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Quantitation of pilsicainide in microscale samples of human biological fluids using liquid chromatography–tandem mass spectrometry

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ABSTRACT

This paper describes a sensitive, reliable method to determine pilsicainide (PLC) levels in microscale sample volumes of human biological fluids using liquid chromatography–tandem mass spectrometry (LC–MS/MS) with electrospray ionization (ESI). PLC and quinidine as an internal standard were extracted with diethylether from 0.1 mL of alkalinized biological fluids. The extract was injected into an analytical column (L-column 2 ODS, 75 mm × 2.1 mm i.d.). The mobile phase for separation consisted of 5 mM ammonium acetate (pH 4.5)/methanol (4:1, v/v) and was delivered at a flow rate of 0.2 mL/min. The drift voltage was 100 V. The sampling aperture was heated at 120 °C and the shield temperature was 260 °C. The ion transitions used to monitor analytes were m/z 273 → m/z 110 for PLC and m/z 325 → m/z 79 for quinidine. The total time for chromatographic separation was less than 8 min. The validated concentration ranges of this method for PLC were 5–2000 ng/mL in plasma, 5–500 ng/mL in ultrafiltered plasma solution, and 25–2000 ng/mL in urine. Mean recoveries of PLC in plasma, ultrafiltered plasma solution, and urine were 93.2–99.7%, 91.4–100.6%, and 93.9–104.7%, respectively. Intra- and interday coefficients of variation for PLC were less than 6.0% and 4.3% in plasma, 6.1% and 3.7% in ultrafiltered plasma solution, and 5.4% and 2.5% in urine at the above concentration ranges, respectively. The lower limit of quantification for PLC in plasma, ultrafiltered plasma solution, and urine were 5 ng/mL, 5 ng/mL, and 25 ng/mL, respectively. This method can be applied to pharmacokinetic study and therapeutic drug monitoring in special populations such as neonates, infants, and the elderly by making effective use of residual samples used for general clinical laboratory testing.

1. Introduction

Pilsicainide (PLC) is a class Ic antiarrhythmic agent derived from lidocaine. It was developed in Japan and is widely used for the treatment of supraventricular and ventricular arrhythmia. An overdose of PLC results in adverse drug reactions including circulatory disorders, mental disorders, neuropathy, ventricular fibrillation, ventricular tachycardia, and syncope [1,2]. As the therapeutic range of PLC (0.2–0.9 μg/mL) in plasma [3] is narrow, therapeutic drug monitoring (TDM) is recommended in the clinical practice when PLC is administered to patients with arrhythmia.

In terms of the pharmacokinetics of PLC in humans, about 90% of orally administered PLC is recovered in urine; 75–86% is recovered as the unchanged form, and about 5% is recovered as 2-hydroxymethylate and minor amounts of other metabolites [4]. The elimination half-life ($t_{1/2}$) of PLC is 4.5–4.9 h. As the prolongation of the $t_{1/2}$ of PLC is related to decreased creatinine clearance in the kidneys [5,6], it is necessary to select the dose of PLC depending on the degree of renal dysfunction. Therefore, because the renal clearance of PLC may need to be estimated in those patients, the urinary concentration of PLC must be determined at first.

PLC also binds to α_1-acid glycoprotein, which is an acute-phase reaction protein, and albumin in plasma [7]. Therefore, the unbound plasma concentration of PLC, which is related to its pharmacological effects, will change with differences in the fractions of plasma proteins in various pathophysiological conditions such as inflammatory disease and kidney disease.

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In patients with such conditions, TDM to detect the unbound plasma concentration of PLC is recommended to ensure the administration of an effective dose and/or avoid adverse drug effects. Generally, however, it is not possible to obtain a sufficient volume of ultrafiltered plasma solution for the measurement of the unbound plasma concentration of PLC because the centrifugal separation of plasma usually must be a volume of less than 10%, reflecting the equilibrium between the unbound and bound fractions of the compound. In addition, in some clinical situations, frequent sampling of large volumes of blood is not possible, as in neonates, infants, and the elderly, when TDM must be performed routinely. In such situations, a sensitive method to determine the plasma concentration of PLC which reuses the residual material for general biochemistry laboratory testing would be both efficient and economical as well as minimally invasive.

The mechanism underlying PLC elimination has yet to be clearly understood because the results of some studies on the mechanism of renal elimination of PLC yielded controversial results, and PLC pharmacokinetics show wide interindividual variation, including hepatic/gastrointestinal metabolism and renal excretion. We previously reported that PLC is excreted via the organic cationic transport system in the renal proximal tubule in humans [8] and the elimination of PLC was not inhibited by verapamil, a potent P-glycoprotein inhibitor, in human and experimental studies [9]. In contrast, Tsuruoka et al. [10] suggested that the excretion of PLC in the kidney is mediated by human multidrug resistance protein 1 based on the results of a pharmacokinetic drug–drug interaction study of PLC. Moreover, a case report suggested that the metabolic rate of PLC was increased by cytochrome p450 induction [11]. Therefore, a quantitative method to investigate the detailed elimination mechanism of PLC is needed.

