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Determination of domperidone in human plasma by LC-MS and its pharmacokinetics in healthy Chinese volunteers

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KEY WORDS domperidone; high pressure liquid chromatography; electrospray ionization mass spectrometry; pharmacokinetics

ABSTRACT

AIM: To determine the concentration of domperidone in human plasma and investigate its pharmacokinetics in Chinese male volunteers following oral administration of a single dose of the medicine 20 mg. **METHODS**: Plasma samples were processed by liquid-liquid extraction and a liquid chromatographic-mass spectrometric assay was developed for the determination of domperidone in human plasma. **RESULTS**: Assay linearity was obtained in the range of 0.52 - 154.5 μg/L (r = 0.9999). The recovery of domperidone from human plasma was more than 75 %. The intraday and interday relative standard deviations (RSD) for the lowest concentration examined (0.52 μg/L) were 9.4 % and 7.6 %, respectively. The method was utilized to determine the concentration of domperidone in Chinese male volunteers. The concentration-time curve was fitted to a two-compartment model. Its main pharmacokinetic parameters were: T_{max} = (0.8 ± 0.7) h, C_{max} = (50 ± 32) μg/L, $T_{1/2}$ = (7.8 ± 1.6) h. **CONCLUSION**: The method described in this report was of high sensitivity and selectivity for accurate determination of the plasma concentration of domperidone in human.

INTRODUCTION

Domperidone, 5-chloro-1-{1-[3-(2, 3-dihydro-2-oxo-1*H*-benzimidazole-1-yl) propyl]-4-piperidinyl}-1, 3-dihydro-2*H*-benzimidazol-2-one, is a potent peripheral dopaminergic antagonist which has been reported to be effective in functional gastrointestinal disorders such as dyspepsia, gastroesophageal reflux, nausea, and vomiting[1]. Moreover, to prevent the common side effects of most Parkinsonian medications, domperidone is usually co-administrated with other dopaminergic antagonists, such as apomorphine, in Parkinson's disease therapy[2-5]. Radioimmunoassay (RIA) method was reported to assay the concentration of domperidone in plasma, but the hydroxylated metabolites showed some cross-reactivities with domperidone[6,7]. Recently, Yamamoto *et al* described quantitative determination method of domperidone by high-performance liquid chromatography with fluorescence detection[8]. In the present study, we established a new method that was highly sensitive and selective for quantification of domperidone in plasma using liquid chromatography-mass spectrometry (LC-MS) and investigated pharmacokinetics of domperidone in healthy Chinese volunteers.

MATERIALS AND METHODS

Instrument and reagents HP1100 LC-MS system (Hewllet Packard Company, USA) equipped with binary pump, on-line vacuum degasser, autosampler, column compartment, diode array detector, mass spectrometry detector of electrospray interface, and HP ChemStation Rev A.06.03. Domperidone was obtained from Hainan Yazhou Pharmaceutical Company Ltd (Haikou, China). The internal standard cisapride was from Shanghai Hefeng Pharmaceutical Company (Shanghai, China). Methanol was of HPLC grade (Tedia Company, USA). Double distilled water was prepared in our laboratory. The stock solutions of domperidone and cisapride were prepared by disolving 20 mg of the free base materials in 100 mL of methanol. Aliquots were subsequently diluted with methanol to yield stock solutions 0.1 g/L. All stock solutions were stored at 4 ℃, and were found to be stable for at least one month.

Liquid chromatographic conditions Mobile

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phase: 0.5 % acetic acid-methanol (60 : 40, v/v); column: Shimadzu VP-ODS, 5 μm, 150 mm × 4.6 mm ID maintained at 25 ℃; flow rate: 1.0 mL/min.

Mass spectrometry detection Electrospray ionization mass spectroscopy (ESI-MS) was carried out on a HP1100 LC-MS series system. The ESI ion source was set in positive ion polasity mode for acquiring all mass spectrometry data. The selective ion mooitoring (SIM) was set at m/z 426 for domperidone and m/z 466 of cisapride. The fragmentor, drying gas flow, drying gas temperature, nebulizing pressure, and capillary voltage were set to 70 V, 10 L/min, 350 ℃, 172 kPa, and 4000 V, respectively.

Extraction procedure Plasma sample or standard 1 mL, the working internal standard solution 10 μL (9.2 ng cisapride), and NaOH solution (2 mol/L) 0.1 mL were added to a test tube. The sample was mixed, then extracted with 5 mL of ethyl acetate using vortex for 3 min, and centrifuged at 1000 × g for 5 min. The top organic layer 4 mL was transferred to another tube, and evaporated to dryness at 50 ℃ under a gentle stream of nitrogen. The residue was reconstituted in 0.1 mL of mobile phase, and centrifuged at 10 000 × g at 4 ℃ for 10 min, then the upper aliquot of this (20 μL) was injected into the LC-MS.

