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Research paper

Therapeutic drug monitoring of ponatinib using a simple high-performance liquid chromatography method in Japanese patients

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ABSTRACT

A simple and highly sensitive high-performance liquid chromatography (HPLC) method was developed for the quantification of ponatinib in human plasma. The developed HPLC method was validated based on International D.S. Food and Drug Administration guidelines. This technique utilized a solid-phase extraction step and required only 200 μL plasma for a single analysis. The lower limit of quantification for ponatinib was 1.0 ng/mL. Coefficients of variation and accuracies for intra- and interday assays were less than 10.8% and within 13.7%, respectively. The precision and accuracy of our HPLC assay was suitable for pharmacokinetic studies of ponatinib. On day 8 after beginning ponatinib therapy with an initial dose of 15 mg, patients having a ponatinib C_0 of less than 23 ng/mL by HPLC may require a dose adjustment to 30 mg to obtain a C_0 of 23 ng/mL of more. The median ponatinib C_0 in 6 Japanese patients taking a 15 mg daily dose was 24.6 ng/mL, which was greater than the target concentration of 23 ng/mL, and that of patients taking 30 mg increased to a plasma concentration of 48.0 ng/mL. This novel treatment strategy using the HPLC method developed herein may be useful for routine ponatinib therapy.

1. Introduction

Ponatinib is a third-generation tyrosine kinase inhibitor (TKI) that was developed to optimally inhibit native and mutated BCR-ABL, including the gatekeeper mutant T315I [1]. Although the daily standard dose of ponatinib was initially 45 mg, the protocol was subsequently amended to reduce the dose of ponatinib following induction due to concerns over the vascular toxicity of ponatinib. The dose of ponatinib is significantly associated with the occurrence of arterial occlusive events [2]. Moreover, higher ponatinib doses are associated with higher rates of most adverse events [2]. In contrast, O'Hare et al. reported that ponatinib suppresses the emergence of single mutations in BCR-ABL at a concentration of 40 nM [1]. Therefore, evaluation of the ponatinib concentration in plasma may be necessary to provide individual treatment through dose adjustment in order to improve efficacy for native and mutated BCR-ABL or avoid adverse events, such as vascular toxicity, and to transition from ineffective treatment to clinical efficacy. Although therapeutic drug monitoring requires determination of the therapeutic target ranges indicating exposure-response (efficacy/toxicity) relationships, the minimum effect concentration (MEC) of ponatinib may be 40 nM (23 ng/mL), and the ponatinib plasma trough concentration (C_0) may be set at above 23 ng/mL [1]. In the present study, we developed a simple, sensitive, specific high-performance liquid chromatography (HPLC) method for the determination of ponatinib concentrations in human plasma. Using our developed HPLC method, after initiation of ponatinib 15 mg, patients with a ponatinib C_0 23 ng/mL or more were maintained at a dose of 15 mg, whereas patients with a C_0 of less than 23 ng/mL were increased to a starting dose of 30 mg to obtain a C_0 of 23 ng/mL or more. This new treatment strategy using our HPLC method is currently being used for routine ponatinib therapy in Japanese patients.

2. Materials and methods

2.1. Chemicals and reagents

Ponatinib and nilotinib as an internal standard (IS) were purchased from Toronto Research Chemicals, Inc. (Ontario, Canada). An Oasis HLB 1 mL extraction cartridge, which contained 30 mg sorbent of 30-μm particle size per cartridge, was purchased from Waters (Milford,

Abbreviations: C_0, plasma trough concentration; CV, coefficient of variation; HPLC, high-performance liquid chromatography; LC–MS/MS, liquid chromatography-tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; HPLC, high-performance liquid chromatography; MEC, minimum effective concentration; TKI, tyrosine kinase inhibitor; UV, ultraviolet

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MA, USA). HPLC-grade acetonitrile and methanol were purchased from Nacalai Tesque (Kyoto, Japan).

Stock solutions of ponatinib and nilotinib were prepared independently on two occasions, i.e., once for the calibration and once for the quality control. The solutions were prepared by dissolving an accurately weighed amount of compound in HPLC-grade methanol to obtain a final concentration of 1.0 mg/mL. Working standard solutions (200, 1000, 5000, 10,000, and 50,000 ng/mL) of ponatinib and that (2000 ng/mL) of nilotinib were prepared by serial dilutions with HPLC-grade methanol and were used to prepare calibration and internal quality control solutions. All the solutions were stored at −20 °C.

2.2. Analytical methods

To 200 μL plasma, 10 μL nilotinib in methanol (20 ng/10 μL) as an IS and 800 μL of HPLC-grade water were added and vortexed for 30 s. This mixture was applied to an Oasis HLB extraction cartridge that had been activated previously with 1.0 mL methanol and then 1.0 mL water. The cartridge was then washed with 1.0 mL water and 1.0 mL of 60% methanol in water and eluted with 1.0 mL of 100% methanol. Eluates were dried by vortex-vacuum evaporation at 70 °C using a rotary evaporator (AS-ONE CVE-2AS, Osaka, Japan). Each extract was reconstituted with 20 μL HPLC-grade methanol and vortexed for 30 s; 20 μL of mobile phase was continuously added to each sample, and samples were vortexed for an additional 30 s. An aliquot of 20 μL of each sample was then injected into the HPLC apparatus. The analysis of plasma samples was performed on a PU-2080 plus chromatography pump equipped with a UV-2075 plus ultraviolet (UV) detector (JASCO, Tokyo, Japan). The HPLC column was a CAPCELL PAK MG II (250 mm × 4.6 mm; Shiseido, Tokyo, Japan), and the mobile phase was 0.5% potassium dihydrogen phosphate (KH_2PO_4; pH 3.5) and acetonitrile (60:40, v/v), which was degassed in an ultrasonic bath prior to use. Before mixing with acetonitrile, the pH of the 0.5% KH_2PO_4 was adjusted with 50% phosphoric acid. The flow rate was 0.5 mL/min at ambient temperature, and detection of peaks was carried out at a wavelength of 250 nm.

