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ELECTRON-CAPTURE GAS CHROMATOGRAPHIC ASSAY FOR METOCLOPRAMIDE IN PLASMA

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SUMMARY

An original electron-capture gas chromatographic assay has been developed for the quantitation of metoclopramide in human plasma. The method involves derivatization with heptafluorobutyryl imidazole after alkaline extraction, acid backwash, and a further alkaline extraction. Plasma levels of metoclopramide as low as 5 μg/l can be measured using 1 ml of plasma, and no interference from related substances or commonly prescribed drugs has been found.

The percentage recovery of drug from plasma ranges from 88% to virtually 100%, and the between-run variation in the assay is 4.3%.

The assay has been used for the study of metoclopramide pharmacokinetics in man following intravenous single-dose administration. The resultant plasma concentration vs. time curve was biexponential, with a terminal half-life of 5.0 h, and a distribution half-time of 0.3 h.

INTRODUCTION

Metoclopramide [Maxolon, 4-amino-5-chloro-N-(2-diethylaminoethyl)-2-methoxybenzamide] is a potent anti-emetic and antispasmodic agent, structurally related to procainamide. Though metoclopramide has been in use for over ten years, and many clinical trials of the drug have been conducted [1—3], detailed human pharmacokinetic studies of metoclopramide have been hampered by the lack of a sufficiently sensitive and specific assay.

Various spectrophotometric and thin-layer chromatographic assays for the drug are available [4,5]; however, such assays suffer both from a lack of sensitivity in the nanogram range required for human studies and from interference from structurally related compounds.

Recently a mass fragmentographic assay for metoclopramide has been de-

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scribed. However, 5-ml sampling volumes were necessary to achieve adequate sensitivity [6]. A recent high-performance liquid chromatographic assay also requires similar large sampling volumes for the same reason [7].

The present paper describes an original electron-capture gas chromatographic (GC) assay for metoclopramide in plasma which is both sensitive and specific enough for pharmacokinetic studies in man; plasma levels of metoclopramide up to 200 μg/l can be measured using 1 ml of plasma, and no interference from related drugs has been noted. This method was developed independently of the somewhat similar method described by Tam and Axelson [8]. Their technique, though simpler, had already proved insufficiently sensitive in our hands while the present method was being developed. Preliminary pharmacokinetic data obtained with the method are included in this report.

EXPERIMENTAL

Reagents

Metoclopramide hydrochloride was provided by Beecham Research Laboratories (Melbourne, Australia) and maprotiline, the internal standard, by Ciba-Geigy (Sydney, Australia). The chemical structures of these compounds are shown in Fig.1.

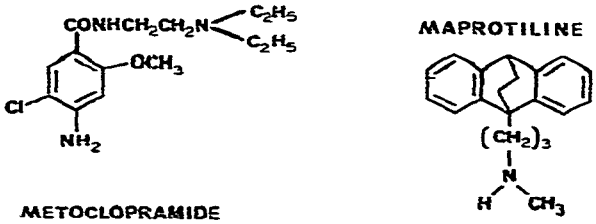

Fig. 1. Chemical structures of metoclopramide and maprotiline, the internal standard.

Stock solutions (1000 μg/l) of both drugs were prepared in absolute methanol and stored at 3—4°C. Chloroform, hexane and methanol were all glass-distilled prior to use. Anhydrous diethyl ether (Mallinckrodt, St. Louis, MO, U.S.A.) was obtained in 450-g cans and used only within 48 h of opening. Bicarbonate—carbonate buffer (pH 10), sodium hydroxide solution (1.0 mol/l) and hydrochloric acid (0.1 mol/l) were prepared in distilled water. The derivatizing reagent, heptafluorobutyryl imidazole (HFBI) Pierce (Rockford, IL, U.S.A.) was obtained in ampoules and stored under nitrogen in glass septum-sealed containers at 4°C after opening. Unused reagent was discarded after 4 days.

