A Novel Method for Determination of Methadone in the Serum by High-Performance Liquid Chromatography with Electrochemical Detection

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Received August 30, 2017; accepted December 25, 2017

In March 2013, the clinical use of oral methadone tablets was initiated in Japan. There are many factors responsible for the change in blood concentrations of methadone, and its pharmacokinetics is very complex. Therefore, a simple and accurate measurement method for methadone blood concentrations was developed using HPLC/electrochemical detector (ECD). An eluent of 10 mM Na₂HPO₄/CH₃CN/CH₃OH (20 : 19 : 3) was used as the mobile phase. The column was used the XTerra® RP18, and the voltage of the ECD was set at 400 to 800 mV. As a result, the calibration curve was linear in the ranges of 10 to 100 ng/mL (r²=0.999). The intra- and inter-day coefficients of variation were <5.2 and <5.8%, respectively. Therefore, this method was considered to be useful for the measurement of methadone blood levels in cancer patients. Also, using this method, blood methadone concentration was measured over time in a patient with cancer-associated pain who was treated with methadone. The estimated clearance (CL/F) and distribution volume (Vd/F) of methadone were 2.84 L/h and 502.8 L, respectively, and took about two weeks to reach steady state.

Key words methadone; HPLC; cancer pain; concentration

MATERIALS AND METHODS

Chemicals Methadone HCL (Lot; 1108001190) was obtained from Teikoku Seiyaku Co., Ltd., Kagawa, Japan. The solvents used for the mobile phase were of chromatographic grade. All other chemicals used were of special reagent grade.

HPLC/ECD Chromatograms were obtained using an eluent containing 10 mM Na₂HPO₄/CH₃CN/CH₃OH (20 : 19 : 3) as the mobile phase at a flow rate of 1.0 mL/min. The voltage of the ECD was set at 400 to 800 mV. Data analysis was performed using ChromNAV 1.17 (Nihon Bunko).

Extraction Procedure Serum samples (1.0 mL) were added to 0.5 mL 4 N NaOH, and extracted using 5 mL butyl chloride. The samples were mixed, centrifuged for 10 min at 3000 rpm, and the supernatant containing butyl chloride (top layer) was transferred to a clean tube. The top layer was then evaporated to dryness. The dried residue was dissolved in 200 µL of the mobile phase, and 40 µL of the solution was injected into the HPLC system.

HPLC/ECD Assay Validation The linearity over the measurement range was assessed with standard curves in the range of 10–100 ng/mL (10, 25, 50, 100 ng/mL) using human serum, and the samples were analyzed using the described HPLC/ECD method. Intra-day (n=10) and inter-day (n=5) reproducibility values were determined by replicate analysis of the methadone samples at high (100 ng/mL) and low (10 ng/mL) concentrations. The extraction rate was calculated based on the absolute calibration curve.

Determination of Methadone in Patient Serum A cancer patient using oral methadone hospitalized at KKR Sapporo Medical Center was tested after obtaining written informed consent for blood sampling. This patient was 81 years old female, with liver and renal function normal, methadone oral tablet started at 5 mg three times a day. There was no combination of inducer and inhibitor for CYP3A4 and CYP2B6.

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Blood samples were collected into heparinized tubes for pharmacokinetic evaluation at trough of 24, 48, 120, 288, and 504 h after initial oral administration. The pharmacokinetic parameters of serum methadone were estimated using a one-compartment model with first-order absorption, and the analysis was performed using the software WinNonlin® (Ver 6.4, Pharsight Corporation, CA, U.S.A.). Measurement of serum methadone concentrations was approved by the ethics committee of KKR Sapporo Medical Center.

RESULTS

Chromatographic Separation and Quantitative Response
In the optimal voltage test, the highest peak for methadone was obtained at 800 mV, and the lowest peak was obtained at 400 mV. Therefore, the voltage of the working electrode of the ECD was set at 400–800 mV.

The methadone peak was detected within 3.8 min and separated well from the serum component (Fig. 1). The recovery rate was 86.5%. A linear regression analysis of the standard curve in the range of 10–100 ng/mL yielded the following equation: 
\[ y = 5012.7x + 1041.1 \] (\( r = 0.999 \), Fig. 2). The lower limit of quantification was 0.5 ng/mL (S/N=3). The intra- and inter-day coefficients of variation were <5.2 and <5.8%, respectively (Table 1).

Determination of Methadone in Patient Serum
This analytical method was applied to determine serum concentrations of methadone in a patient with cancer-associated pain. The methadone trough concentrations after administration were as follows: 33.0 ng/mL at 24 h, 37.9 ng/mL at 48 h, 100.1 ng/mL at 120 h, 192.7 ng/mL at 288 h, and 194.3 ng/mL at 504 h. Since this patient’s methadone serum concentrations was high, it was measured using a calibration curve of 25–250 ng/mL (\( y = 3904.2x \), \( r = 0.991 \)). The estimated clearance (CL/F) and distribution volume (Vd/F) of methadone were 2.84 L/h and 502.8 L, respectively. Figure 3 shows the time course of the increase in methadone concentration in the serum. The QTc (corrected QT interval) before, 2 d after, and 21 d after methadone administration were 0.39, 0.42 and 0.43 s, respectively, and it was within the normal range.

DISCUSSION

Several studies have been reported on measurement of blood concentrations of methadone by the fluorescence polarization immunoassay,\(^{13}\) LC/MS/MS method,\(^{14}\) and GC/MS method.\(^{15}\) We aimed to establish a more sensitive method using HPLC/ECD to determine methadone concentration. Using the LC/MS/MS method,\(^{14}\) methadone concentrations ≥5 ng/mL can be measured. Our HPLC/ECD method has a detection limit of 0.5 ng/mL, which makes it possible to
quantify ≥10 ng/mL, and is comparable to the LC/MS/MS method.14) Also, our method can be measured more quickly in about 2 h from the blood treatment to the completion of measurement. Furthermore, for our method, the intra- and inter-day coefficients of variations were ≤5.8%, and the extraction method is simple. Thus, this measurement method may be highly useful to determine serum methadone concentrations in cancer patients treated for pain.

Blood methadone concentration was measured over time in a patient with cancer-associated pain who was treated with methadone. Steady-state concentration was reached at approximately 2 weeks. The elimination half-life was approximately 123 h, which is longer than the 48 h reported by Rostami-Hodjegan et al.16) This could be attributed to the fact that the patient was 81 years old. The serum creatine levels of this patient ranged from 0.6 to 0.66 mg/dL. The Child–Pugh score was 6, which is in the normal range. Moreover, the QT interval was within the normal range during methadone administration, and methadone was safe for use.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES