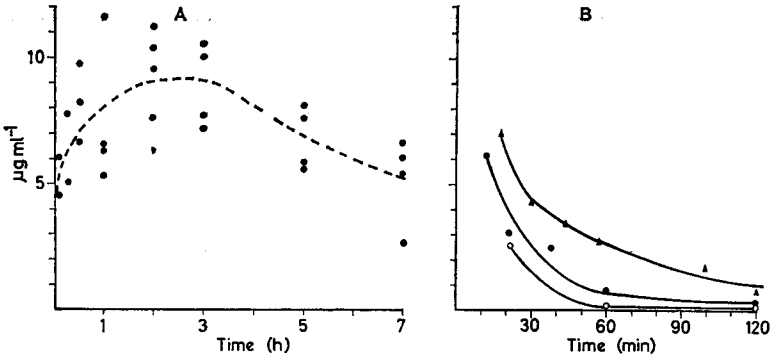


FIG. 2. Concentrations in plasma after oral administration of metoclopramide. A: Metoclopramide in plasma from individual rats killed at different times after giving 100 mg kg^{-1} of the drug by stomach tube. B: Metoclopramide in plasma in rabbits given 50 (○ — ○), 100 (● — ●) and 150 mg kg^{-1} (▲ — ▲) of the drug by stomach tube.

The peak concentrations occurred earlier than 20 min after dosing and the concentration decreased rapidly thereafter. The curves after oral doses of 50, 100 and 150 mg kg^{-1} orally were strikingly similar to those obtained with one tenth these doses given intravenously (Fig. 1B). No metabolites were found in extracts of rabbit plasma. The urine contained large amounts of polar conjugates which were hydrolysed with β-glucuronidase/sulphatase to yield free metoclopramide. Only small quantities of the metabolites I, II and III could be detected after hydrolysis of rabbit urine. The half-lives of the plasma concentration were 17·1 and 45·2 min in the rabbits given 100 and 150 mg kg^{-1}, respectively.

Metoclopramide was rapidly absorbed after its oral administration in aqueous solution to the dog. Peak concentrations occurred earlier than 15 min (Fig. 3) and

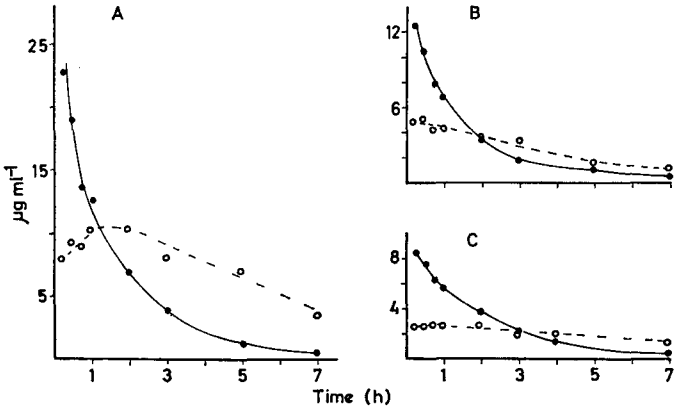


FIG. 3. Concentrations of metoclopramide (● — ●) and of metabolite I (○ - - - ○) in plasma in 3 dogs given different doses of the drug orally. A (F 15·3 kg): 100 mg kg^{-1}; B (F 14·6 kg): 50 mg kg^{-1}; and C (F 23·0 kg): 25 mg kg^{-1}.

the initial plasma concentrations observed in this species were the highest of the three examined. As in the intravenous experiments the disappearance followed first-order kinetics after oral administration. The half-lives of the plasma concentration were 92·2, 76·8 and 68·0 min in the dogs given 25, 50 and 100 mg kg^{-1}, respectively. The corresponding correlation coefficients of linear elimination were 0·990, 0·998 and 0·997.

Chromatography suggested that all of the 3 metabolites found in rat plasma were also present in the plasma of dogs which had received metoclopramide orally (Fig. 4). Of these, only metabolite I attained high concentrations in plasma (Fig. 3). Considerable amounts of this derivative were found 15 min after giving the drug, and its concentration exceeded that of the parent drug after 1 to 3 h. The concentration of the metabolites II and III never exceeded 0·5 μg ml^{-1}.

