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High-performance liquid chromatographic determination of nemonapride and desmethylnemonapride in human plasma using an electrochemical detection

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Abstract

A high-performance liquid chromatographic method using an electrochemical detector (HPLC–ED) was developed for the determination of nemonapride and its active metabolite, desmethylnemonapride in human plasma. Nemonapride, desmethylnemonapride and moperone chloride, which was used as the internal standard (I.S.) in plasma, were extracted by a rapid and simple procedure based on C_{18} bonded-phase extraction, and were separated by C_8 reversed-phase HPLC column. Nemonapride and desmethylnemonapride were detected by high conversion efficiency amperometric detection at +0.84 V. Determination of both nemonapride and desmethylnemonapride were possible in the concentration range at 0.25–5.0 ng/ml, and the limit of detection for each was 0.1 ng/ml. The recoveries of nemonapride and desmethylnemonapride added to plasma were 97.0–98.2% and 96.7–98.8%, respectively, with coefficients of variation of less than 7.2% and 10.3%, respectively. This method is applicable to drug level monitoring in the plasma of schizophrenia patients treated with nemonapride and to the study of pharmacokinetics.

Keywords: Nemonapride; Desmethylnemonapride

1. Introduction

Nemonapride [(+)-*cis*-*N*- (1-benzyl-2-methyl-pyrrolidin-3-yl- 5 -chloro-2-methoxy-4-methyl-aminobenzamide] is a newly developed antipsychotic drug in Japan (Fig. 1). Nemonapride has a higher selective affinity for dopamine D_2 receptors than other drugs [1], so it is used as a specific ligand for dopamine D_2 receptors. The clinical efficacy of nemonapride in the treatment of both positive and negative symptoms of schizophrenia has been reported in several papers [2]. Correlation of D_2-blocking activity and antipychotic effect has been reported in previous papers. Monitoring of plasma levels of nemonapride, therefore, may be useful for the prediction of therapeutic response and/or side effects. Nemonapride was rapidly absorbed after oral administration, reached peak levels within 2 h, and was rapidly metabolized in the liver enzymatically by *N*-demethylation to desmethylnemonapride [3]. Pharmacological activity of desmethylnemonapride is about 10% less than nemonapride. With therapeu-

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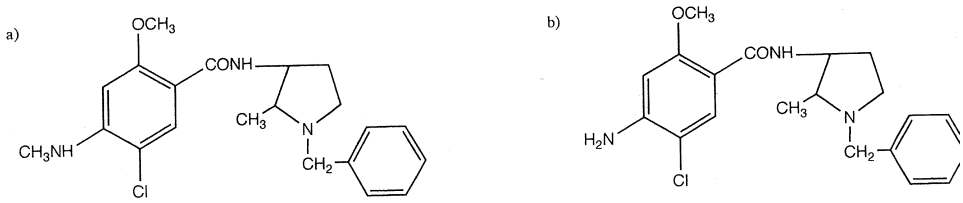

Fig. 1. Chemical structure of (a) nemonapride and (b) desmethylnemonapride.

tic doses of nemonapride, plasma levels of desmethylnemonapride are extremely low. Therefore, a sensitive analytical method for nemonapride needed to be developed for pharmacokinetic studies. A receptor assay for nemonapride in nonhuman biological fluid has been reported [4]. A method for the determination of nemonapride in rat blood and brain by high-performance liquid chromatography (HPLC) using electrochemical detection has been described [5], however determination of nemonapride in human plasma by HPLC method has not been reported. We have reported a simple extraction method of several drugs by using a solid-phase extraction cartridge [6,7]. In the present paper, we describe the development of a highly sensitive HPLC–electrochemical detection method for the simultaneous determination of nemonapride and desmethylnemonapride in human plasma by using this solid-phase extraction method.