Preparation of plasma standards

A 1000 μg/l stock solution of metoclopramide hydrochloride in absolute methanol was prepared and stored at 3—4°C. Appropriate aliquots were removed and transferred to assay tubes. Solvent was removed by gentle evaporation under nitrogen, and immediately, residues were redissolved and equilibrated in 1.0 ml drug-free plasma. These samples were then extracted and assayed by the technique described below. Ten different concentrations of metoclopramide

hydrochloride within the range 5—200 μg/l were used in the preparation of the standard curve. Initial estimates were done in triplicate. The standard curve was checked at least every two weeks using ten single-point estimates over the range 5—200 μg/l. As well, daily checks of the precision and reproducibility of the assay were carried out by including two spiked plasma samples in each group of patient samples being analysed.

Procedure

To a 1.0-ml human plasma sample (spiked or from dosed subjects) in a 20-ml screw-top test-tube were added 250 μl of a 1000 μg/l solution of the internal standard in methanol. 1.0 ml bicarbonate—carbonate buffer (pH 10), and 8.0 ml diethyl ether were also added. The tube was capped and drug and standard were extracted by gentle mixing for 15 min. After centrifugation at 2000 *g* for 4 min, the ether phase was transferred to another screw-cap tube and the aqueous phase re-extracted with a further 8.0 ml ether for 15 min. Following centrifugation, the two ether phases were combined and extracted twice with 2.0 ml 0.1 mol/l hydrochloric acid for 15 min. After centrifugation and subsequent combination of the two aqueous phases in another test-tube, the samples were made alkaline with 1.0 ml sodium hydroxide solution (1.0 mol/l) and extracted into 10.0 ml chloroform for a further 15 min. Layers were separated by centrifugation, and the aqueous phase was discarded, while the organic phase was transferred to a 15-ml glass-stoppered test-tube.

The organic extract was evaporated under nitrogen using gentle heat (50—60°C). To the residue were immediately added 20 μl HFBI, and derivatization proceeded at 75°C for 90 min. After cooling, the sample was alkalinized using 2.0 ml bicarbonate—carbonate buffer (pH 10) and then extracted into 1.0 ml hexane for 2 min.

The hexane phase, after centrifugation, was transferred to a 10-ml centrifuge tube and evaporated to dryness. Immediately prior to chromatography, the residues were reconstituted in 50 μl hexane. A 3—4-μl aliquot was then injected into the gas chromatograph.

Gas chromatographic analysis

The instrument used was a Varian 2700 gas chromatograph fitted with a scandium tritide electron-capture detector. The coiled glass column (1.2 m × 2 mm I.D.) was packed with 3% OV-101 on acid-washed, DMCS treated Gas-Chrom Q (80—100 mesh) (Applied Science Labs., State College, PA, U.S.A.). The column was conditioned at 250°C for 18 h prior to use. Chromatographic conditions were as follows: injector port, foil and oven temperatures were 275°C, 275°C and 200°C respectively; nitrogen carrier gas flow-rate was 37 ml/min; attenuation setting, $128 \cdot 10^{-10}$. Standing current varied between 90% and 50% during the study.

Peak height ratios were calculated by dividing the height of the peak due to metoclopramide by the height of that due to maprotiline. Calibration curves were constructed by plotting peak height ratio as a function of metoclopramide hydrochloride concentration (μg/l of plasma) using known concentrations of metoclopramide hydrochloride in plasma. All assays were carried out in triplicate. Least squares linear regression analysis of the calibration curves was

carried out on a Hewlett-Packard programmable desk calculator. The equation for the regression line was subsequently used to calculate unknown concentrations of metoclopramide hydrochloride in plasma from peak height ratio data.

Derivatization procedure

Aliquots (100 μl) of a 1000 μg/l methanolic solution of metoclopramide hydrochloride were mixed with 250-μl aliquots of a 1000 μg/l solution of maprotiline, and the solvent removed by gentle evaporation. Residues were derivatized with 20 μl HFBI at 75°C for varying time periods up to 5 h, in order to determine the optimal reaction time for derivatization. To stop the reaction instantly, 2 ml bicarbonate—carbonate buffer (pH 10) were added and then the compounds were extracted into hexane. The achievement of optimal conditions was reflected, as will be explained later, in a 1:1 peak height ratio of metoclopramide to maprotiline, as evidenced by GC. Each point in this section of the study was the mean of two determinations.

