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Pharmacokinetic Study of Promethazine in Korean Healthy Subjects Using a Validated HPLC Method

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Abstract – The objective of the present investigation was to study pharmacokinetics of promethazine in Korean healthy subjects using a validated HPLC method. The HPLC analysis was performed on a Capcell Pak CN column with a mixture of acetonitrile-0.02M potassium dihydrogen phosphate (42:58, v/v, pH 6.0) and the analyte was quantified with UV detection at 251 nm. The calibration curve of the drug was linear over the range of 1-40 ng/mL in human serum and the limit of quantification (LOQ) was 1 ng/mL. This analytical method was validated and shown to be specific, accurate, precise and reproducible. This method was applied to pharmacokinetic study of promethazine in Korean healthy volunteers following an oral administration of two 25 mg Himazin® tablets (50 mg promethazine·HCl) after overnight fasting. Serum samples were collected at given intervals over a 36-hour period (12 points) and pharmacokinetic parameters were determined from serum concentration-time profile using WinNonlin program. The estimated $AUC_{0-\infty}$, $AUC_{0-\infty}$, C_{max}, T_{max} and $t_{1/2}$ of promethazine obtained from Korean healthy subjects were 103.84 ± 84.30 ng·hr/mL, 87.94 ± 81.02 ng·hr/mL, 13.43 ± 10.92 ng/mL, 2.00 ± 1.16 hr and 5.88 ± 3.47 hr, respectively.

Keywords □ Promethazine, HPLC, human serum, pharmacokinetic study, validation

INTRODUCTION

Promethazine hydrochloride (N,N,α-trimethyl-10H-phenothiazine-10-ethanamine hydrochloride, Fig. 1 (A)) is a phenothiazine derivative, which has the anti-histamine effect by blocking histamine H_1-receptor off. Promethazine hydrochloride has been used for treatment of various immediate hypersensitivity reactions, for suppressing motion sickness or for sedation (Lackner and Graybiel, 1994; Tarkkila et al., 1995; Terndrup et al., 1991).

A number of analytical methods for the quantification of promethazine in various biological fluids have been reported (Bagli et al., 1994; Fox and McLoughlin, 1993; Song and Putcha, 2001; Pistos and Stewart, 2003; Vanapalli et al., 2001). Also, pharmacokinetics of promethazine after an administration by several routes has been characterized in different ethnic groups (Fox and McLoughlin, 1993; Schwinghammer et al. 1984; Song and Putcha, 2001; Stavchansky et al., 1987; Strenkoski-Nix et al., 2000; Taylor et al., 1983; Zaman et al., 1986). However, pharmacokinetics of promthazine in Korean subjects has not been studied.

Therefore, the objective of this study was to examine the pharmacokinetics of promethazine in Korean healthy volunteers, using the modified HPLC analytical method. In this study, we validated a specific, accurate and reproducible HPLC method for the quantification of promethazine in human serum and applied the method to the pharmacokinetic study of promethazine following an oral dosing in Korean healthy volunteers. Also, the results of this study in Korean could be used for the standard of bioequivalence test of promethazine.

MATERIALS AND METHODS

Chemicals

Promethazine hydrochloride and chlorpromazine (internal standard; I.S., Fig. 1 (B)) were purchased from Sigma (St. Louis, MO, USA). Acetonitrile and potassium dihydrogen phosphate were supplied by Merck (Darmastadt, Germany) and other chemicals were HPLC grade.

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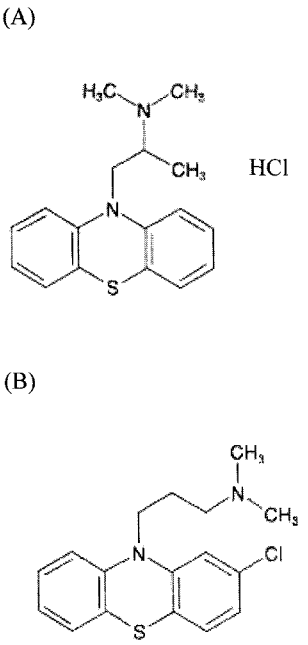

Fig. 1. Chemical structures of promethazine hydrochloride (A) and chlorpromazine (I.S.) (B).