2. Experimental

2.1. Reagents and materials

Nemonapride and its active metabolite, desmethylnemonapride (Fig. 1.) were kindly donated by Yamanouchi Pharmaceutical (Tokyo, Japan) and moperone chloride by Daiichi Pharmaceutical (Tokyo, Japan). The Sep-Pak CN cartridge was purchased from Waters (Milford, MA, USA). All solvents used were of HPLC grade (Wako Pure Chemical Industries, Osaka, Japan). All other reagents and chemicals were purchased from Wako Pure Chemical Industries or Nakarai Tesque (Kyoto, Japan).

2.2. Apparatus

The apparatus used for HPLC was a Jasco Model PU-880 chromatography pump (Jasco, Tokyo, Japan) equipped with a Coulochem Model 5100A electrochemical detector (Environmental Science Association, Bedford, MA, USA). The potential of the electrochemical detector was set at +0.84 V vs. a reference electrode. Test samples were introduced using a Rheodyne Model 7120 injector (Rheodyne, Cotati, CA, USA) with an effective volume of 100 μl. The HPLC column contained Develosil C_8-5 stationary phase (5 μm) (Nomura Chemical, Seto, Japan). A stainless steel analytical column (150×4.6 mm I.D.) was packed in this laboratory by a conventional high-pressure slurry-packing procedure. The mobile phase consisted of 0.5 *M* disodium hydrogen phosphate (pH 3.5)–acetonitrile (72:28, v/v); (the pH was adjusted with 50% phosphoric acid before mixing) it was degassed ultrasonically, and the flow-rate was 1.0 ml/min at ambient temperature.

2.3. Preparation of sample

Moperone chloride (10 ng) in methanol (10 μl) was added to the plasma sample (1.0 or 2.0 ml) as an internal standard (I.S.); the plasma sample was then diluted with 5 ml of 1 *M* NaCl in 0.5% KH_2PO_4 (pH 10), and the solution was mixed and allowed to stand for 10 min at room temperature. The mixture was applied to a Sep-Pak C_{18} cartridge. This cartridge was then washed with 10 ml of water and 5 ml of 50% methanol. Then the desired fraction was eluted with 5 ml of methanol. The eluate was evaporated to dryness in a vacuum at 60°C. The residue was dissolved in 30 μl of methanol and 120 μl of mobile phase and filtered through a membrane filter (0.45-

μm pore size). The filtrate was injected directly onto the HPLC apparatus.

2.4. Calibration graphs

Known amounts of nemonapride and desmethylnemonapride in the range 0.25–5.0 ng/ml were added to blank plasma samples, respectively. These plasma samples were treated in the same manner as previously described. Graphs were constructed of the peak-height ratio of nemonapride or desmethylnemonapride to I.S., and plotted against the concentration of nemonapride and desmethylnemonapride, respectively.

2.5. Preparation of quality control and calibration samples

Duplicate samples were prepared in 1.0 or 2.0 ml of plasma, by adding aliquots of the stock solution of nemonapride and desmethylnemonapride at 0.5, 1.0, 2.0, 3.0 and 5.0 ng/ml, to determine the accuracy and precision of this method. These quality control samples were stored at −40°C. Calibration samples containing 0.5, 1.0, 2.0, 3.0 and 5.0 ng/ml of nemonapride and desmethylnemonapride were prepared. The calibration samples were treated in the same manner as previous described. For each validation run quality control samples were thawed and extracted.

2.6. Recovery experiments

Plasma was spiked by adding a known amount of nemonapride and desmethylnemonapride to drug-free plasma to obtain a total volume of 1.0 or 2.0 ml. These samples were extracted by the procedure described above. Control samples were prepared by adding a known amount of nemonapride and desmethylnemonapride to 1.0 or 2.0 ml of methanol. These control samples were not extracted but were directly evaporated to dryness at 60°C, then the residues were reconstituted in 30 μl of methanol and 120 μl of mobile phase. An external standard instead of the internal standard was added to all of the samples before the samples were evaporated to dryness. The accuracy of recovery was determined by comparison between solid-phase extraction with the nonextraction control.