The derivatives of both metoclopramide and maprotiline were subjected to chemical ionization gas chromatography—mass spectrometry (GC—MS) on a

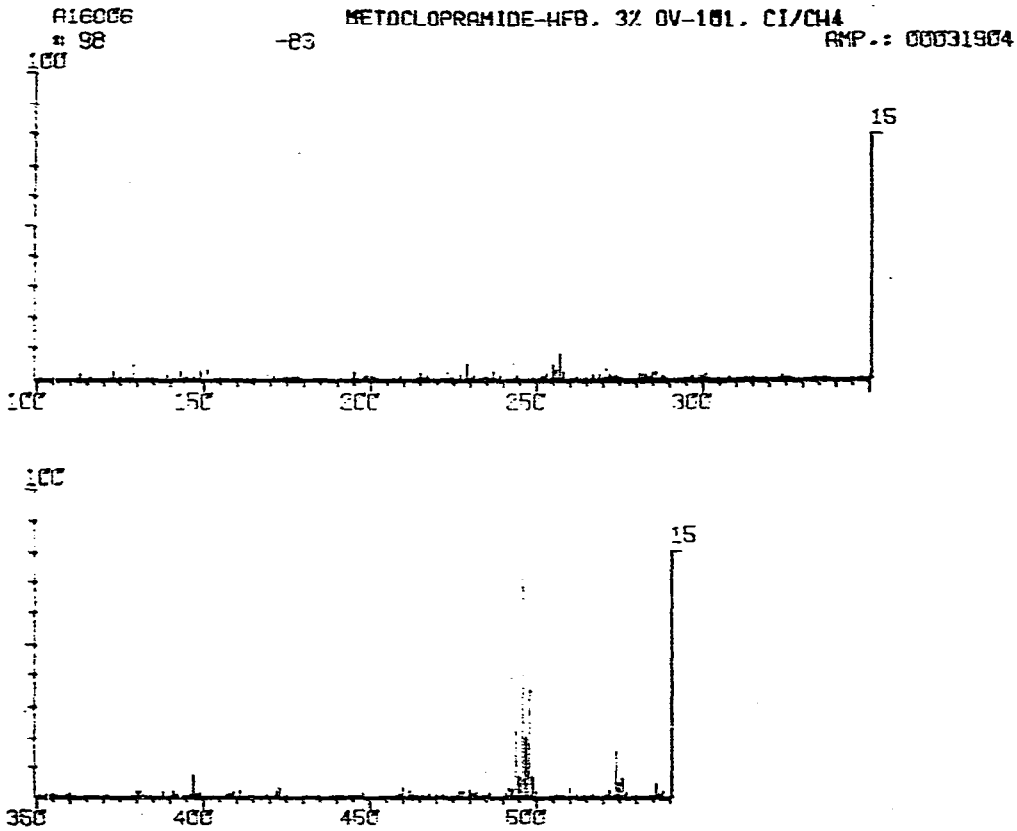

Fig. 2. Chemical ionization mass spectrum of heptafluorobutyric metoclopramide. Carrier gas, methane (20 ml/min); column packing, 3% OV-101 on Gas-Chrom Q (100—120 mesh); oven temperature, 270°C; injector temperature, 250°C; MH^+, *m/e* 496.

Finnigan 3200 gas chromatograph—mass spectrometer and were confirmed as the respective monoheptafluorobutyryl derivatives (Fig. 2).

Precision studies

Samples (1.0 ml) of drug-free human plasma were spiked with appropriate aliquots of a methanolic solution of metoclopramide hydrochloride (1000 μg/l) to give plasma concentrations of 10, 20, 50, 100 and 200 μg/l. Samples were mixed thoroughly and then extracted and assayed as described. Six replicates were analysed at each concentration.

