Journal of Pharmaceutical & Biomedical Analysis
Vol. 5, No. 3, pp. 295–301, 1987
Printed in Great Britain

0731–7085/87 $3.00 + 0.00

Short Communication

Reversed-phase liquid chromatographic determination of meclozine in biological samples

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Keywords: *Meclozine; HPLC determination; dog plasma; human urine; pharmacokinetics.*

Introduction

Meclozine hydrochloride [1-(*p*-chloro-α-phenylbenzyl)-4-(*m*-methylbenzyl)piperazine·2 HCl] is an antihistaminic agent widely used for the management of nausea, vomiting and dizziness associated with motion sickness [1]. Pharmacokinetic studies of this drug in humans and animals require a rapid, sensitive and specific analytical procedure. Various methods for the determination of meclozine hydrochloride have been reported, including polarography [2], fluorometry [3], GLC [4–6] and colorimetry [7]. Most of these methods lack the accuracy, sensitivity and specificity required for the biological assay of the drug. No HPLC method for the determination of meclozine in biological samples appears to be available. The proposed method is sensitive and accurate, and is able to monitor the plasma level versus time profile of meclozine following a single bolus IV dose of 1 mg/kg in a dog. The method was also applied to determine urinary excretion data following a single oral dose of 50 mg in a man.

In view of its sensitivity, accuracy and simplicity the method could be useful for the pharmacokinetic and bioavailability studies of meclozine in man.

Experimental

Chemicals and reagents

Meclozine hydrochloride (Sigma Chemical Co., St. Louis, MO) and hydroxyzine hydrochloride (Sigma Chemical Co.) were used without further purification. Acetonitrile (J.T. Baker Chemical Co.), methanol (J.T. Baker Chemical Co.), *n*-hexane (J.T. Baker Chemical Co.) and water were HPLC grade. All other chemicals were of U.S.P. or A.C.S. quality and were used as received.

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Instrumentation

A modular liquid chromatograph equipped with a variable wavelength UV detector (Spectro Monitor III, LDC/Milton Roy), operated at 230 nm, a fixed volume injector (Rheodyne Model 7010) with a 20 μl sample loop and isocratic eluent supply at ambient temperature was used. A stainless steel reversed-phase octyl column (15 cm × 4.6 mm i.d.) containing 5 μm ultrasphere packing (Beckman Instruments, Inc.) was used with a guard column. Chromatograms were recorded on a strip chart recorder (Varian G-1000) at a speed of 0.3 cm/min.

Mobile phase

The eluent consisting of acetonitrile and water (30:70, v/v) was degassed by bubbling helium gas for 5 min prior to use. Column equilibrium with the eluting solvent was established by pumping (Altex Model 110A HPLC Pump, Altex Scientific) the mobile phase at a rate of 0.2 ml/min overnight. The flow rate was set at 2.5 ml/min during analysis.

Stock solutions

An appropriate quantity of meclozine hydrochloride and hydroxyzine hydrochloride [1-(*p*-chloro-α-phenylbenzyl)-4-(2-hydroxymethoxyethyl)piperazine-2 HCl] internal standard was accurately weighed and dissolved separately in a 100 ml volumetric flask to prepare 1 mg/ml and 0.1 mg/ml solution (as salt), respectively, in methanol. The standard solutions of meclozine hydrochloride in the concentrations between 0.02 and 2.0 mcg/ml and containing 1 mcg/ml of hydroxyzine hydrochloride as internal standard were prepared by diluting the appropriate quantities of stock solutions with the mobile phase. These solutions were used to compare the recovery of the drug from spiked plasma or urine samples.

Extraction procedure

To one ml of dog plasma or human urine samples (dosed or spiked) in a 12 ml glass centrifuge tube were added 10 μl of the stock solution of internal standard and 0.1 ml of 2 M NaOH and vortexed briefly. After addition of 3 ml of *n*-hexane the mixture was vortexed at high speed for 1 min and centrifuged at 3000 r.p.m. for 5 min. The upper organic layer was collected and set aside. The aqueous layer was re-extracted with 3 ml of *n*-hexane. A 5 ml volume of the combined organic layers was then dried under a nitrogen gas stream at ambient temperature. The residue was reconstituted in 200 μl of the eluent. The sample was then shaken on a vortex mixer for 30 s and 20 μl of the resulting solution was injected.