2.7. Method validation

The accuracy of the method was determined by injection of the nemonapride and desmethylnemonapride calibration samples and the four different quality control samples after extraction on six separate days. All calibration curves have a correlation value of at least 0.995. The accuracy was calculated as a percentage of the nominal concentration: Accuracy=(concentration$_{obs}$/concentration$_{nominal}$)·100%. The same data used in the accuracy determinations were used for the calculation of the between-run percentage relative standard deviation (%R.S.D.=(S.D./mean)·100%). The within-run %R.S.D. resulted from analysis of six quality control samples at each concentration with injection on the same day.

2.8. Application to pharmacokinetic studies

Three patients received oral nemonapride (Emilace powder 20 mg/g) at dose amounts of 18, 40 and 90 mg/day at three times in a day for 3 weeks. Blood samples (10 ml) were collected by vein puncture at 0, 1, 2, 4 and 6 h after administration in the morning (AM 8:00). Plasma was separated by centrifugation at 1900 *g* for 15 min and stored at −40°C until analysis. Drug monitoring of nemonapride and desmethylnemonapride was carried out by the previously described methods.

3. Results

The electrochemical detector was used for detection of nemonapride and desmethylnemonapride equipped with coulometric electrodes. Hydrodynamic voltammograms of nemonapride, desmethylnemonapride and moperone chloride as an I.S. were obtained in the HPLC chromatogram. Based on these curves, +0.84 V was the most sensitive potential and half-wave potentials (E1/2) of nemonapride, desmethylnemonapride and I.S. were observed at +0.76 V, +0.82 V and +0.67 V, respectively. A stable and high electrochemical

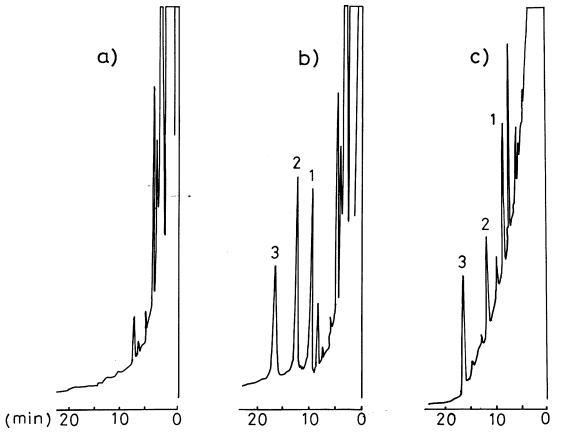

Fig. 2. Typical chromatograms of (a) plasma blank, (b) spiked with nemonapride (2 ng/ml) and desmethylnemonapride (2 ng/ml) in plasma, (c) plasma sample from a patient. Peaks: 1=desmethylnemonapride; 2=I.S.; 3=nemonapride.

response was obtained at +0.84 V. Therefore, the applied potential of this HPLC–electrochemical detection system was set at +0.84 V.

Fig. 2 shows the chromatograms of blank plasma, plasma with added analyte and patient plasma. Low interference from endogenous components in plasma was obtained by this method. Calibration graphs for nemonapride and desmethylnemonapride in human plasma were liner in the range 0.25–5.0 ng/ml (r=0.995) and 0.25–5.0 ng/ml (r=0.986), respectively. The limit of detection for nemonapride and desmethylnemonapride was 0.1 ng/ml (signal-to-noise ratio=5), respectively. The recovery values found for studies are given in Table 1. The recoveries of nemonapride and desmethylnemonapride were determined by adding the following five concentrations to plasma: 0.5, 1.0, 2.0, 3.0 and 5.0 ng/ml of nemonapride and desmethylnemonapride, respectively. The recovery values found for nemonapride and desmethylnemonapride were 97.0–98.2% and 96.7–98.8%, respectively. The relative standard deviation both with intra-day and inter-day assay precision were less than 7.2% and 10.3% at a range of 0.5–5.0 ng/ml, respectively.

