ELSEVIER

Journal of Chromatography B, 720 (1998) 251–255

JOURNAL OF CHROMATOGRAPHY B

Short communication

Quantitative determination of domperidone in rat plasma by high-performance liquid chromatography with fluorescence detection

Koujirou Yamamoto[a,*], Mami Hagino[a], Hajime Kotaki[b], Tatsuji Iga[a]

[a]*Department of Pharmacy, The University of Tokyo Hospital, Faculty of Medicine, The University of Tokyo 3-1, Hongo 7-chome, Bunkyo-ku, Tokyo 113, Japan*

[b]*Department of Pharmacy, The Research Hospital, The Institute of Medical Science, The University of Tokyo 4-6-1 Shiroganedai, Minato-ku, Tokyo 108, Japan*

Received 12 January 1998; received in revised form 7 July 1998; accepted 7 July 1998

Abstract

A sensitive and selective analytical method for the determination of domperidone in rat plasma is described. The procedure involves liquid–liquid extraction followed by reversed-phase high-performance chromatographic analysis with fluorometric detection at 282 nm for excitation and 328 nm for emission. The detection limit was 1 ng ml^{-1} using 1 ml of plasma. This assay procedure should be useful for the pharmacokinetic study of domperidone in small animals such as rats.

Keywords: Domperidone

1. Introduction

Domperidone is a dopamine D2 receptor antagonist which has the properties of increasing lower esophageal sphincter pressure, preventing nausea and vomiting and prompting gastrointestinal motility. Since domperidone does not readily enter the central nervous system, therefore it has a lower propensity to cause extrapyramidal side effects than other dopamine antagonists such as metoclopramide and sulpiride. Though domperidone is now widely used as an antiemetics, information about its pharmacokinetic property is very limited because no practical quantitation method has been available. Radio immunoassay was the only method reported previously [1–4] for the determination of unchanged domperidone in biological samples. Meuldermans et al. separated domperidone from possible metabolites by high-performance liquid chromatographic (HPLC) method with ultraviolet detection [5], however, they could not apply the method to their pharmacokinetic study because its sensitivity is insufficient.

For metoclopramide, another antiemetic agent, several assay methods were available including gas chromatography with nitrogen–phosphorus [6], mass spectrometric [7,8] and electron-capture detection [9–11], HPLC methods with ultraviolet [12–17] and

*Corresponding author. Present address: Department of Clinical Pharmacology, Gunma University School of Medicine, 3-39-15, Showa-machi, Maebashi 371-8511, Japan

0378-4347/98/$ – see front matter
PII: S0378-4347(98)00339-9

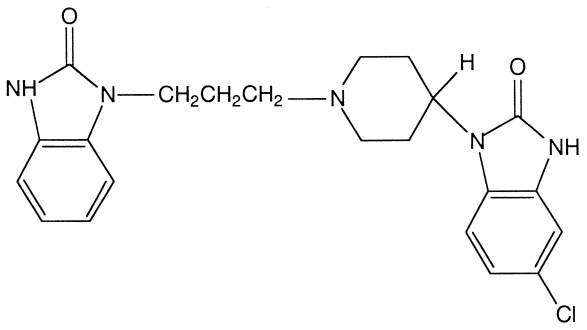

Fig. 1. Chemical structures of domperidone.

fluorescence detection [18,19]. Among these methods, the HPLC method in general seems convenient as minimal sample preparation is required. In the present report, we describe a simple HPLC method with fluorescence detection for the determination of domperidone in rat plasma. The chemical structure of domperidone is shown in Fig. 1.

2. Experimental

2.1. Chemicals

Domperidone was generously supplied from Kyowa Hakko (Tokyo, Japan). All other reagents used were purchased from commercial sources and were of analytical grade. All materials were used without further purification.