HPLC conditions

The HPLC system was composed of a LC-10AD pump, a SPD-10A UV detector and a C-R6A chromatopac data processor (Shimadzu, Tokyo, Japan). The HPLC analysis was carried out on a Capcell Pak CN column (5 μm particle size, 250 mm × 4.6 mm id; Shiseido, Tokyo, Japan) with a mobile phase of acetonitrile and 0.02 M potassium dihydrogen phosphate (42:58, v/v, adjusted to pH 6.0 with 1 M sodium hydroxide) at a flow rate of 0.9 mL/min. The injection volume was 50 μL and the effluent was monitored at a wavelength of 251 nm. All the procedures were performed at room temperature.

Preparation of standard solutions and samples

Stock solutions of promethazine and chlorpromazine (I.S.) were prepared in methanol at concentrations of 1 mg/mL and 0.5 μg/mL, respectively. Both stock solutions were stored at 4°C until analysis. Promathazine stock solution (1 mg/mL) was diluted with human serum to prepare standard solutions at final concentrations of 1, 2, 5, 10, 20 and 40 ng/mL.

The samples were prepared by the method reported previously (Wallace et al., 1981) with modifications. To 1 mL of human serum, 100 μL of I.S. (chlorpromazine, 0.5 μg/mL in methanol) and 500 μL of 1 M sodium hydroxide were added and the sample was vortexed for 3 sec. Then, 4 mL of n-hexane containing 0.8 % 2-propanol was added to the sample followed by vortex-mixing for 90 sec. After centrifugation at 3000 rpm for 10 min, the organic layer (upper layer) of the sample was transferred to a clean test tube. Drug in the organic layer was back-extracted with 200 μL of 0.025 M hydrochloric acid by vortex-mixing for 1 min, and centrifugation at 3000 rpm for 5 min. A 50 μL aliquot of the back-extracted layer (lower layer) was injected into the HPLC system.

Validation of analytical method

Specificity

The interference of endogenous compounds was assessed by analyzing blank human serum and serum spiked with promethazine and I.S. More than ten different sources of human serum were used for the determination of specificity.

Linearity

The calibration curve for promethazine was obtained within a range from 1 to 40 ng/mL. Peak area ratios of promethazine to I.S. versus the promethazine concentrations were used to generate the calibration curve by least-squares linear regression analysis.

Precision and accuracy

Intra- and inter-day precision were determined by the analysis of the samples at four different concentrations of 1, 2, 10 and 20 ng/mL in five replicates within a day or during five consecutive days. The precision was calculated from the ratios of the standard deviation to the mean (coefficient of variation, C.V.). The accuracy was examined by analyzing the samples at four different concentrations of 1, 2, 10 and 20 ng/mL during five consecutive days.

Sensitivity

The sensitivity was assessed by the limit of quantification (LOQ), the lowest concentration of the serum spiked with promethazine with a signal-to-noise ratio of ≥ 5. The acceptable precision and accuracy limits of the LOQ were less than 20% C.V. and 20% bias (80~120% of the theoretical concentrations), respectively.

Recovery

The recovery was determined by comparison of the peak areas from extracted samples spiked with promethazine to those from extracted samples containing water instead of serum. The mean recovery was determined at 2, 10 and 20 ng/mL in triplicates.

Pharmacokinetics of promethazine in Korean subjects

The validated analytical method was applied to measure serum levels of promethazine after a single oral administration of two 25 mg Himazin® tablets (50 mg promethazine · HCl) to 8 healthy male Korean volunteers (23-25 years, 63-85 kg). All the subjects were fasted overnight before drug administration and continued to be fasted for 4 hrs after the dosing. Venous blood samples (8 mL each) were collected just before the dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 36 hrs following drug administration. After centrifugation of blood samples at 3000 rpm for 20 min, the sera were taken and stored at -70 °C until analysis. The maximum serum concentration (C_{max}) and the time required to reach C_{max} (T_{max}) of promethazine were determined from the observed data. Other pharmacokinetic parameters were estimated from serum concentration-time curve by using WinNonlin software (pharsight Corporation, CA, USA) with non-compartmental methods. This study was approved by the institutional review board at the Research Institute of Pharmaceutical Sciences, College of Pharmacy, Ewha Womans University after having received written consents from all the volunteers.

RESULTS

Promethazine and I.S. were well separated from endogenous interferences and the retention times of promethazine and I.S. were 12.8 and 20.2 min, respectively (Fig. 2 (B)).

From nine replicate experiments (Table I), the linearity of the calibration curve was described by straight-line regression equation, $y = 0.0302x + 0.0328$ ($r^2 = 0.9999$, $p<0.01$), where y is the peak area ratio of promethazine to I.S. and x is the concentration of promethazine. As shown in Table II, all the intra- and inter-day coefficients of variation (C.V.) were less than 15 %. The accuracy was varied between 93.62 and 110.00 % at four different concentrations including the limit of quantification (LOQ), which was determined to be 1 ng/mL. The mean recovery for the promethazine was evaluated at low (2 ng/mL), medium (10 ng/mL) and high (20 ng/mL) concentration in triplicate. The mean recovery for promethazine was 94.75 %.

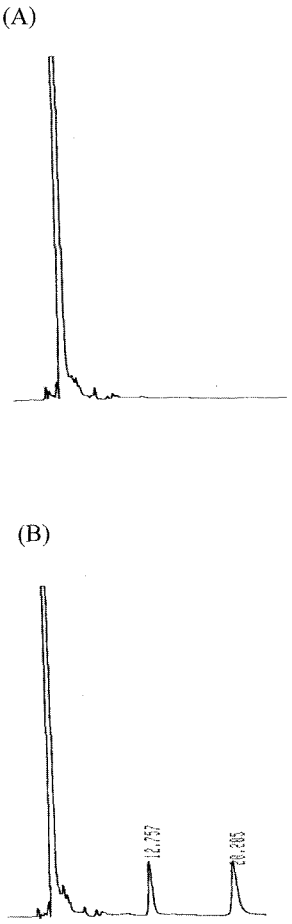

Fig. 2. Representative chromatograms of human blank serum (A) and serum spiked with promethazine and I.S. (B) (Promethazine; 12.757 min, I.S.; 20.205 min).

The validated assay method was successfully applied to the quantitative determination of promethazine in human serum after a single oral dose of 50 mg promathazine to Korean healthy volunteers. The representative chromatograms of a volunteer serum at 0 hr and 3 hr following an oral administration of the drug were shown in Fig. 3. The peaks of promethazine

Table I. Summary of promethazine calibration standards in human serum (n=9).

Conc. (ng/mL)	Peak area ratio of promethazine to I.S.									
	A	B	C	D	E	F	G	H	I	Mean ± S.D.
1	0.07	0.07	0.07	0.06	0.08	0.05	0.06	0.06	0.06	0.06 ± 0.01
2	0.08	0.11	0.09	0.09	0.09	0.08	0.08	0.11	0.10	0.09 ± 0.01
5	0.18	0.19	0.17	0.20	0.17	0.22	0.20	0.17	0.17	0.19 ± 0.02
10	0.33	0.34	0.31	0.38	0.34	0.33	0.30	0.34	0.36	0.34 ± 0.02
20	0.63	0.66	0.68	0.72	0.74	0.57	0.54	0.55	0.59	0.63 ± 0.07
40	1.09	1.25	1.32	1.40	1.33	1.28	0.96	1.17	1.39	1.24 ± 0.14

Table II. Precision and accuracy for the quantification of promethazine in human serum.

Conc. (ng/mL)	Precision C.V. (%)		Accuracy (n=5) (%)
	Intra-day (n=5)	Inter-day (n=5)	
1	14.54	10.18	96.68
2	13.04	14.59	110.00
10	7.01	6.61	96.43
20	6.65	10.54	93.62

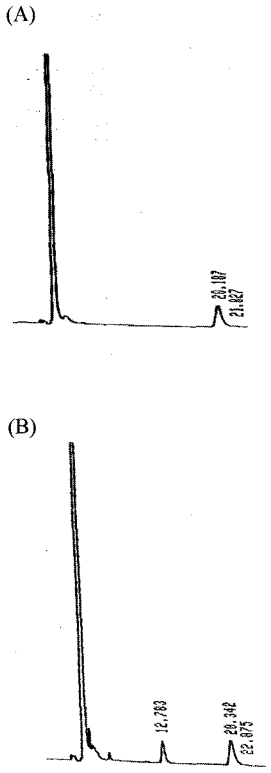

Fig. 3. Representative chromatograms of a volunteer serum at 0 hr (A) and 3 hr (B) after an oral administration of two 25 mg Himazine® tablets (50 mg Promethazine · HCl). (promethazine; 12.783 min, I.S.; 20.187 (A), 20.342 (B) min).

and I.S. were completely separated from endogenous peaks with similar retention times to those obtained from validation studies. Mean serum concentration versus time profile was presented in Fig. 4 and pharmacokinetic parameters of promethazine in Korean subjects were summarized in Table III. The calculated $AUC_{0-\infty}$, AUC_{0-36}, C_{max}, T_{max} and elimination half-life ($t_{1/2}$) of promethazine obtained from Korean subjects were 103.84 ± 84.30 ng · hr/mL, 87.94 ± 81.02 ng · hr/mL, 13.43 ± 10.92 ng/mL, 2.00 ± 1.16 hr and 5.88 ± 3.47 hr, respectively.

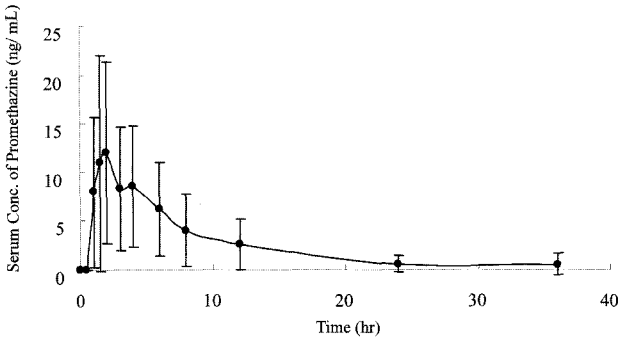

Fig. 4. Mean serum concentration versus time profile following an oral administration of two 25 mg Himazine® tablets (50 mg promethazine · HCl) to eight Korean healthy volunteers.

DISCUSSION

As shown in Fig. 2 (B), the chromatogram exhibited a clear and complete separation among promethazine, I.S. and endogenous substances, suggesting the specificity of the method. The calibration equation from nine replicate experiments, y = 0.0302x + 0.0328 (r^2 =0.9999, $p<0.01$), demonstrated the linearity of the method. According to the bioanalytical validation guide of Korea Food and Drug Administration, the acceptance criteria for intra- and inter-day precision are below 15% C.V. except the concentration of limit of quantification (LOQ) of which allowable range is below 20% C.V.. Also, the acceptable range of accuracy is within 85-115 % of theoretical concentrations except for LOQ (± 20% of theoretical concentration). Based on our results, all the values of precision and accuracy including LOQ were within the specified ranges and therefore acceptable. Thus, the HPLC method for the quantification of promethazine in human serum was validated and shown to be

Table III. Mean pharmacokinetic parameters of promethazine after an oral administration of two 25 mg Himazine® tablets (50 mg promethazine · HCl) to eight Korean healthy subjects (mean ± S.D.).

AUC_{0-36}(ng·hr/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	C_{max}(ng/mL)	T_{max}(hr)	Ke(hr^{-1})	$t_{1/2}$(hr)
87.94 ± 81.02	103.84 ± 84.30	13.43 ± 10.92	2.00 ± 1.16	0.11 ± 0.08	5.88 ± 3.47

AUC_{0-36}: Area under the serum concentration-time curve from zero to 36 hrs
$AUC_{0-\infty}$: Area under the serum concentration-time curve from zero to infinity
C_{max}: Maximum serum concentration
T_{max}: Time required to reach C_{max}
Ke: Overall elimination rate constant
$t_{1/2}$: Elimination half-life

specific, accurate, precise and reproducible.

The validated analytical method was applied to pharmacokinetic study of promethazine in Korean healthy volunteers for the first time. Zaman et al. (1986) previously reported that $AUC_{0-\infty}$, C_{max}, T_{max} and $t_{1/2}$ of promethazine in fifteen American male healthy subjects were 118.00 ± 62.13 ng · hr/mL, 13.99 ± 5.72 ng/mL, 3.00 ± 1.18 hr and 5.89 ± 2.02 hr, respectively.

As shown in Table 3, pharmacokinetic parameters obtained from our study were similar to those reported by Zaman et al. (1986). Also, as reported by other groups (Schwinghammer et al., 1984; Taylor et al., 1983), in the present investigation, high variability in serum concentrations of promethazine was observed after an oral administration, at least in part, due to inter-subject variability of absorption process even at fasting condition. As reported previously (Digregorio and Ruch, 1980; Quinn and Calvert, 1976; Taylor et al., 1983), promethazine significantly binds to plasma protein and undergoes hepatic first-pass metabolism. Therefore, these physicochemical and biological factors influencing oral absorption may change pharmacokinetics of promethazine in human subjects.

ACKNOWLEGMENTS

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