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DETERMINATION OF METOCLOPRAMIDE AND ITS GLUCURONIDE AND SULPHATE CONJUGATES IN HUMAN BIOLOGICAL FLUIDS (PLASMA, URINE AND BILE) BY ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Metoclopramide was determined in human biological fluids (plasma, urine and bile) by reversed-phase ion-pair high-performance liquid chromatography using a newly introduced cyanopropyl column. The method is precise, selective and sensitive: the mean recoveries of metoclopramide from plasma, urine and bile were 74.4, 99.1 and 85.9%, respectively; the mean within- and between-run coefficients of variation were, respectively, 0.8 and 8.5% for plasma and 2.0 and 8.2% for urine at the drug concentration of 100 ng/ml, and 2.3 and 11.2% for bile at the concentration of 20 ng/ml; the lower detection limit was 2 ng/ml for 1 ml of each biological fluid. Enzymic hydrolysis of a urine or bile specimen was used in the identification of metoclopramide, as well as its glucuronide and sulphate conjugates, from the human samples. A preliminary study on metoclopramide determinations from plasma and urine samples of a healthy subject and from bile samples of a patient demonstrated the clinical applicability of the method for therapeutic monitoring and pharmacokinetic studies.

INTRODUCTION

Metoclopramide (MCP) is widely used in the treatment of gastroesophageal reflux, diabetic gastroparesis, and emesis occurring frequently during cancer chemotherapy [1–3]. Its potent antidopaminergic effect on the gastrointestinal smooth muscle and central nervous system (CNS) is considered to be responsible for the pharmacological properties [1–3]. Recent studies have demonstrated that

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an antiemetic effect [4] and CNS-related side-effect(s) caused by MCP [5] may be associated with its concentrations in plasma. Therefore, it would be of clinical value to monitor the drug concentration in patients receiving MCP therapy, not only to optimize the therapeutic efficacy but also to diminish the occurrence of possible side-effect(s).

Previous assay methods for MCP in biological fluids (plasma or serum and urine) have involved thin-layer chromatography [6,7] and high-performance liquid chromatography (HPLC) [8–10]. However, these reported methods require a large sample volume (at least 2 ml) in order to achieve a nanogram-order sensitivity. The lack of a simple, sensitive and specific assay that requires only a small sample volume appears to hamper the routine clinical monitoring of MCP levels. On the other hand, several groups of investigators [11–13] have reported the use of gas chromatography with mass spectrometry (GC–MS) or electron-capture detection (GC–ECD) for assaying MCP in small plasma volumes (0.25–1 ml) with good sensitivity. Obviously, this analytical equipment is costly and may not be applicable to everyday clinical monitoring of MCP.

This report describes a reversed-phase ion-pair HPLC method for the determination of MCP and its glucuronide and sulphate conjugates in human biological fluids (plasma, urine and bile) in 1.0 ml of each sample on a newly introduced cyanopropyl column packed with 4-μm particles. The method is shown to be applicable to pharmacokinetic studies and to clinical use of therapeutic monitoring of MCP. It can also be used to study the enterohepatic behaviour of MCP by measuring the parent drug and its conjugate forms in human bile.

EXPERIMENTAL

Chemicals and reagents

MCP as the base was donated by Fujisawa Pharmaceutical (Osaka, Japan) and disopyramide (DP) phosphate by Searle (Chicago, IL, U.S.A.). β-Glucuronidase (250 000 U per 0.44 g, No. G-3510), sulphatase (10 000 U per 0.5 g, No. S-9626) and *d*-saccharic acid 1,4-lactone (saccharolactone) were purchased from Sigma (St. Louis, MO, U.S.A.). Sodium laurylsulphate (SDS), methanol and acetonitrile (all reagent grade) and dichloromethane (HPLC grade) were purchased from commercial sources. All other chemicals used (reagent grade) were purchased from Wako (Osaka, Japan). A stock solution (1 mg/ml) containing MCP or DP (as an internal standard) was further diluted with methanol to the required concentrations for both compounds (10–250 ng/ml MCP for plasma, 50–200 ng/ml for urine, and 5–30 ng/ml for bile samples, and 10 μg/ml DP), and stored at 4°C until used.