2.2. Sample preparation

Blood samples were collected from rats into a heparinized tube and centrifuged for 3 min at 12 000×*g* to separate plasma. To 0.1 ml of each sample, 3 ng of propranolol as an internal standard in 0.1 ml of water, 0.1 ml of 0.01 *M* NaOH and 5 ml of chloroform was added. The mixture was shaken with a mechanical shaker for 10 min and centrifuged at 1620×*g* for 5 min. Then, 4 ml of the organic phase was transferred to another glass tube and 2.5 ml of 0.01 *M* HCl was added. After the mixture was shaken and centrifuged, 2 ml of the upper aqueous phase was transferred to a new tube and 0.05 ml of 1 *M* NaOH and 5 ml of chloroform was added. The mixture was shaken for 10 min, then centrifuged. A 4 ml volume of the lower organic phase was transferred to another tube and evaporated to dryness under gentle nitrogen stream. The dried residue was dissolved in 100 μl of mobile phase, and 50 μl of the aliquot were injected into the HPLC column.

For the quantitation of domperidone below 10 ng ml^{-1}, 0.1 ml of the internal standard solution and 10 μl of 1 *M* NaOH and 5 ml of chloroform was added to 1 ml of plasma sample, then subjected the same procedure described above. To determine the pH of extraction condition, 100 μl of domperidone solution in methanol at the concentration of 10 μg ml^{-1} was mixed with 1 ml of 0.001, 0.01, 0.1 and 1 *M* NaOH, and 45 min after, 20 μl of the mixture was directly injected to HPLC in preliminary study. The recovery of domperidone from mixture was 109, 109, 87 and 67% for 0.001, 0.01, 0.1 and 1 *M* NaOH, respectively. Therefore, extraction to the organic phase was performed with pH 9–10.

2.3. Chromatographic conditions

The HPLC analysis was performed on a Shimadzu liquid chromatograph system (Shimadzu, Kyoto, Japan) equipped with a LC-10AD pump, SCL-10A system controller, DGU-4A degasser, SIL-10AXL autoinjector and RF-10A spectrofluorometiric detector. The detector was set at 282 nm for excitation and 328 nm for emission wavelength. The column was YMC PACK ODS AP-313 column (YMC, Tokyo, Japan, 250 mm×6 mm I.D., 5 μm particle size, 30 nm poresize) and maintained at 25°C by a CTO-10AC column oven. A guard column C-KGC-324APC-3 (YMC, 23 mm×4.0 mm I.D., 5 μm particle size) was attached before the analytical column. The mobile phase was 0.02 *M* phosphate buffer (pH 3.5)–methanol (45:55, v/v) and pumped at a flow-rate of 1 ml min^{-1}.

2.4. Application to pharmacokinetic study

Three male Wistar rats weighing 260–270 g were used in this study. Domperidone was administered intravenously to rats at a single dose of 2 mg kg^{-1}. Blood samples were drawn at 1, 3, 5, 15, 30, 60, 120, 240 and 360 min after administration, and were

immediately centrifuged to obtain plasma. Plasma samples were stored at −20°C until analysis.

3. Results and discussion

3.1. Specificity and linearity

Typical chromatograms obtained from blank and spiked plasma are shown in Fig. 2. The retention time of domperidone and the internal standard was 8.6 min and 11.0 min, respectively. No endogenous compounds appear to interfere with their peaks. A calibration curve was obtained by plotting the peak-area ratio against the concentration of domperidone in plasma. The calibration curves for plasma were linear in the range of 1–100 ng ml^{-1} (y =.00565·x+0.0538, r=1.000, corrected to the sample volume of 0.1 ml), and 100–10 000 ng ml^{-1} (y=.00651·x−0.410, r=1.000), respectively.

3.2. Extraction recovery

The extraction recovery was evaluated by comparing the peak area obtained from the extract of plasma sample with that resulting from direct injection of a known amount of domperidone. Extraction recoveries at six different concentrations were substantially constant as shown in Table 1. The extraction recoveries at lower concentrations were rather high, which may be due to the interfering peak of endogenous substances or contaminated drugs adsorbed to glass materials. Therefore, we had to prepare another calibration curve at lower concentration.