Human subjects and blood sampling According to the Helsinki Declaration of 1975, eighteen healthy male volunteers, who had given their informed consents, with a body weight of (62 ± 6) kg and age of 20 – 25 a participated in the study. The subjects abstained for medication, alcohol, and cigarette at least one week before and during human study. The subjects were fasted overnight preceding. A standard breakfast was taken 3 h after dosing. Each subject received a single dose of 20 mg (2 × 10 mg per tablet) domperidone and orally took it with a glass of drinking water (200 mL). Blood sampling was conducted consequently at 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, and 24.0 h following dosing using heparinized test tubes. The blood samples were centrifuged at 1000 × g for 10 min. The resultant plasma was frozen at – 20 ℃ until it could be processed for subsequent LC-MS analysis.

Data analysis Data were expressed as $x \pm s$ and PKBP-N1 Pharmacokinetic Program was used to describe the compartment-model of the plasma concentration-time curve of domperidone and calculate main pharmacokinetic parameters.

RESULTS

Assay of domperidone Under the conditions described, the assay was highly specific, and no endogenous plasma materials interfered with the peak of domperidone or cisapride. Domperidone and cisapride were eluted with retention time of 2.6 and 4.9 min, respectively (Fig 1). The calibration curve was linear from 0.52 to 154.5 μg/L ($f = 0.0782C - 0.0295$, $r = 0.9999$, Tab 1). The limit of reliable quantitation was 0.5 μg/L in plasma. The interday and intraday precision was generally good (< 15 %) throughout the assay range. The absolute recoveries of the analytes were more than 70 % within the concentration range of the assay (Tab 2).

Pharmacokinetic study The plasma concentration-time profile of domperidone following a single oral dose of 20 mg was best fitted to a two-compartment

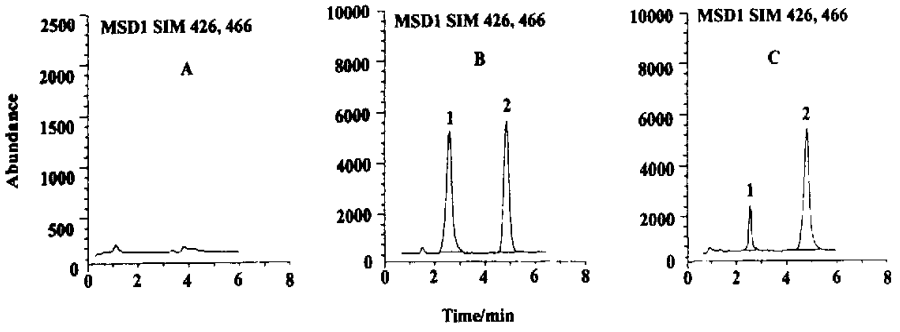

Fig 1. Chromatograms of domperidone using LC-ESI-MS. A) Blank plasma; B) Blank plasma spiked with domperidone and the internal standard; C) Plasma sample after a single oral administration of domperidone tablets. Peak 1: domperidone; Peak 2: internal standard.

Tab 1. Standard curve data of domperidone using LC-MS. $n = 5$.

Concentration/ $\mu g \cdot L^{-1}$	0.52	1.03	2.06	5.15	10.30	25.75	51.50	154.5
A_s/A_{is}	0.0336	0.0769	0.147	0.412	0.718	1.990	3.883	11.48
	0.0363	0.0793	0.149	0.435	0.777	1.923	3.883	11.55
	0.0412	0.0622	0.153	0.424	0.780	1.937	3.910	12.61
	0.0378	0.0651	0.179	0.433	0.770	1.946	4.007	12.16
	0.0326	0.0843	0.170	0.434	0.674	1.910	3.934	12.65
$\bar{x}$	0.036	0.074	0.160	0.428	0.74	1.923	3.92	12.09
s	0.003	0.010	0.014	0.010	0.06	0.019	0.05	0.56

A_s: peak area of domperidone; A_{is}: peak area of internal standard.

Tab 2. Recovery and precision of domperidone in plasma. $n = 5$. $x \pm s$.

Concentration $/\mu g \cdot L^{-1}$	Recovery /%	Precision (RSD)/% Interday	Intraday
0.52	98 ± 15	7.6	9.4
51.5	76 ± 3	4.3	1.3
154.5	85.7 ± 1.3	4.4	4.6

model using PKBP-N1 Pharmacokinetic Program (Fig 2). Domperidone reached its top plasma concentration of (50 ± 32) μg/L at (0.8 ± 0.7) h after dosing, and $T_{1/2}$ was (7.8 ± 1.6) h. Other main pharmacokinetic parameters were listed in Tab 3.