Currently, high-performance liquid chromatography (HPLC) is the main method for determining the concentration of PLC in biological fluids. To the best of our knowledge, only five previous reports described the determination of PLC plasma and/or urine concentrations in humans [4,5,7,8,12]. The limit of detection (LOD) of PLC using solid-phase extraction was reported to be 50 ng/mL in 1-mL sample of both human plasma and urine [4,5], and that using the liquid–liquid extraction method was 10 ng/mL [8] and 50 ng/mL [7] in 0.5-mL sample, respectively. However, while assay methods using a solid-phase extraction column are convenient, they are not economical. Kim et al. [12] used 50 μL of plasma obtained using the deproteination method to assay PLC, although that method cannot measure the unbound PLC concentration.

The liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay has several advantages over HPLC, including high sensitivity and no interference from background peaks due to specific *m*/*z* ion monitoring. The high sensitivity of this assay is suitable for TDM in special populations such as neonates and infants from whom only 100 μL of whole blood can be collected, and/or for measurement of the unbound plasma concentration of PLC since only a microscale volume of sample is required.

Recently, Qui et al. [13] have reported a human pharmacokinetic study of PLC using LC–MS/MS, but this was not a quantitative methodology study. There has been no paper reporting on the quantitative methodology of PLC using LC–MS/MS.

This paper describes a sensitive, reliable method for the determination of PLC in microscale volumes of human plasma, ultrafiltered plasma solution, and urine using LC–MS/MS with an electrospray ionization (ESI) interface, which was validated according to the US Food and Drug Administration (FDA) guidelines [14]. Additionally, this assay is suitable for TDM in hospital and in pharmacokinetic studies in humans following oral administration of PLC hydrochloride (50 mg) because it is more convenient and less time-consuming than other methods.

2. Experimental

2.1. Chemicals

PLC (pK_a 10.2) hydrochloride was kindly provided by Daiichi-Sankyo Pharmaceutical Industry (Tokyo, Japan). Quinidine (pK_a 4.3, 8.4), used as the internal standard (I.S.), was purchased from Wako Pure Chemical Industries (Osaka, Japan). The purity of these materials was greater than 98%. Ammonium acetate, methanol, diethylether, sodium bicarbonate, and water of LC/MS grade were also from Wako Pure Chemical Industries.

2.2. LC–MS/MS conditions

The assay was developed using a 3200 QTRAP LC/MS/MS system (AB SCIEX, Tokyo, Japan), including a LC-20AD prominence liquid chromatograph, DGU-20A3 prominence degasser, CTO-20A prominence column oven, SIL-20AC HT prominence autosampler, and CBM-20A prominence communications bus module (Shimadzu, Kyoto, Japan). The module was controlled by Analyst Software on Windows NT 4.0 and connected to a 3200 QTRAP LC/MS/MS-based mass spectrometer equipped with an ESI source (AB SCIEX). The analytes were separated on an L-column (75 mm × 2.1 mm i.d., particle size 2 μm, CERI, Saitama, Japan), preceded by an L-column precolumn filter. The temperature of the column was maintained at 25 °C. Samples were eluted isocratically using a mobile phase composed of 5 mM ammonium acetate (pH 4.5)/methanol (4:1, v/v) at a flow rate of 0.2 mL/min.

The MS conditions were as follows: curtain gas 40, collision gas 4, ion-spray voltage 5500 V, temperature 700 °C, ion source gas 1–70 psi, ion source gas 2–70 psi, declustering potential 70 V, entrance potential 10 V, collision energy 50 V, and collision cell exit potential 3 V. The positive-ion mode was used, and selected-ion monitoring was performed at *m*/*z* 273 → *m*/*z* 110 for PLC and *m*/*z* 325 → *m*/*z* 79 for quinidine as the I.S.

2.3. Preparation of stock and working solutions

Stock solutions of PLC and the I.S. were prepared by dissolving an appropriate amount of each compound in methanol to yield concentrations of 1 mg/mL for generating standard curves. Working standard solutions of PLC were prepared by serial dilution with 5 mM ammonium acetate (pH 4.5)/methanol (4:1, v/v). The working standard solution of the I.S. (1 μg/mL) was obtained by 1000-fold dilution of the stock solution (1 mg/mL) with 5 mM ammonium acetate (pH 4.5)/methanol (4:1, v/v). Stock solutions were stable at −20 °C for at least 10 months for the purpose of analysis.

2.4. Extraction procedure

A 0.1 mL of I.S. solution (1 μg/mL) and 0.1 mL of Na_2CO_3 (0.1 M) were added to 0.1 mL of human biological fluids (plasma, ultrafiltered plasma solution, or urine) in a 1.5-mL plastic tube. The tubes were vortex-mixed for 10 s, and 1 mL of diethylether was added as an extraction solution. After 30 s of vortex-mixing, the mixture was centrifuged at 9000 × *g* for 5 min at 4 °C, and the organic phase (1 mL) was evaporated to dryness at 40 °C under a stream of nitrogen gas. The residue was dissolved in 250 μL of 5 mM ammonium acetate (pH 4.5)/methanol (4:1, v/v) and vortex-mixed. A 2-μL portion from each sample was injected into the LC–MS/MS system.