Calibration plots

One ml of blank dog plasma or human urine in a 12 ml centrifuge tube was spiked with different amounts of the meclozine hydrochloride stock solution to prepare the standard solutions in the concentration range, 0.02–2.0 mcg/ml for plasma and 0.1–2.0 mcg/ml for urine, respectively, and containing a fixed amount of the internal standard (1 mcg/ml). Reconstituted extracts of the standard samples were chromatographed and calibration plots were obtained by plotting the peak height ratios of meclozine:hydroxyzine versus the concentration of meclozine hydrochloride (meclozine) expressed as microgram per millilitre of plasma or urine. Linear regression analysis was used to determine the slope, intercept and the correlation coefficients.

Quantitation

After subjecting unknown plasma or urine samples to the described extraction and chromatographic procedures, the amount of meclozine was determined by comparing the peak height ratios of meclozine to hydroxyzine obtained from unknown samples with the calibration plots.

Recovery studies

The extraction efficiency of meclozine from dog plasma or human urine was determined by comparing the peak height ratios of the drug to the internal standard obtained after direct injection of the solutions containing known quantities of meclozine hydrochloride (0.02, 0.05, 0.08, 0.1, 0.2, 0.4, 0.8, 1.0, 1.5 and 2.0 mcg/ml) which was directly dissolved in the mobile phase with those obtained after extracting the drug from spiked plasma or urine samples containing equivalent amounts of the drug and the internal standard. At each of the meclozine concentrations used, three to five replicate samples were measured.

Animal study

To demonstrate the applicability of the assay to the quantitation of meclozine in plasma, a single bolus intravenous dose of 1 mg/kg in 1 ml of aqueous ethyl alcohol (50:50, v/v) was administered into the cephalic vein of a greyhound dog weighing 26 kg. Blood samples (5 ml) were withdrawn from the jugular vein using heparinized vacutainer tubes at the intervals of 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 9 and 12 h after dose. Blood specimens were immediately centrifuged and the plasma (2 ml) was stored in a screw-capped plastic vial at −4°C until required for assay, generally within two days.

Urinary excretion in man

The assay was applied for the measurement of the urinary excretion profile of meclozine in a healthy male subject (70 kg) after receiving a single oral dose of 50 mg in an empty stomach. Urine samples were collected prior to dose and at the intervals of 1, 2, 3, 5, 7, 9, 11, 14 and 24 h post-dose. Water (200 ml) was ingested by the subject every 2 h during the day.

Results and Discussion

Chromatograms obtained at the lower limit of sensitivity for drug free plasma or urine extracts showed no interfering peaks at the retention times of meclozine and the internal standard. Figure 1 shows a typical chromatogram for the samples prepared from blank dog plasma (1a), and a dog plasma (1b) containing meclozine (1 mcg/ml) and hydroxyzine. Using the chromatographic conditions described, meclozine and hydroxyzine were well separated and their retention times were 13 and 8 min, respectively. Both peaks were sharp and symmetrical with good baseline resolution, thus facilitating the accurate measurement of the peak height ratios. Hydroxyzine is a good internal standard because of its adequate retention time and similar spectral properties to meclozine. No interferences by the metabolites or normal constituents of plasma or urine were observed.

Standard plots obtained for plasma and urine samples were both highly linear in the concentration range of 0.02–2.0 mcg/ml for plasma and 0.1–2.0 mcg/ml for urine. Linear regression analyses of the standard calibration plots for dog plasma and human urine