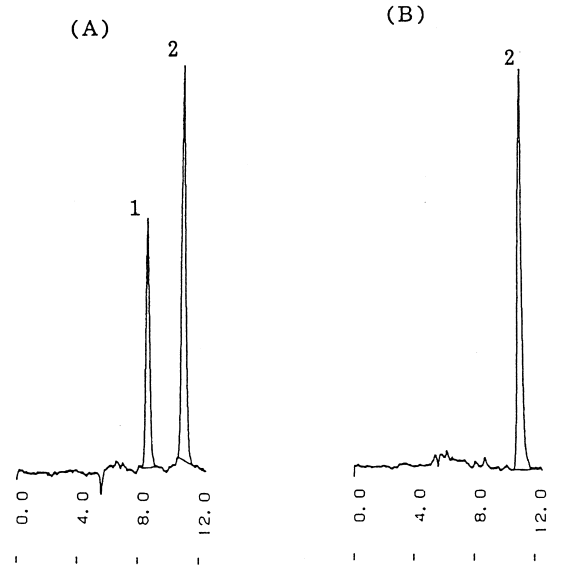

Fig. 2. Chromatograms of rat plasma spiked with 10 ng ml^{-1} of domperidone (A) and drug-free (B). Peaks: 1=domperidone, 2=propranolol. Chromatographic conditions are as described in Section 2.

Table 1
Extraction recoveries of domperidone from plasma

Concentration (ng ml^{-1})	Extraction recovery (n=5, mean±S.D.) (%)
1[a]	97.3±9.3
5[a]	87.1±2.5
10	101.5±5.1
100	83.5±4.2
1000	76.5±8.8
10000	88.3±3.5

[a] Plasma sample size used was 1 ml.

3.3. Reproducibility

The reproducibility of the analysis was determined by repeating the procedure five times for each sample. The coefficient of variation of the peak-area ratio were sufficiently small as shown in Table 2. For the assay of domperidone at lower concentrations, 1 ml of plasma was extracted by similar procedure. The detection limit was 1 ng ml^{-1} with the within-run coefficient of variation and accuracy of less than 5% and 10%, respectively. Huang et al. reported that the concentration of domperidone in human plasma was more than 1 ng ml^{-1} at 12 h after i.v. administration of domperidone at the dose of 20 mg [4],

Table 2
Reproducibility of domperidone analysis in plasma

Concentration (ng ml^{-1})	Coefficient of variation (n=5) (%)
1[a]	5.0
5[a]	3.2
10	9.3
100	3.8
1000	7.9
10000	5.4

[a] Plasma sample size used was 1 ml.

which is clinically relevant dose. Therefore, this method may have enough sensitivity for the pharmacokinetic study in human.

3.4. Application to pharmacokinetics

The analytical method described above was applied to a study of the disposition of domperidone in rats. A chromatogram of real-life sample was shown in Fig. 3. The time course of the plasma concentration and the pharmacokinetic parameters are shown in Fig. 4 and Table 3, respectively. The mean concentration of domperidone in plasma was 15 ng ml^{-1} at 360 min after administration. The apparent elimination half-life from plasma was approximately 73.8 min, which is similar to the results of Heykants et al. [1].

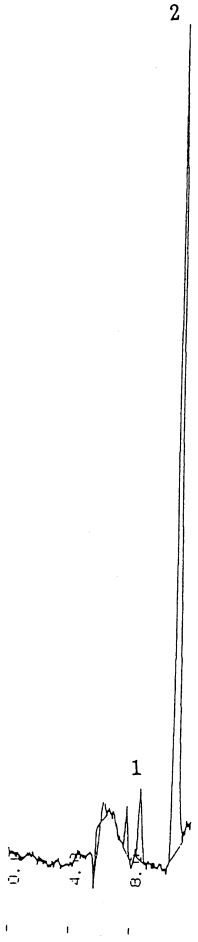

Fig. 3. Chromatograms of rat plasma at 360 min after intravenous administration of domperidone at the dose of 2 mg kg^{-1}. Peaks: 1=domperidone (calculated concentration was 17.5 ng ml^{-1}), 2=propranolol. Chromatographic conditions are as described in Section 2.

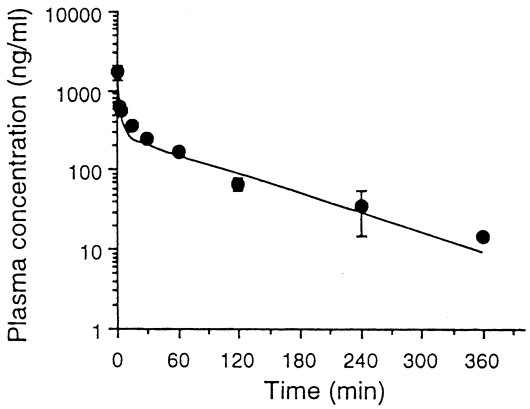

Fig. 4. Time course of domperidone concentration in plasma after intravenous administration of 2 mg kg^{-1} of domperidone to three rats. Each symbol and the vertical bar represents the mean±S.D. The solid line is a fitting line according to a linear 2-compartment model.