2.3. Calibration line

The calibration line was generated by spiking blank plasma from drug-free healthy volunteers with 1.0–250 ng/mL ponatinib. These blank plasma samples were treated as described above. Calibration graphs were constructed from the peak-area ratio of ponatinib to nilotinib as an IS from the HPLC chromatograms. Linear regression intercepts were not forced through zero.

2.4. Extraction recovery

Extraction recoveries from plasma were determined by comparing the peak areas of extracted plasma samples spiked with known amounts of ponatinib according to the above procedure with those of non-extracted quality control samples. Control samples were prepared by mixing solutions containing the same amount of compound that was added to the blank plasma samples; however, this compound was obtained by evaporating to dryness directly, rather than by extraction, and was then reconstituted in methanol.

2.5. Assay validation

The inter-day precision and accuracy of the assay were determined by analyzing quality control samples on 5 separate days, whereas the intraday precision and accuracy were determined by analyzing spiked quality controls that were run in random order 5 times over the course of 1 day. The precision of the method at each concentration was determined by comparing the coefficient of variation (CV) obtained by calculating the standard deviation as a percentage of the calculated mean concentration. The accuracy estimated for each spiked quality control was obtained by comparing the nominal concentration with the assayed concentration. The limit of quantification (LOQ) was determined as the lowest non-zero concentration measured with a CV of less than 20%, as recommended by international guidelines [3], and the limit of detection (LOD) was determined as the lowest concentration with a signal to noise ratio of 3/1.

2.6. Application to pharmacokinetic studies

The HPLC-UV method was used to measure the plasma concentrations of ponatinib in 6 Japanese patients with chronic myeloid leukemia (CML) or Philadelphia chromosome positive acute lymphoblastic leukemia (Ph + ALL). This study was approved by the Ethics Committee of Akita University School of Medicine, and all patients provided written informed consent. Patients were given a 15-mg daily dose of ponatinib (ICLUSIG®, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) as an initial dose. Whole blood samples were collected just prior to oral ponatinib administration (C_0). After beginning ponatinib therapy, measurement of ponatinib C_0 was performed each week for the first month and then once a month until month 6, with appropriate dosage adjustment. Using our developed HPLC method, after initiation of ponatinib 15 mg, patients with a ponatinib C_0 of 23 ng/mL or more were maintained at a dose of 15 mg, whereas patients with a C_0 of less than 23 ng/mL were increased to a starting dose of 30 mg to obtain a C_0 of 23 ng/mL or more. Plasma was isolated by centrifugation at 1900*g* for 15 min and stored at −20 °C until analysis. Plasma samples (200 μL) were then extracted as described above.

3. Results

3.1. Chromatograms

Typical chromatograms obtained for blank plasma (ponatinib free) spiked with nilotinib as the IS (20 ng) and patient plasma on day 8 after 15 mg ponatinib administration are shown in Fig. 1A and B, respectively. There were no interfering peaks from endogenous substances

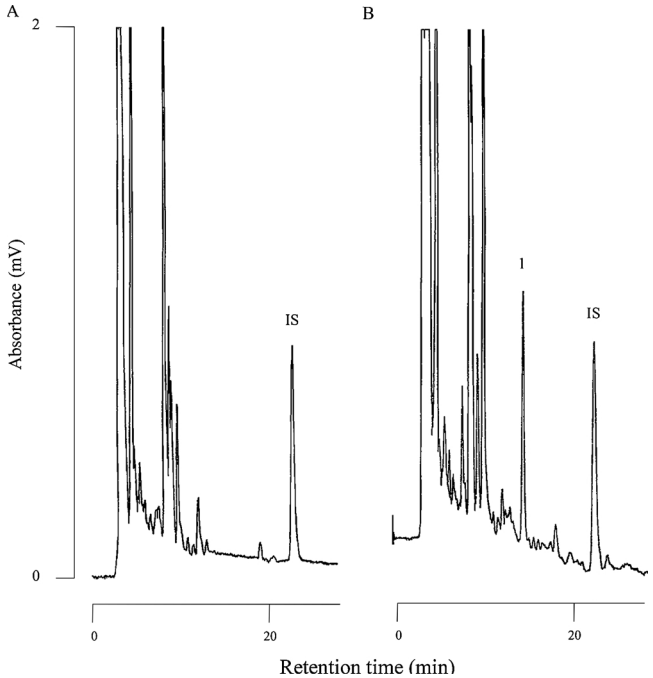

Fig. 1. Typical chromatograms of a blank plasma (ponatinib free) sample (A) and a plasma sample on day 8 after oral administration of 15 mg ponatinib; the plasma concentration of ponatinib was 26.0 ng/mL (B). Peaks: 1 = ponatinib, IS = nilotinib (20 ng).