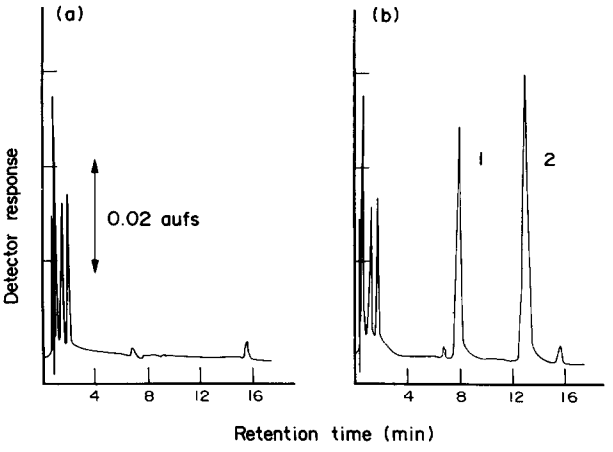

Figure 1

samples were, respectively $y = 0.596x + 0.013$ and $y = 0.612x + 0.0193$, where y and x are the peak height ratio and meclozine concentration, respectively. The small intercepts indicate that the blank plasma has negligible interferences for the drug. The correlation coefficients of both plots were better than 0.999.

The day-to-day reproducibility of the assay for plasma or urine samples was evaluated by comparing the least-square linear regression analyses of the three standard plots obtained from spiked dog plasma or human urine standards at three different days over a one week period. The results of this evaluation are summarized in Tables 1 and 2. The average correlation coefficient was greater than 0.999 and the RSD of the slopes of the three lines was less than 1%. Analysis-of-variance of the data showed no detectable difference in the slopes of the three standard plots for plasma ($F = 2.9$, $P > 0.01$) and urine ($F = 2.65$, $P > 0.01$) samples, respectively. The results thus confirmed excellent linearity of the calibration lines and high reproducibility of the assay. With little variation in the slopes of the standard plots among multiple determinations, the method should be accurate and precise within the assay day as well as between assay days.

The recovery and precision of the assay were assessed by comparing the peak height ratios (meclozine/hydroxyzine) obtained from spiked dog plasma samples of different meclozine concentrations (0.02–2.0 mcg/ml) to the peak height ratios for the samples containing equivalent amounts of the drug and internal standard directly dissolved in the mobile phase. Three to five replicate samples were assayed at each drug concentration and the results are shown in Table 3. The average recovery of the drug was 96.4% and its coefficient of variation was 3.45%. The data indicated that the concentration of the drug in the samples between 0.02 and 2.0 mcg/ml had no detectable effect on recovery. Table 4 shows the standard calibration and recovery of meclozine from spiked human urine between the concentrations of 0.1–2.0 mcg/ml. The average recovery of the drug was 101.8% and its RSD was 2.7%. The sensitivity of the assay defined as the minimum drug concentration corresponding to two times the signal-to-noise ratio was found to be *ca* 20 ng/ml for both plasma and urine samples. The method was applied to monitor the plasma level versus time profile of meclozine in a dog after receiving a bolus IV dose of

Table 1
Regression analysis of data for the standard plots of meclozine in dog plasma

Standard plot*	Slope†	Intercept†	Correlation coefficient†
1	0.596	0.013	0.999
2	0.603	0.009	0.999
3	0.610	0.009	0.999

*Obtained in three different days.
†The mean of 5 determinations at each concentration used.

Table 2
Regression analysis of data for the standard plot of meclozine in human urine

Standard plot*	Slope†	Intercept†	Correlation coefficient†
1	0.612	0.019	0.999
2	0.608	0.023	0.999
3	0.608	0.027	0.999

*Obtained in three different days.
†The mean of 5 determinations at each concentration used.

Table 3
Standard calibration and recovery data of meclozine from spiked dog plasma

Meclozine added (mcg/ml)	Mean peak height ratio	n*	Recovery (mcg/ml)	S.D.	CV† (%)	Recovery‡ (%)
0.02	0.023	3	0.018	0.0007	4.0	90.0
0.05	0.041	3	0.048	0.0018	3.9	96.0
0.08	0.059	3	0.075	0.0026	3.5	94.0
0.1	0.078	5	0.093	0.0036	3.9	92.8
0.2	0.134	5	0.190	0.0072	3.7	97.7
0.4	0.250	5	0.390	0.0135	3.5	96.8
0.8	0.477	5	0.800	0.0230	2.9	100.0
1.0	0.605	5	0.980	0.0270	2.8	97.6
1.5	0.921	5	1.450	0.0450	3.15	96.9
2.0	1.200	5	2.000	0.0640	3.2	100.0

*Number of replicates.
†Average CV = 3.46%.
‡Average recovery = 96.3%.
$y = 0.596x + 0.012$, $r = 0.999$.