Recovery

Plasma standards of metoclopramide hydrochloride were prepared using appropriate aliquots of a methanolic solution of metoclopramide hydrochloride (1000 μg/l); the solvent was evaporated and the residues reconstituted in 1.0 ml drug-free plasma. These standards were extracted and assayed as described. The plasma standards were then compared with aqueous standards (prepared similarly, with the residues being reconstituted in 1.0 ml distilled water) and with derivatized standards, directly injected (i.e. not extracted). Concentrations of 5, 20, 50, 100, 150 and 200 μg/l were studied in triplicate.

Interference

Over 30 substances, including commonly prescribed drugs, were tested for interference in the assay procedure. Plasma samples (1.0 ml) from treated patients, or plasma samples spiked with therapeutic concentrations of the substances, were extracted and assayed as described above, or methanolic solutions of some compounds at appropriate concentration levels were derivatized with HFBI (after removal of solvent) and chromatographed as described previously.

Pharmacokinetic studies

A 10-mg dose of metoclopramide hydrochloride was administered by intravenous injection over 1 min to a volunteer. The subject, a 37-year old male weighing 67 kg, had been fasting prior to dosing. A PTFE catheter was inserted into an antecubital vein for the collection of blood samples. Twenty 10-ml blood samples were collected at appropriate intervals over a 24-h period, with six samples being taken in the first hour. Samples were centrifuged immediately, the plasma being removed and stored at −20°C prior to analysis.

RESULTS AND DISCUSSION

Acetylation has often proved to be a useful derivatization procedure prior to GC, and with the advent of the fluorinated acylimidazoles as derivatizing agents, the potential applications of such reactions have increased enormously. Several investigators have employed these reagents to facilitate quantitation of phenols, and primary and secondary amines, especially by electron-capture GC [8—10].

Derivatization with HFBI has been used in the present study to quantitate metoclopramide in plasma. The addition of seven fluorine atoms to both metoclopramide and its internal standard, maprotiline, provides excellent sensitivity

for electron-capture GC; as well, the derivatization procedure overcomes problems associated with excessive adsorptive losses on column.

Fig.3 illustrates the time dependency of the derivatization procedure. The relative molar electron-capture response of the derivatives of metoclopramide and maprotiline is such that 100 ng of metoclopramide and 250 ng maprotiline are responsible for a 1:1 peak height ratio upon chromatography. Plateau conditions are reached at 75 min; 90 min was the time chosen for optimal reaction conditions. Monoheptafluorobutyryl derivatives of both the drug and its internal standard are formed (as determined by GC—MS).