Apparatus and chromatographic conditions

The chromatographic system consisted of a Shimadzu LC-6A pump (Kyoto, Japan), a 20-μl loop (Rheodyne, Cotati, CA, U.S.A.), and a Shimadzu SPD-6AV variable-wavelength UV detector set at 275 nm with a range of 0.005 a.u.f.s. A Shimadzu C-R3A Chromatopac was used for integration. A NOVA-PAK cartridge, a cyanopropyl (CN) column (4 μm particle size, 10 cm$\times$8 mm I.D.) sup-

plied by Waters Assoc. (Milford, MA, U.S.A.) was used with a mobile phase of 35% acetonitrile and 1% SDS in 0.61 *M* sodium acetate solution, adjusted with acetic acid to pH 5.0. The flow-rate was 1.0 ml/min, and the separation was performed at ambient temperature.

Sample preparation and assay procedure

Urine. A 1-ml aliquot of 100-fold diluted urine was transferred to a 10-ml glass-stoppered centrifuge tube, and 1 ml of 1 *M* sodium hydroxide, 0.5 ml of DP (10 μg/ml) as the internal standard and 6 ml of dichloromethane were added. The tube was vigorously shaken for 10 min, and then centrifuged (1761 *g* for 5 min). A 5-ml aliquot of the organic layer was then evaporated to dryness at 50°C under a stream of nitrogen. The residue was reconstituted with 100 μl of methanol, and a 20-μl aliquot was then injected onto the HPLC system.

Plasma and bile. To a 1-ml volume of plasma or bile were added 1 ml of 1 *M* carbonate buffer (pH 10.2), 0.5 ml of DP (10 μg/ml) and 5 ml of dichloromethane. After mixing, the aqueous phase was removed and the remaining organic layer was back-extracted with 6 ml of 0.1 *M* phosphate buffer (pH 3.2). Following centrifugation (1761 *g* for 5 min), 5 ml of the aqueous solution was alkalinized with 1 ml of 1 *M* sodium hydroxide and re-extracted with 5 ml of dichloromethane. After centrifugation (1761 *g* for 5 min) and aspiration of the aqueous layer, the organic solution was evaporated and then assayed in the same way as the urine sample.

Deconjugation by enzyme hydrolysis

A 0.5-ml aliquot of 100-fold diluted urine or non-diluted bile was transferred to a glass-stoppered centrifuge tube. Approximately 1000 U of β-glucuronidase were added to each tube for glucuronide deconjugation, or 10 U of sulphatase were added for sulphate deconjugation in the presence of 2 mg of saccharolactone (β-glucuronidase inhibitor). The tubes were incubated at 37°C for 24 h (the plateau reached at ca. 16 h) for glucuronide deconjugation and for 48 h (near the post-incubated plateau time) for sulphate deconjugation. The samples were then assayed for MCP as described earlier.

Quantification

We prepared standards by adding known amounts of MCP and DP to drug-free human biological samples to give final required concentrations (20, 40 and 60 ng/ml for plasma; 50, 100 and 200 ng/ml for urine; and 10, 20 and 30 ng/ml for bile samples). Each volume of biological fluids used was 1 ml, to which 0.5 ml of DP dissolved in methanol were added. The assay of each sample was performed as described above. Calibration curves were constructed for each sample assay by using the analyte-to-internal standard peak-height ratios. The ratios for an unknown sample to the MCP concentrations were converted by the use of the corresponding calibration curves. Linear regression analyses were performed within the drug concentration ranges as described above.

Application

One healthy subject received a 10-mg intravenous dose of MCP (Primperan® parenteral, Fujisawa Pharmaceutical) over ca. 3 min and a 20-mg oral dose of MCP from the same manufacturer on two separate occasions, two weeks apart, after an overnight fast. Blood samples were collected into heparinized tubes at 0 (just prior to the dose), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 h post-dose. Blood was centrifuged (880 *g* for 15 min) and the plasma was separated. Urine was collected into plastic containers at intervals 0–0.25, 0.25–0.75, 0.75–1.25, 1.25–1.75, 1.75–2.25, 2.25–3, 3–5, 5–7, 7–9, 9–11 and 11–13 h after the dose. In a separate study, the excretion of MCP and its conjugates into bile was examined in a patient with cholangioma, who had an external bile drainage because of his biliary obstruction and showed normal liver function tests. This patient eventually required MCP as an antiemetic. A 10-mg dose of MCP (same parenteral formulation as administered to the normal subject) was infused over 1 h. Bile samples were collected at intervals 0–12, 12–18, 18–28, 28–36 and 36–42 h post-dose.