Table 4
Standard calibration and recovery data of meclozine from spiked human urine samples

Meclozine added (mcg/ml)	Mean peak height ratio	n*	Recovery (mcg/ml)	S.D.	CV† (%)	Recovery‡ (%)
0.10	0.084	5	0.1	0.0035	3.5	100.0
0.20	0.140	5	0.2	0.0061	3.0	100.0
0.40	0.278	5	0.42	0.0116	2.8	105.0
0.80	0.496	5	0.83	0.0257	3.1	103.9
1.00	0.620	5	1.0	0.0250	2.5	100.0
1.50	0.940	5	1.48	0.0340	2.3	99.0
2.00	1.250	5	2.1	0.0437	2.1	105.0

*Number of replicates.
†Average CV = 2.7%.
‡Average recovery = 101.8%.
$y = 0.612x + 0.0193$, $r = 0.999$.

meclozine hydrochloride (1 mg/kg) and the urinary excretion profile in a human subject who received a single oral dose of a 50 mg tablet.

According to Fig. 2, the disposition of meclozine in the dog follows the two-compartmental pharmacokinetic model as represented by:

$$C = Ae^{-\alpha t} + Be^{-\beta t},$$

where C is the plasma meclozine concentration, A and B are the intercepts on the concentration axis, and α and β are the first order hybrid rate constants for the rapid and slow disposition phases, respectively [8]. The apparent half-life for the α phase was very short (<10 min) and the half-life for the terminal elimination phase was 3 h. From 0 to 12 h post-dose, the plasma levels of meclozine in the dog were approximately in the

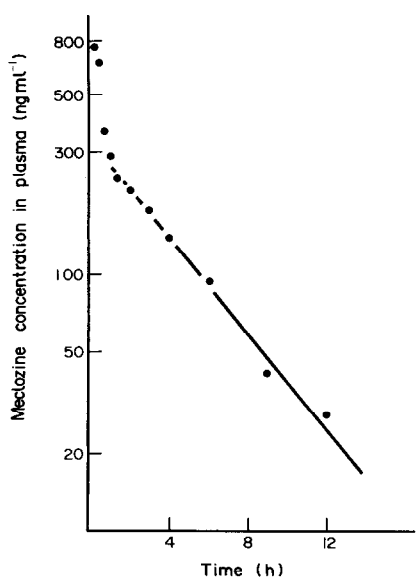

Figure 2

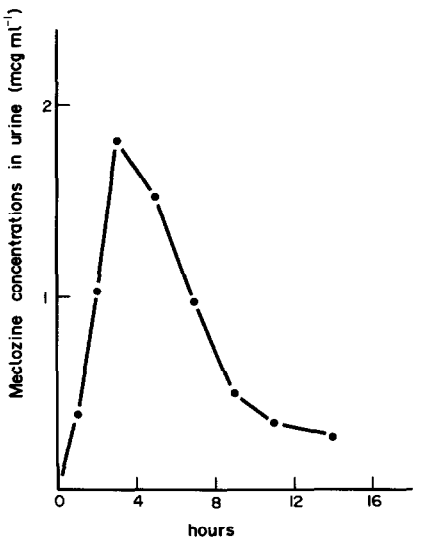

Figure 3

range of 0.3–0.03 mcg/ml. The volume of distribution at the β phase (*Vd*) was *ca* 64 l [9], indicating that the drug was extensively distributed into the tissues.

Urinary excretion data in a male subject (70 kg) showed that the percentage of dose recovered in urine as meclozine in the interval of 24 h was *ca* 3.2% of the administered dose, suggesting that the drug was extensively biotransformed. Figure 3 shows the time course of meclozine concentrations in urine in this subject.

In conclusion, the new reversed-phase HPLC method developed in this study is simple, sensitive and specific, and could be used for pharmacokinetic and bioavailability studies of meclozine in man.

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[*First received for review 1 November 1985; revised manuscript received 28 May 1986*]