The patients who received the therapeutic treatment of nemonapride at 18, 40, and 90 mg/day in divided doses three times a day over 3 weeks had been diagnosed with schizophrenia. The plasma concentration of nemonapride and desmethylnemonapride was determined at 0, 1, 2, 4 and 6 h after the patients received an oral dose of 6, 13.3 and 30 mg of nemonapride in the morning. These results are shown in Figs. 3 and 4. The time required to reach the maximum concentration of nemonapride and desmethylnemonapride was about 1 and 2 h. The maximum concentrations of nemonapride and desmethylnemonapride were 0.6, 0.9, 1.8 ng/ml, and 0.9, 1.9, 3.3 ng/ml at oral doses of 6, 13.3 and 30 mg of nemonapride, respectively. Thus, nemonapride and desmethylnemonapride profiles in plasma showed dose-dependency.

Table 1
Accuracy and precision of determination of nemonapride and desmethylnemonapride in human plasma

Added	Found (mean±S.D.)	Recovery (%)	C.V. (%)	
			Between-day	Within-day
Nemonapride (ng/ml)				
0.5	0.49±0.04	98.2	7.2	6.4
1.0	0.97±0.06	97.4	5.8	4.2
2.0	1.95±0.07	97.6	3.4	4.9
3.0	2.91±0.12	97.0	4.1	4.2
5.0	4.92±0.14	98.2	6.2	4.8
Desmethylnemonapride (ng/ml)				
0.5	0.48±0.06	96.7	10.3	7.4
1.0	0.99±0.09	98.8	8.5	4.8
2.0	1.95±0.10	97.3	4.9	4.2
3.0	2.93±0.13	97.6	4.4	4.1
5.0	4.95±0.16	98.6	5.6	4.6

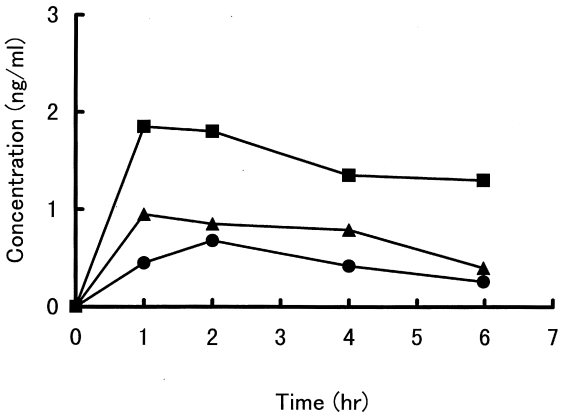

Fig. 3. Plasma concentration–time profile of nemonapride after oral administration in three patients. Nemonapride 6 mg (●); nemonapride 13.3 mg (▲); nemonapride 30 mg (■).