DISCUSSION

Earlier investigations have shown that metoclopramide is metabolized by *N*-deethylation to yield *N*-(ethylaminoethyl)-2-methoxy-4-amino-5-chlorobenzamide in the rabbit and in the rat (Arita & others, 1970a; see Donatelli, 1971). The R_F values in 4 solvent systems as reported by Arita & others (1970a) for this derivative isolated from rabbit urine, were indistinguishable from those observed with metabolite I from

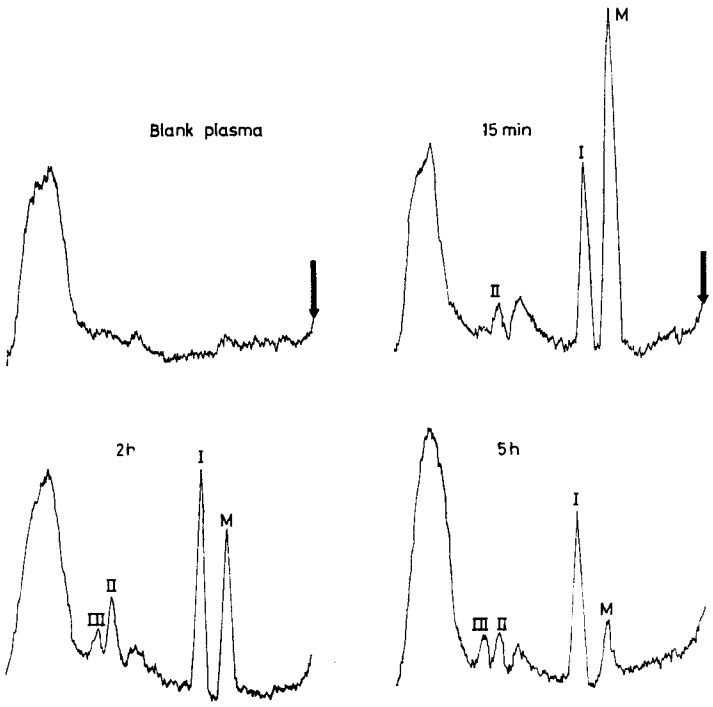


FIG. 4. Scanning of thin-layer chromatograms (solvent 1) of extracts of plasma obtained at various times after an oral dose of 100 mg kg^{-1} of metoclopramide in a dog (F 15·3 kg). M = Metoclopramide; I, II and III = Metabolites (see text).

the rat and the dog in this laboratory (Segura & Bakke, unpublished results). The present study demonstrated that this derivative is a major metabolic product in the latter two species.

The colour reactions obtained with the spray reagent used suggest that there are two additional metabolites which retain the aromatic amino group. The findings also indicate that conjugation is a major pathway, especially in the rabbit. This species excretes large amounts of glucuronides and/or sulphate conjugates of metoclopramide in the urine and 3–5% of an intramuscular dose of 40 mg of the drug appears as conjugates in the bile (Arita & others, 1970b).

With intravenous administration of metoclopramide, the plasma concentrations observed did not reveal any major interspecies variation. In all of the animals a high apparent volume of distribution (>1·1 kg^{-1}) was observed. The half-lives of the plasma concentration were short in all 3 species but the main free metabolite (I) was not detected in plasma in the rat and in the rabbit, and only found in small amounts in dog plasma after intravenous injection.

The rapid appearance of peak concentrations of metoclopramide in plasma after oral administration in the rabbit and in the dog suggests that the drug is well absorbed from the gastrointestinal tract. However, comparison of the areas under the concentration-time curves following intravenous injection in rabbits (Fig. 1B) and after giving 10-fold the intravenous doses orally (Fig. 2B) shows that the systemic availability of metoclopramide administered by the oral route is low in this species. Only approximately 10% of a dose of metoclopramide given orally seems to reach the systemic circulation as the unchanged drug.

In the absence of any indications of incomplete absorption of the drug when given in solution, the most likely cause of the low systemic availability is uptake and metabolism during the first pass of metoclopramide through the gut wall and the liver. As reviewed by Rowland (1973), a number of drugs are now known to exhibit a "first-pass" effect with a high hepatic extraction ratio.

Rapid appearance in the plasma of large amounts of a metabolite after oral administration is another indication that the liver may play an active role in reducing the systemic availability of the unchanged drug (Rowland, 1973). It is noteworthy that metabolite I attained high concentrations in plasma 15 min after oral administration of metoclopramide in the dog. However, the assessment of the extent of first-pass metabolism in this species is complicated by the difference in dose and elimination half-life following oral and intravenous administration.

The mechanism of the slow elimination of metoclopramide from plasma after oral administration to rats is not known. However, in rats given metoclopramide by intramuscular injection the drug is more rapidly eliminated when enterohepatic circulation is prevented by the creation of biliary fistulae (see Donatelli, 1971). Retention is a well known pharmacokinetic effect of enterohepatic recycling which is particularly important in the rat and in the dog (Smith, 1971; Rowland, 1973). After oral administration the liver is flushed with drug during the absorption phase. It is, therefore, conceivable that a larger fraction of metoclopramide enters an enterohepatic circulation when this route is used.

The recommended dose of metoclopramide in adult patients is 10–20 mg regardless of the route of administration. However, no studies have appeared comparing the blood concentration of unchanged metoclopramide and the metabolism of the drug after oral and parenteral administration in man. Due to the considerable interspecies variation, the present findings cannot be extrapolated to other species. A more sensitive method is needed for the analysis of low concentration of unchanged metoclopramide and metabolites in body fluids after therapeutic doses of the drug in man.

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