Table 3
Mean pharmacokinetic parameters of domperidone after intravenous administration in three rats

Parameters		
$t_{1/2\beta}$	(min)	73.8
CL_{tot}	(ml min^{-1} kg^{-1})	59.4
V_1	(l kg^{-1})	1.06
V_{ss}	(l kg^{-1})	6.39

4. Conclusion

A simple and specific HPLC method with fluorescence detection was established for the determination of domperidone in plasma. Our method would be useful in the pharmacokinetic studies of domperidone, since the domperidone concentration in plasma could be precisely determined at 1 ng ml^{-1}.

References

[1] J. Heykants, A. Knaeps, W. Meuldermans, M. Michiels, Eur. J. Drug Metab. Pharmacokin. 6 (1981) 27.
[2] J. Heykants, R. Hendriks, W. Meuldermans, M. Michiels, H. Scheygrond, H. Reyntjens, Eur. J. Drug Metab. Pharmacokin. 6 (1981) 61.
[3] M. Michiels, R. Hendriks, J. Heykants, Eur. J. Drug Metab. Pharmacokin. 6 (1981) 37.
[4] Y.C. Huang, J.L. Colaizzi, R.H. Bierman, R. Woestenborghs, J.J. Heykants, J. Clin. Pharmacol. 26 (1986) 628.
[5] W. Meuldermans, R. Hurkmans, E. Swysen, J. Hendrickx, M. Michiels, W. Lauwers, J. Heykants, Eur. J. Drug Metab. Pharmacokin. 6 (1981) 49.
[6] R. Saller, D. Hellenbrecht, L. Briemann, A. Hellstern, H. Hess, P. Mitrou, M. Hodgson, G. Achtert, P. Brockmann, H.J. Hausleiter, Clin. Pharmacol. Ther. 37 (1985) 43.
[7] D.N. Bateman, C. Kahn, K. Mashiter, D.S. Davies, Br. J. Clin. Pharmacol. 6 (1978) 401.
[8] K.W. Riggs, A. Szeitz, D.W. Rurak, A.E. Multib, F.S. Abbott, J.E. Axelson, J. Chromatogr. B 660 (1994) 315.
[9] Y.K. Tam, J.E. Axelson, J. Chromatogr. 170 (1979) 157.
[10] L.M. Ross-Lee, M.J. Eadie, F. Bochner, W.D. Hooper, J.H. Tyrer, J. Chromatogr. 183 (1980) 175.
[11] K.W. Riggs, J.E. Axelson, D.W. Rurak, D.A. Hasman, B.A. McErlane, M. Bylsma-Howell, G.H. McMorland, R. Ongley, J.D.E. Price, J. Chromatogr. 276 (1983) 319.
[12] C. Graffner, P. Lagerstrom, P. Lundborg, Br. J. Clin. Pharmacol. 8 (1979) 469.
[13] G.B. Biship-Freudling, H. Vergin, J. Chromatogr. 273 (1983) 453.
[14] W.B. Taylor, S.J. Proctor, D.N. Bateman, Br. J. Clin. Pharmacol. 18 (1984) 679.
[15] H. Takahashi, H. Ogata, H. Echizen, T. Ishizaki, J. Chromatogr. 419 (1987) 243.
[16] A.P. DeJong, A.J. Wittebrood, W.M. Duchantinier, J. Bron, J. Chromatogr. 419 (1987) 233.
[17] D.C. Buss, A.D. Hutchings, S. Scott, P.A. Routledge, Ther. Drug Monitor. 12 (1990) 293.
[18] F. Albani, R. Riva, M. Contin, A. Baruzzi, Biomed. Chromatogr. 2 (1987) 135.
[19] H. Vergin, R. Krammer, V. Nitsche, M. Miczka, K. Strobel, H. Schimmel, Arzneim. Forsch. Drug Res. 40 (1990) 679.