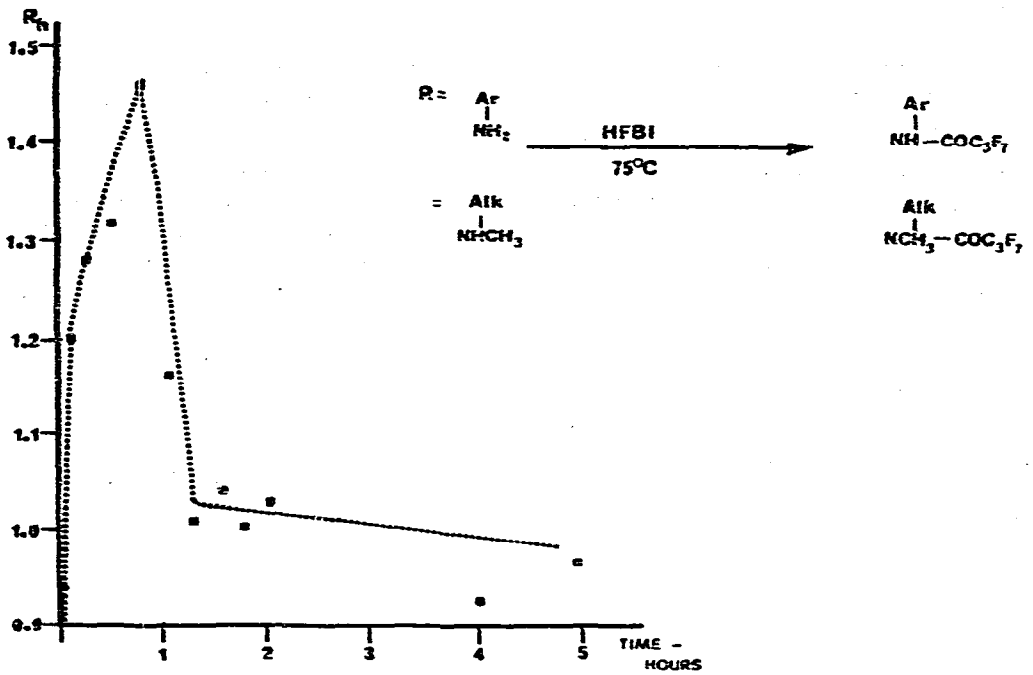

Fig. 3. Time dependency of the formation of the HFB-amides of 100 ng metoclopramide and 250 ng maprotiline, shown as the peak height ratio of the derivatives of the two substances.

The derivatizing agent is unstable in the presence of moisture and every effort must be made to ensure as anhydrous an environment as possible during the actual derivatization procedure. The derivatized samples themselves do not have an extremely long bench-life and must be chromatographed within 2—3 days of preparation.

The extraction procedure described consists essentially of an alkaline extraction, followed by an acid backwash, then a further alkaline extraction. The final organic phase is evaporated to dryness and the residues derivatized. An alkaline wash is then used to remove excess reagent and to convert any diperfluoroacyl derivatives to monoperfluoroacylamines. A second extraction step is employed in the early stages to maximize recovery of the drug.

A typical gas chromatogram obtained with the method is illustrated in Fig.4. Excellent resolution of peaks due to maprotiline and metoclopramide is achieved at the operating conditions described, and there is no interference

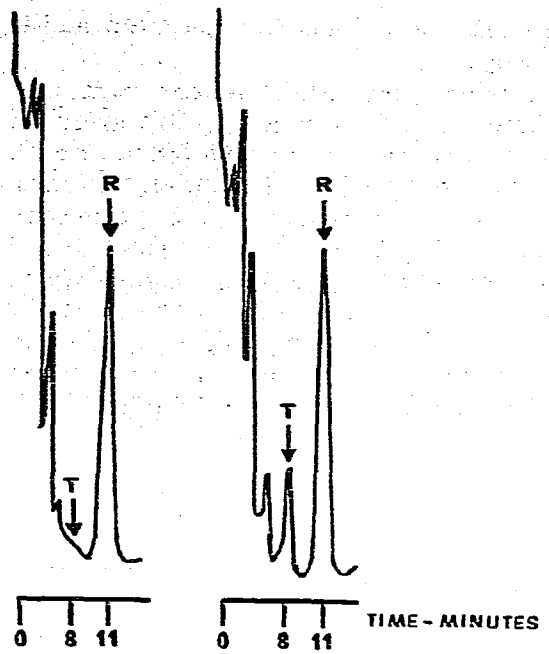

Fig. 4. Gas chromatograms obtained with extracts from 1.0-ml plasma samples containing no metoclopramide (left) and 30 μg/l metoclopramide (right). 250 ng maprotiline was used per ml of plasma. Derivatized metoclopramide (T) is eluted at 8 min and derivatized maprotiline (R) at 11 min.