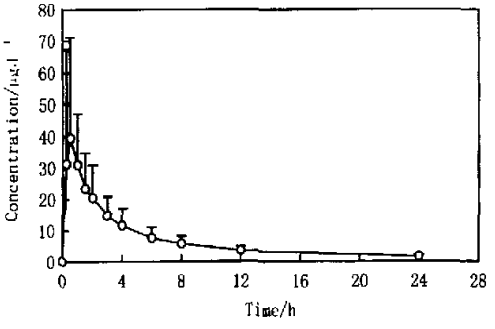

Fig 2. Plasma concentration-time profile after a single oral dose of 20 mg domperidone in Chinese male volunteers. $n = 18$ volunteers. $x \pm s$.

DISCUSSION

Domperidone is generally given with low dose for treatment of gastrointestinal disorders. The plasma concentration of domperidone was too low to be detected by conventional detectors. Therefore, HPLC-UV method was hard to reach the required limit of quantification (LOQ).

Tab 3. Pharmacokinetic parameters of domperidone following a single oral dosing (20 mg) to 18 Chinese male volunteers.

№	k/h^{-1}	$T_{1/2}$/h	C_{max}/ $\mu g \cdot L^{-1}$	T_{max}/h	AUC_{0-24}/ $\mu g \cdot L^{-1} \cdot h^{-1}$	CL/F $L \cdot h^{-1}$
A	0.08	8.2	25.3	1.0	101.6	171.4
B	0.11	6.2	39.2	0.5	157.3	119.5
C	0.11	6.5	66.3	0.5	157.1	118.9
D	0.09	8.1	23.2	2.0	107.8	167.0
E	0.10	7.0	31.6	0.5	100.7	184.1
F	0.09	7.9	63.2	0.3	175.9	102.1
G	0.08	8.5	39.1	0.5	160.4	110.1
H	0.07	10.1	40.3	0.3	107.3	160.0
I	0.09	7.5	62.2	0.5	233.5	77.1
J	0.09	7.6	31.5	1.0	118.4	151.6
K	0.06	11.2	20.9	0.5	96.4	166.3
L	0.12	5.6	51.8	0.5	157.3	119.6
M	0.12	5.7	48.4	0.5	231.8	82.1
N	0.06	10.8	13.6	3.0	124.6	128.2
O	0.10	6.8	52.8	1.5	179.3	102.3
P	0.09	7.8	39.2	0.3	148.2	121.9
Q	0.09	7.8	144.0	0.3	330.9	54.8
R	0.10	7.0	110.5	0.5	332.1	56.1
$\bar{x}$	0.09	7.8	50	0.8	168	122
s	0.02	1.6	32	0.7	72	39

The first pharmacokinetic study on domperidone was reported by Heykants and the colleagues using radio-immunoassay (RIA) to determine the plasma level of the medicine. The hydroxylated metabolites, however, exhibited some cross-reactivities with domperidone[6,7,9-11]. Recently, Yamamoto *et al* described a quantitative method for determination of domperidone by high-performance liquid chromatography with fluorescence detection. The detection limit of was reported to be 1 μg/L with the plasma sample of 1 mL[8].

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In the study presented here, we established a new analytical method using LC-ESI-MS. The interference in the blank plasma chromatograph was greatly cut down in SIM mode, ie, m/z 426 for domperidone and m/z 466 for cisapride, respectively. Baseline separation of domperidone and internal standard was achieved within 6 min. The LOQ of 0.5 μg/L was achieved using this method, which was sensitive enough for determination of domperidone concentration in human plasma. Moreover, the sample extraction procedure was quite simple and stable.

In conclusion, the LC-MS method described in this report was highly sensitive and specific enough for accurate determination of the plasma level of domperidone.

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NOTE After submission of this manuscript, authors noticed the work of Zavitsanos AP *et al* (J Chromatogr B Biomed Sci Appl 1999; 730: 9-24).

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液相色谱-质谱联用法测定人血浆中多潘立酮及其在健康中国志愿者中的药物动力学

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关键词 多潘立酮; 高压液相色谱法; 电子喷雾离子化质量光谱法; 药物动力学

目的: 测定人血浆中多潘立酮的浓度, 并研究在中国志愿者中单剂量口服 20 mg 后的药物动力学过程. **方法**: 用液-液萃取法提取血浆中药物. 采用液相色谱-质谱联用分析手段, 建立测定人血浆中多潘立酮浓度的分析方法. **结果**: 多潘立酮在 0.52-154.5 μg/L 浓度范围内线性良好($r=0.9999$), 其在人血浆中的回收率大于 75 %, 低浓度(0.52 μg/L)的日内、日间差异分别为 9.4 %和 7.6 %. 多潘立酮的血药浓度-经时曲线符合二房室模型, 其主要药物动力学参数为: $T_{max}=(0.8\pm0.7)$ h, $C_{max}=(50\pm32)$ μg/L, $T_{1/2}=(7.8\pm1.6)$ h. **结论**: 本方法专属性强, 灵敏度高, 可准确测定多潘立酮在人血浆中的浓度.

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