Plasma, urine and bile samples were stored at −20°C until analysed.

RESULTS

With our chromatographic system, MCP and the internal standard exhibited symmetrical peaks with baseline resolution. We observed no interfering peak when blank samples of plasma and urine from a normal person or of bile from a patient were analysed (Fig. 1). The retention time was 7.8 min for MCP and 9.1 min for the internal standard, DP. The total elution time per assay was less than 10 min.

Calibration curves for MCP showed an excellent linearity ($r = 0.999$ or better) and passed through the origin over the ranges of 10–250 ng/ml for plasma, 50–200 ng/ml for urine and 5–30 ng/ml for bile specimens.

The detection limit of the assay method may depend on the sample size. With 1 ml of plasma urine or bile, as used in the present study, the detection limit of the assay, defined as thrice the level of baseline noise, was 2 ng/ml for all three samples.

The precision data are summarized in Table I. The within-run precision, expressed as the coefficient of variation (C.V.), was less than 3.5% for all the samples, while the between-run C.V. ranged from 5.1 to 8.5% and 8.2 to 12.2% over the concentration range 50–200 ng/ml for plasma and urine samples, respectively, and from 5.5 to 11.2% over the concentration range 10–30 ng/ml for bile samples.

Analytical recovery data are given in Table II. These data were collected by adding known amounts of MCP to drug-free human biological fluids and taking aliquots of them through the complete procedure, then comparing the peak heights of extracted drug with those of pure standard. The overall recoveries (mean ± S.D.) from plasma urine and bile over the concentration ranges as given in Table II were 74.4 ± 8.2, 99.1 ± 5.5, and 85.9 ± 9.2%, respectively.

We are not so sure with which drug(s) MCP would be coadministered. However, H_2-receptor blocking drugs are widely used in upper gastrointestinal disorders and may occasionally be indicated for clinical conditions under which MCP

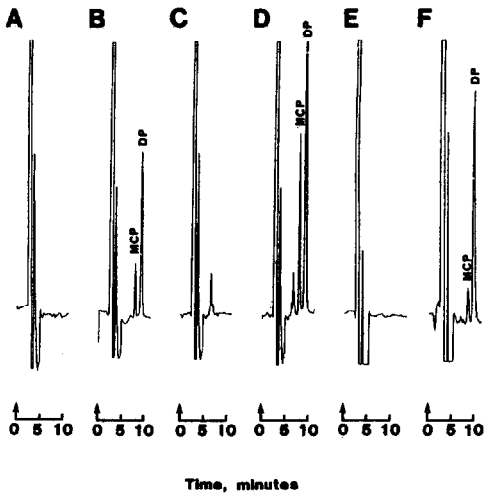

Fig. 1. Chromatograms showing resolution of metoclopramide (MCP) and the internal standard, disopyramide (DP). Extracts were obtained from (A) blank plasma, (B) plasma sample of a normal subject taken at 2 h after an oral 20-mg dose of MCP, (C) blank urine, (D) urine sample of a normal subject collected during a 1.75–2.25 h period after an oral 20-mg dose of MCP, (E) blank bile and (F) bile sample of a patient collected during a 0–12 h period after an intravenous 10-mg dose of MCP infused over 1 h. The measured concentrations correspond to 57.0, 133.7 and 50.0 ng/ml in B, D and F, respectively.

is also given. Therefore, we searched for possible interferences from three H_2-receptor blocking drugs (cimetidine, ranitidine and famotidine) in plasma assay: we found none. Furthermore, no interference was observed in plasma taken from patients receiving any of these three H_2-receptor blockers for the treatment of peptic ulcers.

The plasma concentration–time and cumulative urinary excretion–time curves

TABLE I

ANALYTICAL PRECISION IN THE DETERMINATION OF METOCLOPRAMIDE IN PLASMA, URINE AND BILE SAMPLES

Sample	MCP concentration (ng/ml)	Coefficient of variation (%)	
		Between-run assay ($n=5$)	Within-run assay ($n=5$)
Plasma	50	5.9	1.2
	100	8.5	0.8
	200	5.1	0.3
Urine	50	8.8	3.0
	100	8.2	2.0
	200	12.2	1.7
Bile	10	5.5	3.5
	20	11.2	2.3
	30	8.0	1.7

TABLE II

ANALYTICAL RECOVERIES OF METOCLOPRAMIDE FROM PLASMA, URINE AND BILE SAMPLES

Sample	MCP concentration (ng/ml)	Recovery (mean ± S.D., $n=5$) (%)
Plasma	20	75.0 ± 11.4
	50	72.0 ± 5.7
	100	76.1 ± 7.6
Urine	50	100.3 ± 9.1
	100	98.1 ± 2.7
	200	98.8 ± 3.3
Bile	10	84.1 ± 4.9
	20	89.1 ± 8.2
	30	84.5 ± 1.7

of MCP obtained from a normal subject, who received an intravenous 10-mg and an oral 20-mg dose of MCP on two separate occasions, are illustrated in Fig. 2. The total area under the MCP concentration–time curve to infinite time (AUC_0^∞) was calculated by the trapezoidal rule. The total drug clearance (Cl) and volume of disatribution (V_d) after the intravenous dose were calculated as Cl=intravenous dose/AUC_0^∞ and $V_d = Cl/\beta$, respectively. The apparent elimination half-life ($t_{\frac{1}{2}\beta}$) was calculated as $t_{1/2\beta} = 0.693/\beta$, where β is the apparent terminal rate constant. The renal clearance (Cl_R) of MCP was estimated from $Cl_R = Ae_0^\infty / AUC_0^\infty$, where Ae_0^∞ is the cumulative amount of MCP excreted in

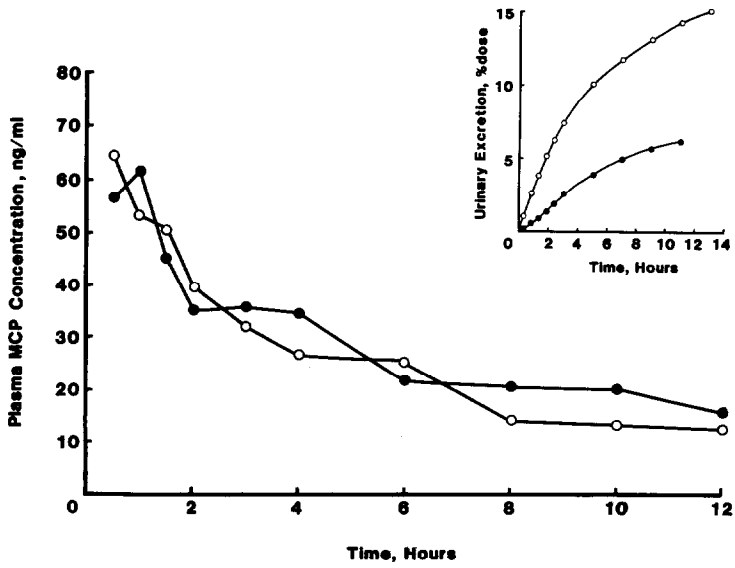

Fig. 2. Plasma concentration–time and cumulative urinary excretion–time curves of metoclopramide in a normal subject receiving an intravenous (○) 10-mg and oral (●) 20-mg dose on two separate occasions.

TABLE III

BILIARY EXCRETION DATA ON METOCLOPRAMIDE AND ITS CONJUGATES

Bile samples were collected from a patient who received an intravenous dose of 10 mg of MCP infused over 1 h. The total recovery from bile over the post-dose 42-h period was 150.2 μg as MCP equivalent, being ca. 1.5% of the administered dose. Values in parentheses are μg MCP equivalent.