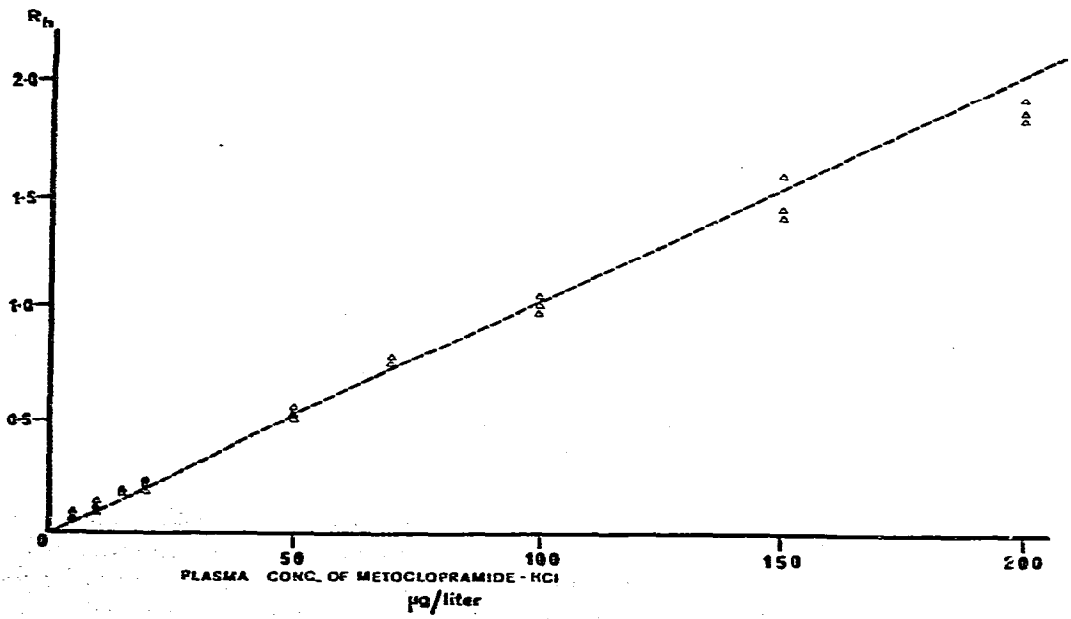

Fig. 5. Calibration curve for the quantitation of metoclopramide hydrochloride in plasma from 5 to 200 μg/l. Peak height ratio of drug to standard is related to plasma concentration of metoclopramide hydrochloride by $R_h = 0.009$ (concn.) + 0.032 ($r^2 = 0.998$).

from extracted plasma components. The retention times for metoclopramide and maprotiline are 8 and 11 min respectively.

The assay is quantitated by expressing the peak height ratio of metoclopramide to maprotiline as a function of plasma concentration of metoclopramide hydrochloride. Standard curves are linear up to 200 μg/l with a minimal detectable concentration of 5 μg/l based on 1.0-ml sampling volumes. Coefficient of determination (r^2) values, as determined by linear regression analysis, have ranged from 0.985 to 0.999 (see Fig. 5). Within-run (Table I) and between-run precision studies have proved the reliability and reproducibility of this method; the average variation from day to day is 4.3%. As well, a study conducted to assess percentage recovery from plasma, compared with directly injected derivatized standards, and with recovery from distilled water, indicated that mean percentages recovered ranged from 88% to virtually 100% (Table II).

TABLE I

WITHIN-RUN PRECISION

n = 6 at each concentration studied.

Concentration added (μg/l in plasma)	Mean concentration recovered (± S.D.) (μg/l)
10	10.15 ± 1.95
20	21.77 ± 2.87
50	53.15 ± 2.36
100	100.08 ± 7.94
200	208.20 ± 8.28

TABLE II

RECOVERY (%)

n = 3 at each concentration studied.

Concentration (μg/l)	Recovery (%)*	Recovery (%)**
5	101.4	101.3
20	108.8	105.9
50	106.3	94.9
100	100.1	89.5
150	102.7	90.5
200	104.1	100.7

*Compared with directly injected, derivatized standards.
**Compared with recovery from distilled water.

Specificity of the assay was established for over 30 drugs (Table III) that were thought likely to interfere, or which might possibly be taken concurrently with metoclopramide. No such interference was observed at normal therapeutic concentrations of these drugs. This method, in our experience, proved superior to that of Tam and Axelson [8] which we found insufficiently sensitive for pharmacokinetic studies in man. The assay for metoclopramide developed personally, uses a different internal standard, maprotiline, which is added prior

TABLE III

DRUGS AND METABOLITES TESTED FOR INTERFERENCE IN ASSAY

Acetylsalicylic acid	Methdilazine
Carbamazepine	Methysergide
Chlordiazepoxide	Nitrazepam
Chlorimipramine	Nortriptyline
Clonazepam	Oxazepam
Cyproheptadine	Phenobarbitone
Desipramine	Phenytoin
Desmethyldiazepam	Prednisolone
Diazepam	Prednisone
Dicyclomine	Procainamide
Ergotamine	Prochlorperazine
Ethosuximide	Propranolol
Fluphenazine	Salicylic acid
Haloperidol	Trifluoperazine
Imipramine	Valproic acid
Levodopa	

to the first extraction, rather than prior to derivatization. It also uses a double extraction technique which results in higher recoveries and better sensitivity. HFBI, the derivatizing agent, also gave higher yields of metoclopramide-HFB and maprotiline-HFB than did heptafluorobutyric anhydride.