4. Discussion

Our goal in conducting this study was to develop an efficient chromatographic method for the determination of nemonapride and desmethylnemonapride levels in human plasma which would be clinically useful for monitoring patients who are being treated with nemonapride and which would be experimentally useful for the study of nemonapride pharmacokinetics. In a previous paper, Imamura et al. [5] reported a method for the determination of nemonapride levels in rat blood and brain using HPLC with electrochemical detection. However, a method for determining plasma levels of nemonapride and desmethylnemonapride in humans and the profile analysis of nemonapride and desmethylnemonapride have not been previously reported. Patients being treated with nemonapride should be monitored for both nemonapride and desmethylemonapride plasma levels because desmethylnemonapride has 10% of the pharmacological activity of nemonapride. Imamura et al. [5] have described a liquid–liquid extraction method of nemonapride from rat blood and brain. The liquid–liquid extraction, however, was time-consuming and not applicable to HPLC with electrochemical detection because many interfering peaks of endogenous substances appeared on the chromatogram. We therefore directed our project towards establishing a simple extraction method for nemonapride, desmethylnemonapride and I.S. in plasma. In our preliminary experiments, higher recovery was obtained using a solid-phase extraction method with Sep-Pak C_{18} or Sep-Pak C_8 cartridges to which was applied alkaline solution rather than acidic or neutral solutions, and high ionic strength solvents rather than low ionic strength solvents. Therefore, high ionic and alkaline solutions (1 *M* NaCl in 0.5% KH_2PO_4, pH 10) were used for the adsorption of plasma nemonapride, desmethylnemonapride and I.S. on Sep-Pak stationary phases. Lower interference from endogenous components of plasma was obtained by using Sep-Pak C_{18} rather than for Sep-Pak C_8. From among several analytical columns which we tested, we found that a reversed-phase C_8 (150×4.6 mm I.D., 5 μm) column was the most efficient for separation of nemonapride, desmethylnemonapride and I.S. from interfering endogenous substances in human plasma.

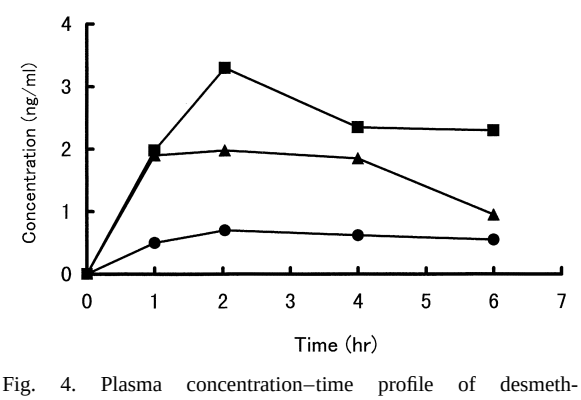

Fig. 4. Plasma concentration–time profile of desmethylnemonapride after oral administration in three patients. Nemonapride 6 mg (●); nemonapride 13.3 mg (▲); nemonapride 30 mg (■).

Good resolution of each compound from each other and interfering substances from plasma was achieved with the present HPLC method. The retention times of desmethylnemonapride, nemonapride and I.S. were approximately 10, 13 and 17 min, respectively. The chromatographic run times were approximately 20 min. The linear relation of calibration graphs for both nemonapride and desmethylnemonapride in human plasma were obtained within a range 0.25–5.0 ng/ml. Ordinary apparatus using 2 ml of plasma sample achieved a high-sensitive detection limit of low concentration. The sensitivity and the calibration range of the present

method were appropriate for therapeutic drug monitoring of nemonapride and desmethylnemonapride in patients. We have found this proposed method to be efficient, accurate and precise.

After oral administration of nemonapride in dosages of 6, 13.3, and 30 mg, the time required to reach maximum concentration of nemonapride was about 1 h and the respective concentrations were 0.6, 0.9, and 1.8 ng/ml. Likewise, after oral administration of nemonapride in dosages of 6, 13.3, and 30 mg, the time required to reach maximum concentration of desmethylnemonapride was about 2 h, later than t_{max} of nemonapride and the respective concentrations were 0.9, 1.9, and 3.3 ng/ml. The fact that the concentration of desmethylnemonapride was higher than nemonapride in all three patients who received nemonapride orally shows that nemonapride was rapidly metabolized by *N*-demethylation to desmethylnemonapride. The concentrations of nemonapride in patient's plasma in the present paper are lower than those reported by Okajima. et al. [3].

The results of the present study indicate that a sensitive and precise method for simultaneous determination of nemonapride and desmethylnemonapride could be applied to pharmacokinetic studies in patients receiving nemonapride treatment. In the future, we are planning to conduct a fixed-dose study of nemonapride in schizophrenic patients to investigate and correlate the relationship between therapeutic effects and side effects of the drug with plasma concentrations of drug and prolactin.

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