Post-dose bile collection period (h)	Bile volume (ml)	MCP (μg)	MCP sulphate (μg)	MCP glucuronide (μg)
0–12	200	16.8	124.0 (98.0)	12.0 (7.6)
12–18	130	1.4	4.3 (3.4)	3.3 (2.1)
18–28	185	1.4	6.6 (5.2)	0 (0)
28–36	150	0.9	6.1 (4.8)	7.0 (4.4)
36–42	110	0	2.2 (1.7)	4.0 (2.5)
Total	775	20.5	143.2 (113.1)	26.3 (16.6)

urine, projected to infinite time. The absolute bioavailability (F) was calculated from F = oral AUC_0^∞ · intravenous dose/intravenous AUC_0^∞ · oral dose. The kinetic data derived from using the above equations were: $\beta = 0.061\ h^{-1}$, $t_{1/2\beta} = 11.4$ h, $V_d = 5.0$ 1/kg, $Cl = 263$ ml/min, and $Cl_R = 67.0$ ml/min (ca. 25% of Cl) after the intravenous dose; $\beta = 0.072\ h^{-1}$, $t_{1/2\beta} = 9.6$ h, $Cl_R = 56.2$ ml/min and $F = 32\%$ after the oral dose. The observed values for $t_{1/2\beta}$ and Cl in this subject appear to be longer and smaller than the respective values reported previously [14]. However, the values for F and V_d estimated in this study are in agreement with the range of values reported previously [2].

The biliary excretion data on MCP and its conjugates obtained from a patient, who received an intravenous dose of 10 mg of MCP infused over 1 h, are given in Table III. The biliary excretion of sulphate conjugate was much greater (five to six times as MCP equivalent) than that of non-conjugated MCP or glucuronide conjugate. The total amount recovered from bile over 42 h was 150.2 μg as MCP equivalent, being ca. 1.5% of the administered dose.

DISCUSSION

Various analytical procedures have been introduced for the determination of MCP in biological fluids [6–13]. However, these methods appear to have some drawbacks with regard to their sensitivity (i.e. with thin-layer chromatography or conventional HPLC methods) and some clinical disadvantages (e.g. cost and rapidity in GC–MS or GC–ECD) in terms of routine use [11–13]. An improvement of sensitivity has been noted when a recently introduced 4-μm cyanopropyl column is used, compared with a conventional CN or C_{18} column with 10-μm particles.

In order to select the most suitable extraction solvent, dichloromethane, carbon tetrachloride, diethyl ether, chloroform, *n*-hexane, cyclohexane, benzene,

toluene and ethyl acetate were examined. Among these solvents, dichloromethane had the highest extraction recovery for MCP and the internal standard, DP.

In order to attain the optimal separation between MCP and endogenous component(s) as well as the internal standard, 0.01, 0.1 and 1% of sodium pentanesulphate (C_5), sodium hexanesulphate (C_6) or SDS (C_{12}) were examined as a counter-ion. Since the concentration and/or carbon number of the counter-ions is proportional to the retention time, 1% SDS was selected as the ideal counter-ion.

To eliminate possible interferences from plasma component(s), the extract of drug-free plasma was analysed at a UV detection wavelength of 308 nm, at which the least interference had been suggested by previous workers [9]. However, we found that the use of either 275 or 308 nm for detecting MCP in plasma with the maximum sensitivity did not show any appreciable difference in the interference. Therefore, we adopted the wavelength of 275 nm.

To determine the optimal concentrations as well as the pH of the aqueous solutions for backwash, pH 2.6 phosphoric acid (0.005 *M*), pH 3.2 phosphate buffer (0.1 *M*), and pH 2, 3 and 4 acetate buffers (0.1 *M* each) were tested. Of these, 0.1 *M* phosphate buffer was selected because of its greatest efficiency for not only extracting MCP but also eliminating the interference(s) of endogenous component(s).

Since the kinetic data of MCP were obtained from only one subject and remain highly preliminary, an exact comparison of the data from our assay method with those observed with other assay methods [14] would be injudicious. However, to our knowledge, this report is the first to show the excretory profiles of MCP and its two conjugates in human bile. The analysis of the bile specimen from a patient suggested that not only MCP but also its sulphate and glucuronide are not so extensively circulated enterohepatically (i.e. total biliary excretion of MCP and its conjugates over 42 h was only ca. 1.5% of the administered dose). Therefore, we are tempted to speculate that the enterohepatic recycling of MCP is not so active in humans, in contrast to the rather active enterohepatic circulation previously observed in rabbits [13].

In conclusion, our findings (Tables I–III; Fig. 2) indicate that the assay method is useful both for therapeutic monitoring of MCP and for pharmacokinetic studies of MCP and its conjugates in humans.

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