The assay has been used in preliminary pharmacokinetic studies in man; it appears sufficiently sensitive to determine blood levels of metoclopramide after an intravenous dose of 10 mg. The relevant plasma concentration—time profile is shown in Fig.6, and the pharmacokinetic parameters calculated from these data are tabulated in Table IV.

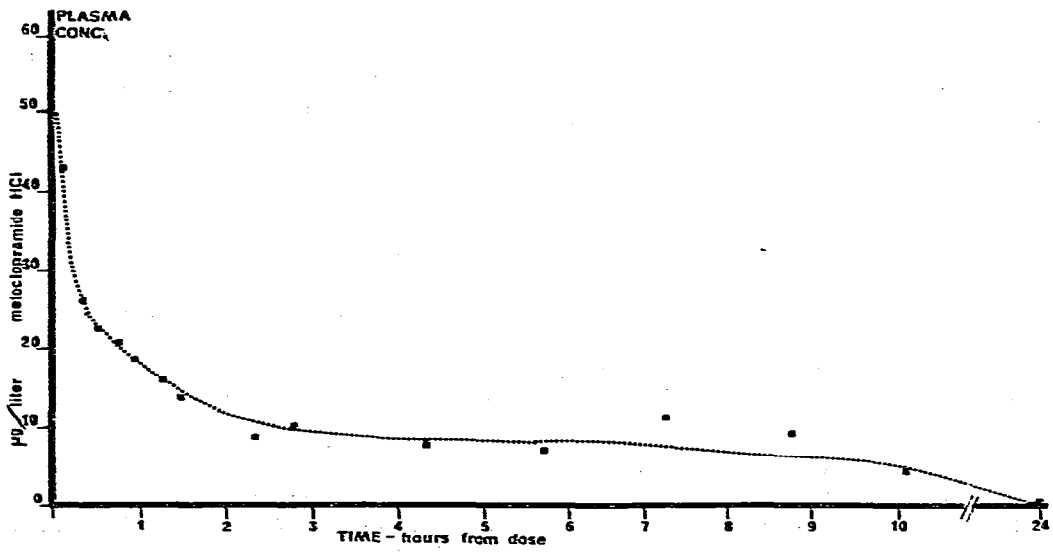

Fig.6. Concentrations of metoclopramide hydrochloride in human plasma after administration of a 10-mg intravenous dose to a healthy male volunteer. Plasma levels were followed for 24 h.

TABLE IV

PHARMACOKINETIC PARAMETERS IN ONE SUBJECT

Subject I (intravenous metoclopramide 10 mg)	
$t_{\frac{1}{2}}$ (β)	5.0 h
k_β	0.14 h^{-1}
Clearance (area)	0.94 l/kg/h
Volume of distribution	6.79 l/kg
AUC (trapez)	158.21 μg/l·h
$t_{\frac{1}{2}}$ (α)	0.33 h
k_α	2.1 h^{-1}

The plasma concentration—time profile was biexponential; data were found to fit a two-compartment open model with terminal half-life of the order of 5 h. This would appear to differ from preliminary work published by Teng et al. [7] which suggested that data were best fitted to a one-compartment model with half-life of about 4.0 h, though further pharmacokinetic studies are required.

From the results collected in Table IV, it would appear that metoclopramide is fairly rapidly and extensively distributed throughout the body. Clearance is high, indicating rapid elimination of this drug, most likely by conjugation and N-dealkylation [4,11,12].

Side effects experienced by the subject included dry mouth, irritability and mild disorientation (all in first 60 min) and very marked sedation at about 3 h after dosing. No attempt was made to correlate these effects with metoclopramide plasma levels.

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